Efficacy of Four Species of Zingiberaceae Extract Against Vectors of Dengue, Chikungunya and Filariasis

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Abstract. Laboratory bioassays on insecticidal activity of hexane crude extract derived from four species of *Zingiber*: *Zingiber officinale* var. *rubrum* (HZOR), *Zingiber montanum* (HZM), *Zingiber spectabile* (HZS) and *Zingiber zerumbet* (HZZ) were carried out against three mosquito larvae vector: *Ae. albopictus*, *Ae. aegypti*, and *Cx. quinquefasciatus*. GC/MS analysis revealed 43, 82, 50, and 51 compounds from HZO, HZM, HZS, and HZZ, respectively. The major principal constituents found in HZO extract were zingerone (14.92%) and benzaldehyde dimethyl thiol acetal (11.61%); HZM extract were dimethyl 4-methylphthalate (12.64%) and carbendazim (12.62%); HZS extract had 1,1'-ethylenebisdecalin (42.52%) and 1-pentadecyne (11.5%); and HZZ extract were humulene epoxide II (20.84%) and zerumbone (60.4%). Assessment of larvicidal efficacy demonstrated good larvicide effects towards all the crude hexanes. The mortality was observed after 24h exposure. The highest larvicidal mortality of *Ae. albopictus* larvae was found in HZOR, HZM, and HZS (LC$_{50}$= 96.86, 99.04 and 93.35 mg/L; LC$_{90}$= 168.65, 153.77, and 168.65 mg/L) respectively. HZM and HZZ were effective against *Ae. aegypti* larvae with LC$_{50}$= 84.95 and 82.05 mg/L and LC$_{90}$= 134.85 and 121.05 mg/L, respectively. HZZ showed the most effective extract against *Cx. quinquefasciatus* larvae with LC$_{50}$= 49.28 mg/L and LC$_{90}$= 83.87 mg/L. No mortality was recorded in the control. Results from studies suggest that bioassay-guided effective extracts of *Z. officinale* var. *rubrum*, *Z. montanum*, *Z. spectabile* and *Z. zerumbet* are potential larvicidal candidates for controlling *Ae. albopictus*, *Ae. aegypti*, and *Cx. quinquefasciatus*.

INTRODUCTION

Over 2 billion people in the tropics have been infected by mosquito-borne diseases, such as malaria, chikungunya, dengue fever, lympatic filariasis, yellow fever, Japanese encephalitis etc. (Govindarajan *et al.*, 2011). *Aedes aegypti*, *Aedes albopictus* and *Culex quinquefasciatus* are three medically important vectors in Malaysia. *Aedes* species are known as vectors transmitting dengue and chikungunya virus, while *Cx. quinquefasciatus* is a vector that transmits Japanese encephalitis (Vinayachandra *et al.*, 2011).

Chemical control using synthetic insecticides have been used so far and it was favourable because of their speedy action and easy to employ. However, the major problems using the chemicals for controlling the mosquitoes are the development of resistance by the mosquito, damaging environment and also human (Rahuman *et al.*, 2008). Fortunately, plants which are rich in bioactive chemicals probably can be alternative insecticide to control the mosquitoes. Because of this, much effort has been focused on plant extracts or phytochemicals as potential sources of commercial mosquito control agents for the interruption of the transmission of mosquito-borne diseases at the individual as well as at the community level (Govindarajan *et al.*, 2011).

The family *Zingiberaceae* occur chiefly in the tropical regions, especially in the Indo-Malaysian region of Asia with approximately 53 genera and 1,300 species (Larsen *et al.*, 1999). It has several medicinally important genera including *Zingiber*. *Zingiber officinale* var. *rubrum* is morphologically similar to the common ginger (*Zingiber officinale*), but the rhizomes of this variant are smaller, have a stronger and more pungent smell, red on the outside but is yellowish pink in cross section (Ibrahim *et al.*, 2008, Figure 1). *Zingiber montanum* is a perennial, clumping herb (Figure 2). The rhizomes are horizontal creeping, tuberous, cylindrical to ovoid, irregular, palmately and profusely branched, laterally compressed and strongly aromatic with yellow flesh colour (Lim, 2016). *Zingiber spectabile* is a perennial herb, robust, growing up to more than 3 m tall (Figure 3). The inflorescence is cylindrical, cone-shaped and only a few flowers are produced at any one time. Inflorescence bracts form open pouches at the apex. When young, the bracts are yellow turning to deep yellow or orangish-red on maturity. Corolla lobes light yellow, lip dark purple with yellow blotches (Ibrahim *et al.*, 2008). The rhizome of *Zingiber zerumbet* is thick, aromatic, pale to bright yellow in cross section, bitter taste (Figure 4). The inflorescence bracts are green when young turning to red on maturity. The pale yellow or white flowers emerge from the lowest bract first, and when exhausted, the flower dried and falls away. After flowering, the bracts change colour until the entire inflorescence is bright crimson. (Ibrahim *et al.*, 2008). More than 85 species of herbs under this genus are mostly distributed in East Asia and tropical Australia and many species have been used as food and traditional medicine for a variety of ailments, such as in traditional Chinese and Islamic medicine, the fresh rhizome of

Figure 1. The rhizome of *Z. officinale* var. *rubrum* (Picture by Prof Halijah Ibrahim)

Figure 2. The rhizome of *Z. montanum* (Picture by Prof Halijah Ibrahim)

Figure 3. The bract of *Z. spectabile*

Figure 4. The rhizome of *Z. zerumbet* (Photo by Prof. Halijah Ibrahim)

Zingiber officinale is used for colds, headache, vomiting, cough, flatulence and the dried rhizome is used for stomach ache, lumbago diarrhea, and digestion problems (Pushpanathan *et al.*, 2008).

Some studies have been reported for insecticidal activity on Zingiberaceae, for example: Bandara *et al.* (2005) who studied the compound 4-(3',4'-dimethoxyphenyl)buta-1 ,3-diene isolated from rhizome dichloromethane extract of *Zingiber purpureum* showed insecticidal activity against bruchid (Coleoptera: Bruchidae) and larvicidal activity against the second instar of *Ae. aegypti* (L) (Diptera: Culicidae). Crude hexane, ethyl acetate and methanol extracts of eight plants, viz *Aristolochia indica* L., *Cassia angustifolia* Vahl., *Dyospiros melanoxylon* Roxb., *Dolichos biflorus* L., *Gymnema sylvestre* (Retz) Schult, *Justicia procumbens* L., *Mimosa pudica* L., and *Zingiber zerumbet* L., have been tested for adulticidal, repellent and larvicidal activity against adult and early fourth instar larvae of *Cx. gelidus* Theobald and *Cx. quinquefasciatus* Say (Diptera: Culicidae), and revealed that hexane extract of *Z. zerumbet* has the highest larvicide effects against fourth instar larvae of *Cx. quinquefasciatus* (Kamaraj *et al.*, 2010). Madhu *et al.* (2010) studied the efficacy of petroleum ether extract of *Curcuma aromatica* against the larvae of filariasis vector mosquitoes, *Cx. quinquefasciatus*. The essential oils derived from *Zingiber officinale* investigated by Thavara *et al.* (2007) showed moderate repellent activity (85%) against three cockroach species *Periplaneta americana*, *Blatella germanica*, and *Neostylopyga rhombifolia* under laboratory conditions while Pushpanathan *et al.* (2008) found that the essential oils extracted by steam distillation from *Zingiber officinale* was effective for larvicidal and repellent activity against the filarial mosquito *Cx. quinquefasciatus*. Little is known about the insecticidal activity of the *Zingiber* species used in this study. Hence, this present study aims to evaluate the larvicidal potential of the hexane extracts of *Zingiber officinale* var. *rubrum*, *Zingiber montanum*, *Zingiber spectabile* and *Zingiber zerumbet* against three medically important mosquito species namely *Ae. albopictus*, *Ae. aegypti* and *Cx. quinquefasciatus*.

MATERIALS AND METHODS

Plant materials and extraction process

Dry and fresh rhizomes of *Z. officinale* var. *rubrum*, *Z. montanum* and *Z. zerumbet* were purchased from the local market and *Z. spectabile* was collected at Pengajian Luar Universiti Malaya, Gombak and all species were authenticated by Prof. Dr. Halijah Ibrahim. The hexane extraction of plant samples were prepared by soaking 500 g of fine powder of *Z. officinale* var. *rubrum*, *Z. montanum*, *Z. spectabile* and *Z. zerumbet* separately into ± 2.5 L of hexane solvent for three days. After that, the extracts were filtered and were evaporated using rotary vacuum evaporator. Each crude extract was transferred into vial, covered with aluminum foil and kept at -4ºC until further test.

Mosquito cultures

Larvae of *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus* were obtained from the mosquito-rearing laboratory of the Insectary of Medical Entomology Unit, Institute of Medical Research (IMR), Kuala Lumpur. The larvae of *Aedes* species were fed with small chunks of half cooked liver while larvae of *Culex* species were fed with mice chew. The culture was maintained at 25ºC with the humidity between 50% – 70%. The late third instar and early fourth instar were used for the bioassay experiment.

Gas chromatography–mass spectrometry (GC-MS) analysis

The hexane extracts of *Z. officinale* var. *rubrum*, *Z. montanum*, *Z. spectabile* and *Z. zerumbet* were analyzed on an Agilent 6890 GC equipped with 5973 N mass selective detector and an HP-5(5% phenyl methylpolysiloxane) capillary column. The oven temperature was programmed from 50ºC to 280ºC at the rate of 4 C/min and held at this temperature for 5 min. The injector and interface temperatures were 250ºC and 280ºC, respectively. The carrier gas was

helium at a flow rate of 1.0 ml/min (constant flow). The sample (2.0 µl) was injected with a split of 20:1. Electron impact mass spectrometry was carried out at 70 eV. The ion source and quadrupole temperatures were maintained at 230ºC and 150ºC respectively.

Larvicidal bioassays

The mosquito larvicidal bioassays were carried out under laboratory conditions by a slight modification of the standard protocol by WHO (2005). Late third instar and early fourth instar larvae of *Ae. albopictus*, *Ae. aegypti* and *Cx. quinquefasciatus* were tested with HZOR, HZM, HZS and HZZ separately. A total of 0.6 g of the hexane crude extract was diluted in 100 ml of methanol (MeOH) in order to prepare a serial dilution of test concentrations. Three concentrations were prepared for each extract: 70 mg/L, 100 mg/L and 200 mg/L. In the experiment, appropriate concentration of serial dilution was added to 200 ml of distilled water in a 250 ml glass cup and waited for 15-30 minutes to ensure a homogeneous test solution. Each cup was filled with 25 larvae and methanol served as control. All cups were kept at room temperature (25 ± 2ºC; humidity: 80% ± 10% RH). Knockdown (KD) were observed at 6 hours exposure and mortality of larvae were recorded after 24 hours. Observations were also made on the behavior of larvae. Larvae were considered as dead when they did not respond to the stimuli such as probing with a needle in the siphon or cervical region. Moribund larvae were those that were incapable of rising to the surface of the water (within a reasonable period of time) or showing a characteristic diving reaction when the water was disturbed. Some larvae display discolouration, unnatural positions, tremors, incoordination or rigor. The number of moribund and dead larvae in each concentration were calculated and expresses as percentage mortalities. Experiments were done in three replicates and the percentage mortality was reported from the average of three replicates.

Data analysis

The average larval mortality of *Ae. albopictus*, *Ae. aegypti* and *Cx. quinquefasciatus* were recorded. In cases where the control mortality was between 5%–20%, the observed percentage mortality (%M) was corrected by Abbott's formula (Abbott 1925):

$$\% \, M = \frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \, x \, 100\%$$

Due to not normally distribution of this variable, Generalized Linear Model (GLZ) was applied with three source of variations and interactions: *Zingiber* species, concentrations and time exposure. Least Significant Difference (LSD) Fisher test was used for post hoc analyses. Results with $P<0.05$ were considered to be statistically significant different. The LC_{50} & LC_{90} values (with 95% confidence limits) were calculated by probit analysis using Statistical Package for Social Science (Version 21).

The compounds in this present study will be categorized according to Cheng *et al.* (2003) where the compounds with $LC_{50}>100$mg/L were considered not active, compounds with $LC_{50}<100$mg/L were considered active and those with $LC_{50}<50$mg/L were considered highly active.

RESULTS

The yield of the hexane extracts for *Zingiber officinale* var. *rubrum*, *Zingiber montanum*, *Zingiber spectabile* and *Zingiber zerumbet* were 1.72 g, 2.47 g, 1.72 g and 4.33 g, respectively. The chemical compositions of the extracts were analyzed using GC-MS and the results are shown in Table 1. The chemical analysis showed that 43 compounds were identified from HZOR, 82 compounds from HZM, 50 compounds found in HZS, and 51 compounds identified from HZZ.

Table 1. Chemical compositions of the hexane extracts of *Z. officinale* var. *rubrum*, *Z. montanum*, *Z. spectabile* and *Z. zerumbet*

Compounds	Composition (%)			
	HZOR	HZM	HZS	HZZ
Terpinen-4-ol	2.77	2.86		
alpha-curcumene	5.63			
alpha-Bergamotene	2.69			
Zingiberene	8.6			
alpha-sinensal	2.69			
(E)-Beta-Ocimene	2.69			
Bisabolene	2.88			
Beta-Cedrene		1.98		
Beta-sesquiphellandrene	5.81	1.98		
Caryophyllene oxide				1.38
Camphene				3.25
Humulene epoxide II				20.84
cis-decahydro Naphthalane				20.84
6,7-Dimethoxyquinoxaline		15.49		
Zerumbone				60.4
Benzoic acid		4.17		
E-geranyl acetone	1.36			
Neryl acetone	1.36			
Dimethyl 4-methylphthalate		4.64		
2,4,5-trichlorophenol		10.32		
Benzaldehyde dimethyl thiol acetal	11.61			
Zingerone	14.92			
1.1'-Ethylenebisdecalin			41.3	
Allopregnanolone			1.28	
Gingerol	7.2			
2-monopalmitin			1.03	
Contortadiol			1.37	
Linoleyl chloride			1.57	
3-Methoxy-L-tyrosine	5.43			
Allomadendrene oxide-(1)			1.37	
Noscapine		5.17		
Dihydrojasmone	1.98			
[6]-paradol	1.98			
Flufylline		1.26		
Campesterol			1.36	
Echimidine		3.13		
gamma-sitosterol	1.66		6.57	1.64
Beta-Sitosterol	1.66		14.33	1.64

High percentage of zingerone (14.92%) and benzaldehyde dimethyl thiol acetal (11.61%) were recorded in HZOR. In HZM, 2,4,5-trichlorophenol (10.38%) and dimethyl 4-methylphthalate (12.64%) were the most abundant compounds. The major compounds for HZS were 1,1'-Ethylenebisdecalin (42.52%) and 1-Pentadecyne (11.5%) while for HZZ, zerumbone (60.4%) and humulene epoxide II (20.84%) were the most abundant.

Overall results in this study showed that hexane extracts of *Z. officinale* var. *rubrum*, *Z. montanum*, *Z. spectabile* and *Z. zerumbet* exhibit good mortality activity on *Ae. albopictus*, *Ae. aegypti* and *Cx. quinquefasciatus* larvae. At 6h exposure, HZOR showed the most effective in inhibiting *Ae. albopictus* larvae with mortality percentage ranging from 20% to 80%. Overall, the HZZ was more effective than other extracts in controlling the *Ae. aegypti* larvae with 96% mortality after being exposed for 6h. Similarly, HZZ possess good larvicidal activity with more than 97% mortality against *Cx. quinquefasciatus* larvae with the same period of exposure. After 24h exposure to the plant extracts, all extracts tested showed high mortality activity that is 20 times higher than that observed for the 6h exposure against all the mosquitoes tested. The control or untreated group did not exhibit any mortality within 6h and 24h exposure.

The results of Generalized Linear Models (GLZ) (Table 2) carried out on mortality of different mosquito larvae using different *Zingiber* species, different time periods and different concentrations and their interaction as variable, revealed significant difference in larval mortality (p<0.05). Following the GLZ ANOVA, means of groups were compared and results showed that the hexane extracts of four *Zingiber* species were not statistically different between HZS and HZZ against larvae of *Ae. albopictus*, while were statistically different against larvae of *Ae. aegypti* and *Cx. quinquefasciatus* (Figure 5).

Table 2. The results of Generalized Linear Models (GLZ) for all factors and interactions effects

| Mosquito species	Source of variations	F value	df	P-value
Ae. albopictus	Time (T)	552.14	1	<0.05
	Concentrations (C)	1092.56	2	<0.05
	Plant species (P)	91.93	3	<0.05
	T x C	89.75	2	<0.05
	C x P	75.26	6	<0.05
	T x P	81.25	3	<0.05
	T x C x P	45.37	6	<0.05
Ae. aegypti	Time (T)	475.02	1	<0.05
	Concentrations (C)	948.04	2	<0.05
	Plant species (P)	300.08	3	<0.05
	T x C	66.54	2	<0.05
	C x P	141.04	6	<0.05
	T x P	37.30	3	<0.05
	T x C x P	116.71	6	<0.05
Cx. quinquefasciatus	Time (T)	807.51	1	<0.05
	Concentrations (C)	914.88	2	<0.05
	Plant species (P)	1113.88	3	<0.05
	T x C	14.17	2	<0.05
	C x P	194.17	6	<0.05
	T x P	206.59	3	<0.05
	T x C x P	372.05	6	<0.05
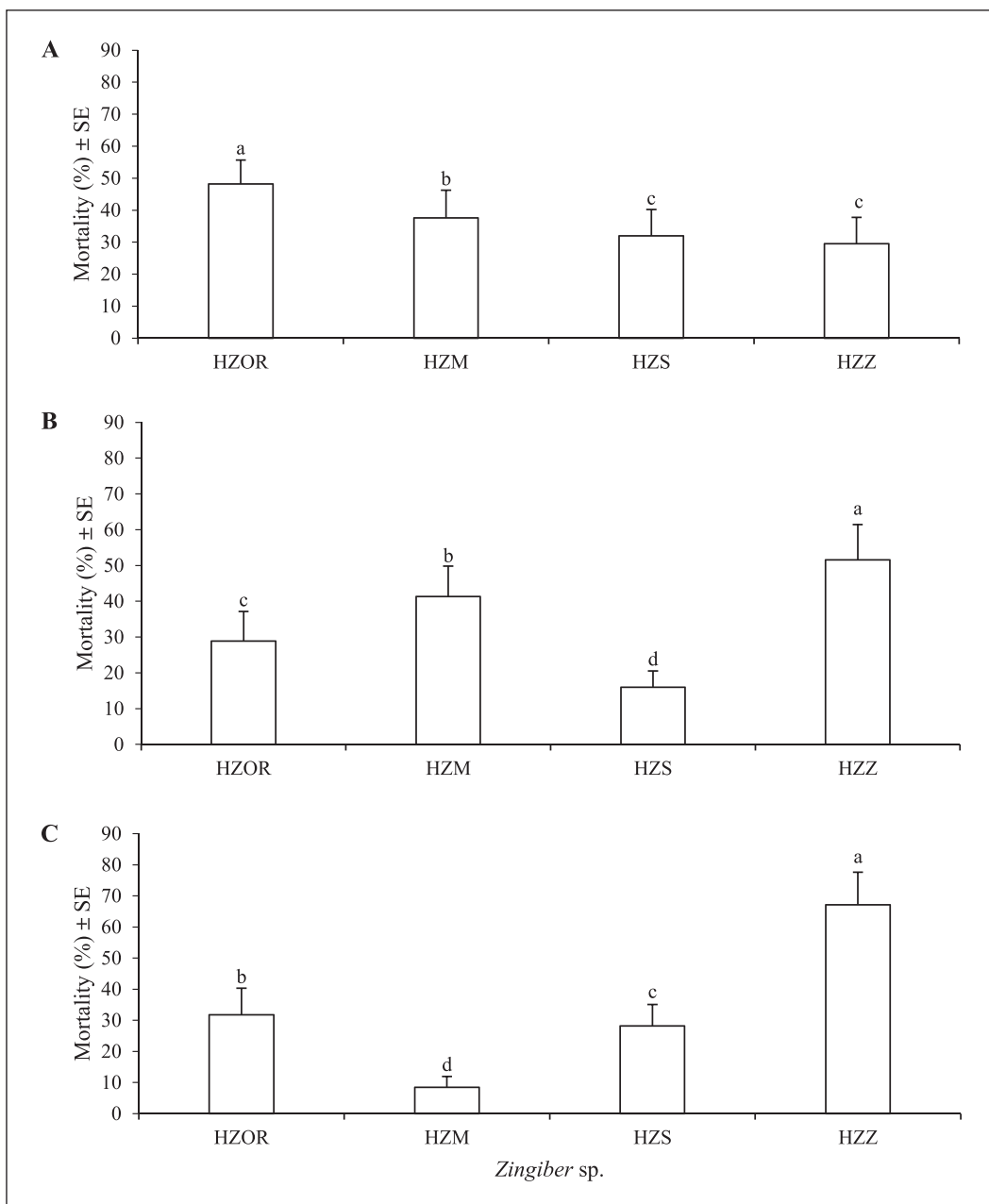
Figure 5. The average of percentage mortality of *Ae. albopictus* (**A**), *Ae. aegypti* (**B**), and *Cx. quinquefasciatus* (**C**) when exposed to four *Zingiber* sp. Means with *same letters above bars* indicate no significant difference (p<0.05).

According to Cheng *et al.* (2003), the compounds with $LC_{50}>100mg/L$ were considered not active, compounds with $LC_{50}<100mg/L$ were considered active and those with $LC_{50}<50mg/L$ were considered highly active. Therefore, among the *Zingiber* species tested, the present results showed that HZS, HZOR and HZM were noted to be active against the larvae of *Ae. albopictus* ($LC_{50}=$ 93.51, 96.86, 99.04 mg/L; $LC_{90}=$ 168.65, 168.65, 153.77 mg/L, respectively) (Table 3). The HZZ and HZM were recorded

Table 3. Log-probit analysis of larvicidal activity of hexane extracts of four *Zingiber* species against larvae of *Ae. albopictus*

Zingiber species	Concentrations	Time of exposure (h)	Mortality (%) ± SD	LC_{50} (mg/L)[a]	95% Confidence limits		LC_{90} (mg/L)[b]	R^2 values
					Lower	Upper		
Z. officinale var. *rubrum*	70 mg/L	6	21.33 ± 8.33	96.86	84.07	111.18	168.65	0.90
		24	33.33 ± 6.11					
	100 mg/L	6	20.00 ± 10.58					
		24	36.00 ± 10.58					
	200 mg/L	6	80.00 ± 4.00					
		24	98.67 ± 2.31					
Z. montanum	70 mg/L	6	0.00 ± 0.00	99.04	87.39	114.87	153.77	0.88
		24	21.33 ± 8.33					
	100 mg/L	6	2.67 ± 2.31					
		24	41.33 ± 20.53					
	200 mg/L	6	60.00 ± 4.00					
		24	100.00 ± 0.00					
Z. spectabile	70 mg/L	6	4.00 ± 6.93	93.51	78.34	108.84	168.65	0.91
		24	32.00 ± 20.78					
	100 mg/L	6	4.00 ± 4.00					
		24	48.00 ± 4.00					
	200 mg/L	6	8.00 ± 4.00					
		24	96.00 ± 4.00					
Z. zerumbet	70 mg/L	6	0.00 ± 0.00	106.57	99.35	115.35	162.77	0.85
		24	17.33 ± 2.31					
	100 mg/L	6	0.00 ± 0.00					
		24	29.33 ± 6.11					
	200 mg/L	6	30.67 ± 2.31					
		24	100.00 ± 0.00					

[a]LC_{50} Lethal concentration that kills 50% of the larvae
[b]LC_{90} Lethal concentration that kills 90% of the larvae

to be active against larvae of *Ae. aegypti* (LC_{50}= 82.05, 84.95 mg/L; LC_{90}= 121.05, 134.85 mg/L, respectively) (Table 4). Overall, HZZ was noted to be highly active against larvae of *Cx. quinquefasciatus* (LC_{50}= 49.28 mg/L; LC_{90}= 83.87 mg/L) (Table 5).

DISCUSSION

Plant extracts and phytochemicals have potential as mosquito control agents because many of them are easily available, bio-degradable and can be applied to mosquito breeding places as alternative insecticides (Ali *et al.*, 2013). David *et al.* (2000) found that phytochemicals primarily affect the midgut epithelium and secondarily affect the gastric caeca and the Malphigian tubules in mosquito larvae. In addition, Maurya *et al.* (2007) found that the crude extracts may be more effective compared with the individual active compounds, due to natural synergism that discourages the development of resistance in the vectors.

The percentage mortality is dependent on the concentration of the tested extracts. Thus, higher inhibition of mosquito larvae was observed in the 200 mg/L compared to 70 mg/L of the extract. As a result, very low concentrations of the tested extracts resulted in low mortality rates. In Table 3, the percentage mortality of the hexane extract of *Z. officinale* var. *rubrum* against *Ae. albopictus* larvae increased from 33.33% at lower concentration to 36.00% mortality at the higher concentration (100 mg/L). Later 98.67% mortality was recorded at the

Table 4. Log-probit analysis of larvicidal activity of hexane extracts of four *Zingiber* species against larvae of *Ae. aegypti*

Zingiber species	Concentrations	Time of exposure (h)	Mortality (%) ± SD	LC_{50} (mg/L)[a]	95% Confidence limits		LC_{90} (mg/L)[b]	R^2 values
					Lower	Upper		
Z. officinale var. *rubrum*	70 mg/L	6	1.33 ± 2.31	120.60	112.03	130.29	187.85	0.91
		24	5.33 ± 2.31					
	100 mg/L	6	1.33 ± 2.31					
		24	29.33 ± 22.03					
	200 mg/L	6	41.33 ± 12.22					
		24	94.67 ± 2.31					
Z. montanum	70 mg/L	6	1.33 ± 2.31	84.95	72.42	97.26	134.85	0.83
		24	24.00 ± 10.58					
	100 mg/L	6	10.67 ± 9.24					
		24	76.00 ± 6.93					
	200 mg/L	6	38.67 ± 8.33					
		24	97.33 ± 2.31					
Z. spectabile	70 mg/L	6	0.00 ± 0.00	155.93	129.29	300.91	315.79	0.90
		24	13.33 ± 6.11					
	100 mg/L	6	1.33 ± 2.31					
		24	16.00 ± 0.00					
	200 mg/L	6	10.67 ± 6.11					
		24	54.67 ± 2.31					
Z. zerumbet	70 mg/L	6	6.67 ± 4.62	82.05	70.93	92.79	121.05	0.86
		24	30.67 ± 14.05					
	100 mg/L	6	2.67 ± 4.62					
		24	73.33 ± 15.14					
	200 mg/L	6	96.00 ± 0.00					
		24	100.00 ± 0.00					

[a]LC_{50} Lethal concentration that kills 50% of the larvae
[b]LC_{90} Lethal concentration that kills 90% of the larvae

higher concentration used in this study (200 mg/L). These findings showed that the concentrations of test substances affected degree of toxicity, mortality speed and mortality rates. The symptoms in larvae treated with each of the *Zingiber* species were dependent on dosage and not affected by different periods of exposure. The symptoms observed showed abnormal behaviors such as restlessness, sluggishness, and coiling movement, and subsequently settled at the bottom of the cup with abnormal wagging, tremors, convulsions, and paralysis, and later died slowly. Therefore, the results clearly showed that larvicidal activity was dose dependent.

The results of the present study are also comparable to earlier reports on the larvicidal activities of plant extracts. Studies by Kamaraj *et al*. (2010) on the larvicidal activity of hexane extracts of *Zingiber zerumbet*, ethyl acetate extracts of *Dolichos biflorus* and methanol extracts of *Aristolochia indica* showed highest larval mortality against *Cx. gelidus* (LC_{50}= 26.48, 33.02, and 12.47 mg/L; LC_{90}= 127.73, 128.79, and 62.33 mg/L) and *Cx. quinquefasciatus* (LC_{50} = 69.18, 34.76, and 25.60 mg/L; LC_{90} = 324.40, 172.78, and 105.52 mg/L). Results of Kamaraj *et al*. (2010) showed higher larval mortality compared to the present study. Studies on insecticidal activity using hexane extracts of *Zingiber* species are limited. However, hexane extracts from other plants, such as hexane extracts of the kernel of *Knema attenuata* exhibited least toxicity with LC_{50} value of 239 mg/L against *Ae. albopictus* larvae (Vinayachandra *et al*., 2011). Warikoo

Table 5. Log-probit analysis of larvicidal activity of hexane extracts of four *Zingiber* species against larvae of *Cx. quinquefasciatus*

Zingiber species	Concentrations	Time of exposure (h)	Mortality (%) ± SD	LC_{50} (mg/L)[a]	95% Confidence limits		LC_{90} (mg/L)[b]	R^2 values
					Lower	Upper		
Z. officinale var. *rubrum*	70 mg/L	6	0.00 ± 0.00	130.58	53.72	313.62	213.81	0.88
		24	9.33 ± 10.07					
	100 mg/L	6	2.67 ± 4.62					
		24	28.00 ± 6.93					
	200 mg/L	6	52.00 ± 14.42					
		24	98.67 ± 2.31					
Z. montanum	70 mg/L	6	1.33 ± 2.31	176.35	73.11	288.51	275.97	0.85
		24	2.67 ± 4.62					
	100 mg/L	6	1.33 ± 2.31					
		24	2.67 ± 2.31					
	200 mg/L	6	4.00 ± 4.00					
		24	38.67 ± 12.22					
Z. spectabile	70 mg/L	6	0.00 ± 0.00	107.78	87.90	128.31	228.83	0.99
		24	20.00 ± 13.86					
	100 mg/L	6	2.67 ± 2.31					
		24	53.33 ± 12.86					
	200 mg/L	6	17.33 ± 6.11					
		24	76.00 ± 4.00					
Z. zerumbet	70 mg/L	6	2.67 ± 2.31	49.28	40.71	58.59	83.87	0.60
		24	93.33 ± 4.62					
	100 mg/L	6	9.33 ± 2.31					
		24	100.00 ± 0.00					
	200 mg/L	6	97.33 ± 2.31					
		24	100.00 ± 0.00					

[a]LC_{50} Lethal concentration that kills 50% of the larvae
[b]LC_{90} Lethal concentration that kills 90% of the larvae

et al. (2012) studied the larvicidal and irritant activities of hexane leaf extracts of *Citrus sinensis* against dengue vector *Ae. aegypti* L. The study found that the citrus leaf extracts from hexane possessed moderate larvicidal efficiency. The bioassay resulted in an LC_{50} and LC_{90} value of 446.84 and 1 370.96 mg/L, respectively after 24 h of exposure. Traboulsi *et al.* (2002) reported that the non-polar phyto products such as hexane extract from plants possess high larvicidal activity.

Compounds identified as 4-gingerol and (6)-dehydrogingerdione and (6)-dihydrogingerdione, isolated from the petroleum ether extract of the rhizome of *Z. officinale* showed good results on larvicidal activity with LC_{50} values of 4.25, 9.80 and 18.20 mg/L on *Ae. aegypti* and 5.52, 7.66 and 27.24 mg/L on *Cx. quinquefasciatus*

respectively (Rahuman *et al.*, 2008). Dua *et al.* (2013) also found that caryophyllene oxide extracted from essential oil of *Psoralea corylifolia* Linn. seeds showed good larvicidal activity against *Cx. quinquefasciatus* with LC_{50} of 63 mg/L and LC_{90} of 99 mg/L. Zerumbone and α-humulene are the main constituents identified from essential oil of *Zingiber zerumbet* exhibited good larvicidal activity against *Ae. aegypti* with LC_{50} of 48 mg/L and LC_{90} of 62 mg/L (Sutthanont *et al.*, 2010). Larvicidal investigation of *Zingiber officinale* essential oil and their major compounds, including zingiberene, citronellol, and β-sesquiphellandrene, demonstrate effective activity against larvae of *Ae. aegypti* (LC_{50}= 46 mg/L; LC_{90}=84 mg/L) (Moon *et al.*, 2011).

The essential oils of *Cupressus macrocarpa* aerial parts and their constituents, including sabinene, α-pinene, and terpinen-4-ol, provided good larvicidal effect against *Ae. albopictus*, with LC_{50} of 54 mg/L and LC_{90} of 84 mg/L (Giatropoulos *et al.*, 2013). The toxicities of β-caryophyllene and α-humulene identified in *Copaifera multijuga* were evaluated against *Ae. aegypti* larvae and found that LC_{50} values were lower than 30 mg/L (Tavares *et al.*, 2013). Compounds detected in essential oils of *Guarea humaitensis* and *Guarea scabra* which are humulene epoxide II and α-trans-bergamotene, effective against *Ae. aegypti* larvae with LC_{50} values of 48 and 98 mg/L; LC_{90} values of 80 and 158 mg/L, respectively (Magalhaes *et al.*, 2010). Qualitative and quantitative variations of the chemical constituents of essential oils result in variations in toxicity against mosquito species (Sukumar *et al.*, 1991). Interestingly, the active larvicidal compounds in these studies, including, zingiberene, zerumbone, β-sesquiphellandrene, humulene epoxide II, terpinen-4-ol, neral, geranial, (E)-β-ocimene, and caryophellene oxide as stated above, were also detected in the hexane extract of *Zingiber* species investigated in the present study. Hummel-brunner & Isman (2001) stated that even minor constituents or other constituents that do not cause mortality if mixed, can result significant effect. Hence, other compounds such as zingerone, gingerol, and etc identified in the hexane extracts of *Zingiber officinale* var. *rubrum*, *Zingiber montanum*, *Zingiber spectabile* and *Zingiber zerumbet* should not be neglected.

In conclusion, this study has added new knowledge on the effects of *Zingiber officinale* var. *rubrum*, *Zingiber montanum*, *Zingiber spectabile* and *Zingiber zerumbet* hexane extracts against *Ae. albopictus*, *Ae. aegypti* and *Cx. quinquefasciatus* which are vectors of dengue, chikungunya and filariasis diseases, respectively. Larvicidal assays clearly demonstrated the toxicity of the hexane extracts of *Z. officinale* var. *rubrum*, *Z. montanum*, *Zingiber spectabile* and *Zingiber zerumbet* against *Ae. albopictus*, *Ae. aegypti* and *Cx. quinquefasciatus*, even at low dosages. Therefore, further studies need to be carried out to investigate the active compound(s) that might possess the larvicidal properties, and subsequently to develop a commercial formulation or product to be used as a mosquitocidal.

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