POTENTIAL OF NATURAL ESSENTIAL OILS AND CINNAMALDEHYDE AS INSECTICIDES AGAINST THE DENGUE VECTOR *AEDES AEGYPTI* (DIPTERA: CULICIDAE)

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Abstract. In order to search and develop a new and more efficacious natural alternative to synthetic chemicals for mosquito control, plants belonging to families known to possess insecticidal properties were selected for investigation of their lethal potential to adult stage. Essential oils isolated from eight indigenous plants using steam distillation were screened individually at a discriminating dosage (15 μg/mg insect) for topical toxicity towards adult female *Aedes aegypti*, Mueang Chiang Mai-susceptible (MCM-S) strain. Dose-response bioassays of the effective oils indicated *Cinnamomum verum* bark oil, LD_{50} value of 3.37 μg/mg female insect, as the most effective agent against MCM-S *Ae. aegypti*. Chemical analysis by gas chromatography-mass spectrometry revealed 16 different compounds, constituting 98.3% of *C. verum* oil composition, the most abundant being cinnamaldehyde (90.2%), followed by 2-propanyl benzene (4.2%) and 3-phenylpropanal (1.2%). LD_{50} value of *C. verum* oil and cinnamaldehyde against adult female MCM-S *Ae. aegypti* was 3.37 and 3.49 μg/mg, respectively, and 3.27 and 3.73 μg/mg, respectively, against adult female Pang Mai Dang-resistant (PMD-R) *Ae. aegypti*, over 1,000 folds less potent than of permethrin, with LD_{50} value of 0.43 and 3.72 ng/mg female against MCM-S and PMD-R strain, respectively. Although permethrin was more effective than *C. verum* oil (and cinnamaldehyde) against adult female *Ae. aegypti*, the former similar effectiveness against both MCM-S and PMD-R strains indicate the potential of developing *C. verum* oil and/or its main bioactive comstituents as natural alternative insecticides to synthetic chemical agents currently employed against adult female *Ae. aegypti*, a vector of dengue virus.

Keywords: *Cinnamomum verum*, *Aedes aegypti*, cinnamaldehyde, essential oil, insecticidal activity

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INTRODUCTION

Dengue fever (DF), together with associated dengue hemorrhagic fever (DHF), are currently considered the most prominent mosquito-borne viral disease

affecting humans worldwide, primarily in developing countries of tropical and subtropical regions (Horstick *et al*, 2015; WHO, 2017). Almost half of the world's population live in dengue-affected areas, resulting in approximately 50-100 million new infections annually, with 500,000 severe dengue cases and around 25,000 dengue-attributed deaths (WHO, 2012).

In Thailand, the incidence rate of dengue diseases is on an increasing trend, with cyclic outbreaks occurring every 2-3 years. In the year 2015, a total of 142,925 dengue cases with 141 deaths were notified throughout Thailand (Ministry of Public Health, 2016). As a vaccine against dengue has been registered only recently in the country (but not as yet approved for general use) and no curative medical treatment is available, prevention and control of disease transmission depend mainly on environmental measures and mosquito management, particularly application of insecticides (WHO, 2009, 2016; Durbin, 2016). Temephos and pyrethroids, which have historically been used as synthetic larvicide and adulticide, respectively, are still the current compounds of choice for controlling mosquito vector populations throughout the country (Chareonviriyaphap *et al*, 2013). In addition to the adverse effects on human health, beneficial non-target organisms and the environment as a whole, the extensive, repeated and indiscriminate use of synthetic insecticides have led also to increased insecticidal resistance of mosquito vectors (Ranson *et al*, 2010; Plernsub *et al*, 2016). The worldwide spread of resistance to conventional chemical insecticides, which have caused the failure of many mosquito control programs, is responsible in part for the current global resurgence of vector-borne infectious diseases, including dengue (Gubler, 2002; Corbel, 2016).

Investigation of insecticide susceptibility in two main species of mosquitoes responsible for DF and DHF in Thailand, namely, *Aedes aegypti* and *Aedes albopictus*, revealed prevalence of insecticidal resistance throughout the country. Resistance to DDT and pyrethroids, including permethrin and deltamethrin, was observed in field-collected populations of *Ae. aegypti* and *Ae. albopictus* from northern Thailand (Somboon *et al*, 2003). In central Thailand, field-caught *Ae. aegypti* was also found to be resistant to deltamethrin (Yaicharoen *et al*, 2005). Testing of susceptibility status by Jirakanjanakit *et al* (2007) demonstrated that while resistance to pyrethroids or together with organophosphate and/or carbamate is documented in most wild-caught *Ae. aegypti* from the northern, central, northern-central, northeastern, and eastern regions of Thailand, no resistance to any of the test insecticides is observed in *Ae. albopictus*. However, recent investigations of three field strains of *Ae. albopictus* collected from Rayong, Koh Chang and Pong Nom Ron revealed varying levels of resistance to five different pyrethroids available in Thailand, namely, bifenthrin, cypermethrin, α-cypermethrin, deltamethrin, and permethrin, and in Pong Nom Ron *Ae. albopictus* is resistant to all pyrethroids tested (Thanispong *et al*, 2015). The spread of pyrethroid resistance in both vectors of dengue virus, *Ae. aegypti* and *Ae. albopictus*, in most areas of Thailand, indicates a possible limitation of new pyrethroid candidates for use in controlling these mosquito species, particularly on resistant populations. Thus, development and application of alternative choices of chemicals or other control strategies should be carried out. Biologically active insecticides of herbal origin, which do not present cross-resistance to current insecticides are also needed as

promising products to aid in the control of mosquitoes.

In recent decades, a growing number of plant-based products, especially essential oils (EOs) and their constituent compounds have been recognized as potential ovicides, larvicides, pupicides, adulticides, antifeedants, and repellents against various species of mosquitoes (Chaiyasit *et al*, 2006; Ebadollahi, 2011; Zoubiri and Baaliouamer, 2011, 2014; Ali *et al*, 2014; Liu *et al*, 2014; Intirach *et al*, 2016). EO-derived insecticides are also now popular among products of natural origin preferred by consumers, which are affordable and eco-friendly. In addition to being available in a wide selection and biodegradable, EOs that contain a multitude of active ingredients with a wide array of modes of action leading to slow development of resistance in mosquito populations are attractive integrated vector control tools (Okumu *et al*, 2007; Pereira *et al*, 2014).

Hence, this study was designed to screen EOs isolated from different plant genera for topical toxicity towards adult female *Ae. aegypti*. The adulticidal potential of the most effective oil and its main constituent also were investigated and compared with that of a synthetic pyrethroid, permethrin, against both pyrethroid-susceptible and -resistant strains of *Ae. aegypti*. The resulting knowledge should be useful in future employment of the EO and/or its major bioactive compound as natural alternatives for improving vector management specifically of populations of mosquitoes fully resistant to a large variety of conventional synthetic chemical insecticides.

MATERIALS AND METHODS

Chemicals

Synthetic permethrin and cinnamaldehyde of analytical standard were purchased from Sigma-Aldrich (St Louis, MO); silicone oil- and pyrethroid-impregnated papers, permethrin (0.75%), λ-cyhalothrin (0.03%), and WHO adulticide test kits from the Vector Control Research Unit, Universiti Sains Malaysia, Penang, Malaysia, which produces the WHO insecticide test kits under the auspices of WHO Pesticide Evaluation Scheme; and all other chemicals and reagents (of analytical grade) from local agencies in Chiang Mai Province, Thailand.

Mosquito maintenance

The mosquito populations available for adulticidal bioassay were free-mating laboratory *Ae. aegypti*, comprising Mueang Chiang Mai-susceptible (MCM-S) and Pang Mai Dang-resistant (PMD-R) strains. The MCM-S strain was established from local specimens collected in areas with pools of stagnant water at various places of Mueang Chiang Mai District, Chiang Mai Province (Sutthanont *et al*, 2010). The PMD-R strain, resistant to permethrin, was established from field specimens collected at Ban Pang Mai Dang, Mae Tang District, Chiang Mai Province (Prapanthadara *et al*, 2002). The MCM-S and the PMD-R *Ae. aegypti* strain was maintained continuously since 1995 and 1997, respectively, in the insectary of the Department of Parasitology, Faculty of Medicine, Chiang Mai University (CMU), Chiang Mai Province. In order to maintain the pyrethroid resistance level, PMD-R strain was reared under drug pressure (Chareonviriyaphap *et al*, 2002).

Each strain of *Ae. aegypti* was colonized separately in a laboratory free of exposure to pathogens at 25 ± 2°C and 80 ± 10% relative humidity under a 14:10 hour light:dark photoperiod cycle. Under these conditions, full development from egg to adult took about 3-4 weeks. Approximately 200 larvae were reared

in plastic trays containing tap water and fed ad libitum on sterilized grounded dog biscuits until pupae emerged. Pupae were transferred to a cup containing tap water and placed in humidified rearing cages (30 × 30 × 30 cm) for adult emergence. Adults were provided regularly with 10% sucrose plus 10% multivitamin solution. Female mosquitoes were fed blood periodically for egg production. Two to five day-old non-blood fed females of each *Ae. aegypti* strain were randomly selected for adult bioassays, including evaluation of susceptibility to pyrethroid insecticides and determination of adulticidal activity of plant EOs.

Evaluation of mosquito susceptibility to pyrethroid insecticides

Susceptibility of *Ae. aegypti* female mosquitoes to synthetic pyrethroids was performed using standard WHO test kits with discriminating doses of 0.75% permethrin and 0.03% λ-cyhalothrin under controlled conditions as described above (WHO, 1998). In brief, four exposure tubes each containing 25 mosquitoes for each test group and placed in a horizontal position were exposed for 1 hour. Then the mosquitoes were provided with a 10% sucrose plus 10% multivitamin solution and kept under observation for 24 hours. Percent mortality in each test group was determined after a 24-hour holding period. The bioassays were performed in four replicates along with negative controls exposed to silicone oil-impregnated filter paper, and the results reported as mean of individual tests.

Plant materials

Eight species of indigenous plants (Table 1) were selected based on the literature on insecticidal properties (Sukumar *et al*, 1991; Shaalan *et al*, 2005; Maia and Moore, 2011; Ghosh *et al*, 2012). The selected plants were purchased from local herb suppliers or collected from different places in Chiang Mai Province. Scientific identification of these plants was determined by Mr James Franklin Maxwell, botanist at the CMU Herbarium, Department of Biology, Faculty of Science, Chiang Mai University (CMU); and Ms Wannaree Charoensup, scientist at the Department of Pharmaceutical Science, Faculty of Pharmacy, CMU. A voucher specimen of each plant was deposited at the Department of Parasitology, Faculty of Medicine, CMU.

Extraction of EOs

Plant specimens were shade-dried separately for 5-10 days in an open area with ventilation at ambient temperature of 30 ± 5°C to remove moisture content prior to extracting the EOs. Coarsely ground dry material of each plant was subjected to steam distillation for at least 3 hours until no more EO could be obtained. The resulting EOs were dried over anhydrous sodium sulfate and kept in an airtight bottle at 4°C until used. In each case, the yield of EO was averaged over three experiments and reported as yield per dry weight of the plant material.

Adulticidal bioassay

Preliminary adulticidal activity of EOs was conducted individually on MCM-S *Ae. aegypti* females at a discriminating dose of 15 µg/mg female using topical application according to a slightly modified version of the standard WHO susceptibility test (WHO, 1996). Each EO was dissolved in ethanol or acetone and tested on a group of 25 non-blood-fed female mosquitoes (2-5 days old). Mosquitoes were weighed individually after being anesthetized temporarily for 30 seconds with carbon dioxide. A 0.1 µl aliquot of EO solution was applied to the

Table 1
Ethnobotanical data, physical characteristics, percent yield, and adulticidal activity against *Aedes aegypti* pyrethroid susceptible (MCM-S) strain of essential oils obtained from eight selected plant species.

Family/species	Common name	Voucher specimen	Part used	Physical characteristic		% Yield	% Mortality[a]
				Appearance	Density (g/ml)		
Cyperaceae							
Cyperus rotundus Linn. Ssp. Rotundus	Nut grass	PARA-CY-001-Rh/3	Rhizome	Pale yellow liquid	0.96	0.12	76.0
Lauraceae							
Cinnamomum verum J. Presl.	Ceylon cinnamon	PARA-CI-007-Ba/1	Bark	Pale yellow liquid	1.02	0.48	100
Rutaceae							
Citrus reticulata Blanco.	Mandarin orange	PARA-CI-004-Pe/2	Peel	Pale yellow liquid	0.84	6.70	4.0
Umbellifereae							
Coriandrum sativum Linn.	Coriander	PARA-CO-002-Fr/4	Fruit	Pale yellow liquid	0.89	0.72	35.0
Zingiberaceae							
Alpinia galanga (Linn.) Willd. var. Galanga	Galanga	PARA-AL-001-Rh/2	Rhizome	Pale yellow liquid	0.89	0.36	84.0
Amomum uliginosum Koenig.	Bustard cardamom	PARA-AM-002-Fr/4	Fruit	Colorless liquid	0.95	0.94	35.0
Kaempferia pandurata Roxb.	Fingerroot	PARA-KA-001-Rh/1	Rhizome	Colorless liquid	0.97	0.25	11.0
Zingiber montanum (Koenig) Link ex Die	Cassumunar ginger	PARA-ZI-007-Rh/2	Rhizome	Pale yellow liquid	0.90	2.29	20.0

[a]100 mosquitoes exposed to 0.1 µl of the test sample for 24 hours.

upper part of the immobilized female's pronotum and solvent-treated mosquitoes served as the negative control group. Mosquitoes in all groups were transferred into plastic cups and provided with 10% sucrose plus 10% multivitamin solution for 24 hours, after which mortality was confirmed by lack of response to mechanical stimuli, and recorded. Each bioassay was carried out under controlled conditions as described above. Four replicates were performed simultaneously for each EO, and the results reported as mean percent mortality of the pooled data.

A dose-mortality response bioassay was carried out on the *Ae. aegypti* MCM-S strain according to the screening protocol (WHO, 1996). In brief, each plant oil was diluted serially in ethanol or acetone to prepare a graded series of 4-7 concentrations. A group of 25 individual females was treated with each concentration, using at least four different concentrations covering a range of mortality from 10% to 90%. Every bioassay was carried out at controlled conditions as described above in four replicates together with controls. Mortality was recorded after a 24-hour holding period and reported as the mean of four replicates. Four experimental treatments were repeated for each test sample using different batches of mosquitoes, and the results were pooled for calculating percent mortality to determine lethal dose (LD) value. The most effective EO, with potentially significant adulticidal efficacy, was subjected to chemical analysis and adulticidal bioassay against both MCM-S and PMD-R *Ae. aegypti* strains.

Chemical analysis of EO

EO chemical profile was determined by gas chromatography-mass spectrometry (GC-MS) at the Science and Technology Service Center, CMU employing a Hewlett-Packard 7890A gas chromatographer (Agilent Technology, Wilmington, DE) equiped with a DB-5MS column (30 m × 0.25 mm ID × 0.25 μm film thickness). and a MSD 5975C (EI) (Agilent Technology). The total GC-MS running time was 20 minutes. The injector and transfer line temperature was 250ºC and 280°C, respectively; the oven temperature programmed from 50º to 250° at 10°C/minute; carrier helium gas at 1.0 μl/minute (constant flow); injection of 0.2 μl [1/10% (v/v), in CH_2Cl_2], and the split ratio of 100:1. An electron impact mass spectrometry with ionization energy of 70 eV was used for GC-MS detection. Data were acquired over a range of 50-550 amu with a scan rate of 2.91 scan/second. The relative constituents content were expressed as normalized percent peak area. Identification of EO constituents was based on their retention indices, determined in relation to a homologous series of n-alkanes (C_8-C_{40}) under the same operating conditions, with computer matching of a database (Wiley 8NO8). The molecular formula and structure of the identified compounds ascertained from the mass spectra were confirmed also by comparison with available authenticated samples.

Data analysis

The 24-hour percent mortality was calculated and corrections made when necessary using Abbott's formula (Abbott, 1925). The corrected data were analyzed by regression analysis using computerized statistical program SPSS (Version 19.0; IBM, Armonk, NY). LD_{50}, LD_{90} and LD_{95} values were calculated using probit analysis with 95% confidence interval (95% CI). Chi-square values were calculated for each bioassay to assess significance and measurement of difference among the test samples, and p-value <0.05

Table 2
Percent mortality of female *Aedes aegypti* MCM-S and PMD-R strains at 24-hour exposure to 0.75% permethrin (PER) and 0.03% λ-cyhalothrin (LAM).

Experiment[a]	Treatment	% Mortality	
		MCM-S strain	PMD-R strain
I	PER	100	58
	LAM	100	82
	Control	0	0
II	PER	100	52
	LAM	100	84
	Control	0	0
III	PER	100	64
	LAM	100	80
	Control	0	0
IV	PER	100	67
	LAM	100	85
	Control	0	0
Mean ± SEM (Range)	PER	100	60 ± 7 (52-67)
	LAM	100	83 ± 2 (80-85)
	Control	0	0

[a]100 female mosquitoes per group. Control, vehicle.

is considered significant. Resistance ratio (RR) was estimated at the LD_{50} level using the formula (RR = LD_{50} of resistant strain/ LD_{50} of susceptible strain), where RR > 1 indicates resistance and RR ≤ 1 susceptible (Ramkumar and Shivakumar, 2015).

RESULTS

Prior to testing with EOs, female *Ae. aegypti* MCM-S and PMD-R strains were evaluated for susceptibility to two commonly used conventional pyrethroids, 0.75% permethrin and 0.03% λ-cyhalothrin, resulting in complete mortality to the MCM-S strain, but mortality of 52-67% and 80-85% after exposure of PMD-R strain to permethrin and λ-cyhalothrin, respectively (Table 2). No mortality was observed in the negative controls of both *Ae. aegypti* strains.

The EOs isolated from eight selected plant species using steam distillation showed a large variation in yield ranging in dry weight from 0.12% (v/w) for *Cyperus rotundus* to 6.70% (v/w) for *Citrus reticulata* (Table 1). The EOs obtained were colorless or pale yellow liquid, less dense than water, except for that of *Cinnamomum verum*. Preliminary bioassay of the EOs via topical application on test mosquitoes revealed a wide variety of toxicity. All EOs were capable of killing *Ae. aegypti* MCM-S females, with a range of 4.0-100% mortality after a 24-hour treatment with 15 μg/mg female (Table 1). Dose-response bioassays revealed highest efficacy from *C. verum* EO (LD_{50} value = 3.37 μg/mg female), followed by that of *Alpinia galanga* (LD_{50} value = 7.97 μg/mg female) and *C. rotundus* (LD_{50} value = 10.05 μg/mg female) (Table 3). No mortality was

Table 3
Adulticidal activity of essential oils derived from eight selected plant species against pyrethroid susceptible (MCM-S) strain of *Aedes aegypti*.

Essential oil dosage (μg/mg female)	% Mortality[a] (mean ± SE)	Adulticidal activity (95% CI) (μg/mg female)			χ^2	df	SE	Regression coefficient
		LD_{50}	LD_{90}	LD_{95}				
Cyperus rotundus								
4.8	17 ± 2	10.05	17.52	19.63	0.34	3	0.02	0.17
6.7	29 ± 2	(9.37-10.78)	(16.00-19.76)	(17.78-22.41)				
9.6	48 ± 2							
11.5	60 ± 1							
14.4	76 ± 1							
Cinnamomum verum								
2.5	20 ± 2	3.37	4.84	5.26	14.46	3	0.04	0.87
3.1	46 ± 2	(3.08-3.61)	(4.43-5.63)	(4.75-6.26)				
3.6	57 ± 3							
4.1	69 ± 2							
4.6	87 ± 2							
Citrus reticulata								
25.2	29 ± 2	34.99	54.89	60.53	1.36	2	0.01	0.06
35.6	47 ± 2	(32.64-37.03)	(51.13-60.63)	(55.81-67.88)				
42.0	66 ± 1							
50.4	86 ± 1							
Coriandrum sativum								
8.9	28 ± 2	19.27	40.82	46.93	0.38	5	0.01	0.06
13.3	36 ± 2	(17.44-20.93)	(37.37-45.76)	(42.57-53.25)				
17.6	46 ± 1							
22.2	57 ± 2							
26.7	65 ± 1							
31.1	77 ± 1							
35.6	84 ± 1							
Alpinia galanga								
7.1	34 ± 1	7.97	10.77	11.57	0.12	2	0.07	0.46
8.1	52 ± 1	(7.61-8.25)	(10.18-11.81)	(10.81-12.92)				
8.9	65 ± 1							
9.8	80 ± 1							
Amomum uliginosum								
12.5	20 ± 2	14.49	17.51	18.36	6.05	3	0.04	0.42
13.5	37 ± 1	(13.71-15.17)	(16.53-19.58)	(17.18-20.98)				
15.2	53 ± 1							
16.1	74 ± 0.5							
17.1	91 ± 2							
Kaempferia pandurata								
19.4	37 ± 1	26.2	52.54	60.01	0.44	2	0.01	0.05
29.1	57 ± 1	(22.43-29.05)	(47.72-60.30)	(53.81-70.24)				
38.8	71 ± 1							
48.5	87 ± 1							
Zingiber montanum								
9.0	28 ± 1	15.56	28.78	32.53	0.58	2	0.01	0.1
13.5	39 ± 1	(14.18-16.92)	(25.76-33.87)	(28.76-38.95)				
18.0	60 ± 1							
22.5	75 ± 1							

[a]400 female mosquitoes (4 replicates) exposed to 0.1 μl of the test sample for 24 hours.

Table 4
Chemical composition of *Cinnamomum verum* bark essential oil.

No.	Chemical constituent	RT (min)	KI	Composition (%)
1	Benzaldehyde	5.1	970	0.10
2	α-Limonene	6.06	1036	0.03
3	1,8-Cineole	6.13	1041	0.23
4	α-Phellandrene	7.06	1112	0.04
5	3-Phenylpropanal	8.06	1171	1.23
6	Isoborneol	8.24	1182	0.15
7	P-Cymene	8.34	1189	0.21
8	Cinnamaldehyde	9.83	1293	90.17
9	L-Calamenene	11.1	1387	0.98
10	2-Propynyl benzene	11.94	1453	4.20
11	γ-Muurolene	12.36	1485	0.04
12	Zizanene	12.65	1508	0.12
13	Cis-Calamenene	12.95	1533	0.44
14	Torreyol	14.42	1657	0.18
15	Guaiazulene	14.56	1670	0.05
16	Cadalin	15.35	1740	0.11
	Total			98.28
	Phenylpropanoids			91.4
	Simple aromatic compounds			4.3
	Hydrocarbon sesquiterpenes			1.92
	Oxygenated monoterpenes			0.38
	Hydrocarbon monoterpenes			0.28

RT, Retention time; KI, Kovats index relative to n-alkanes (C_8-C_{40}) on a DB-5MS column.

observed in the negative controls.

Thus *C. verum* EO was chosen for investigation of its chemical composition by GC-MS analytical technique that showed the presence of 16 compounds, accounting for 98.28% of the *C. verum* EO composition (Table 4). The main constituent was cinnamaldehyde (90.17%), together with minor amounts of 2-propynyl benzene (4.2%) and 3-phenylpropanal (1.2%) (Fig 1). Phenylpropanoids (91.4%) were the major class of compounds in *C. verum* bark oil. The relative amounts of the remaining 13 compounds ranged from 0.03% to 0.98%. The adulticidal activity of *C. verum* oil, cinnamaldehyde and permethrin against adult female MCM-S and PMD-R *Ae. aegypti* was shown in Table 5. LD_{50} values of *C.verum* against MCM-S and PMD-R strains were 3.37 and 3.27 μg/mg female, respectively (RR=0.97). Cinnamaldehyde also provided comparable effectiveness on MCM-S and PMD-R strains, with LD_{50} values of 3.49 and 3.73 μg/mg female, respectively (RR=1.07). On the other hand, permethrin was exquisitely more effective against MCM-S (LD_{50} value = 0.43 ng/mg female) and PMD-R (LD_{50} value = 3.72 ng/mg female) strains (RR = 8.65) (Table 5).

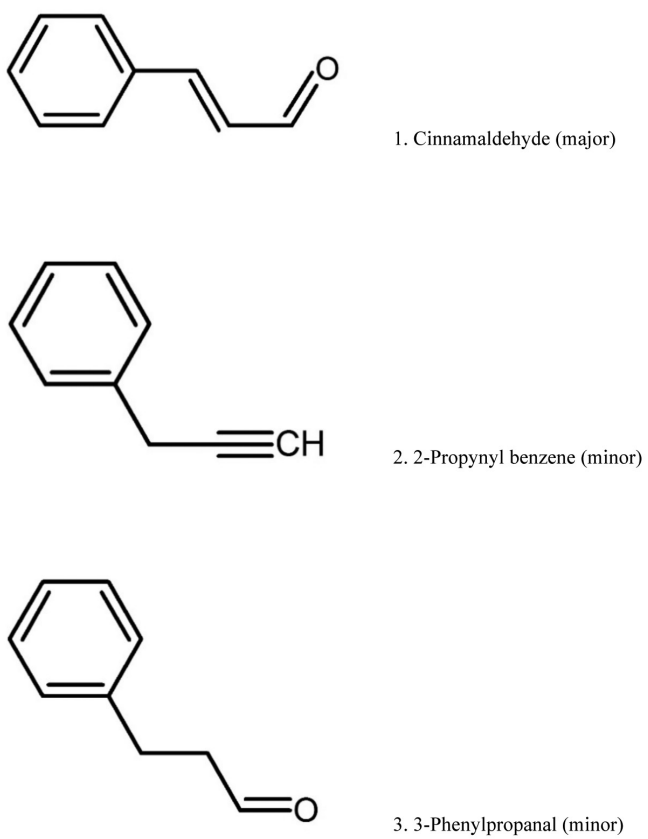

Fig 1-Chemical structures of the major and minor constituents of *Cinnamomum verum* bark essential oil.

DISCUSSION

This study shows that among eight Thai local plants, *C. verum* EO and its main constituent cinnamaldehyde exhibit the best adulticide activity equally against female *Ae. aegypti* MCM-S and PMD-R strains from northern Thailand. However, these agents were 1,000 folds less potent against PMD-R strain than permethrin, and to which MCM-S strain was about 8,000 times less sensitive.

Varied degrees of susceptibility to pyrethroid insecticides observed in this study indicate *Ae. aegypti* PMD-R strain proved to be highly resistant to permethrin but moderately resistant to λ-cyhalothin, based on the WHO criteria for resistance (WHO, 1998). Similar results obtained from the study of Chaiyasit *et al* (2006) demonstrated different susceptibility to pyrethroids in two populations of *Ae. aegypti*, laboratory and field strains, collected in Mueang Chiang Mai District, Chiang Mai Province. While resistance (51-66% mortality) and mild susceptibility (82-88% mortality) were observed in the natural field strains of *Ae. aegypti* exposed to 0.25% permethrin and 0.03% λ-cyhalothrin, respectively, the laboratory-reared *Ae. aegypti* is completely susceptible to these insecticides, as evidenced by 100% mortality in all the cases. All 32 populations of *Ae. aegypti* collected from different provinces throughout Thailand show evidence of incipient resistance (62.5% mortality) or levels of survival deem resistant (37.5% mortality) to permethrin (Chuaycharoensuk *et al*, 2011). High resistance to 0.75% permethrin was observed in *Ae. aegypti* collected from the northern provinces, including Chiang Mai, Kamphaeng Phet and Lampang, with mortality of 44.59%, 50.0% and 43.54%, respectively. However, all the collected *Ae. aegypti* are still 100% susceptible to 0.05% λ-cyhalothrin. Srisawat *et al* (2012) also reported that all field-collected *Ae. aegypti* from the central provinces of Thailand, including Bangkok, Prachin Buri and Ratchaburi, are highly resistant to 0.75% permethrin, with mortality of 0-63%, 35%, and 51%, respectively. The resistance of *Ae. aegypti*

Table 5
Adulticidal activity of *Cinnamomum verum* essential oil, cinnamaldehyde and permethrin against pyrethroid-susceptible (MCM-S) and -resistant (PMD-R) strains of *Aedes aegypti*.

Test material	% Mortality[a] (mean ± SE)	Adulticidal activity (95% CI, µg or ng/mg female)			χ^2	df	SE	Regression coefficient
		LD_{50}	LD_{90}	LD_{95}				
C. verum oil (µg/mg female)								
MCM-S								
2.55	20 ± 2	3.37	4.84	5.26	14.46	3	0.04	0.87
3.06	46 ± 2	(3.08-3.61)	(4.43-5.63)	(4.75-6.26)				
3.57	57± 3							
4.08	69 ± 2							
4.59	87 ± 2							
PMD-R								
2.55	23 ± 2	3.27	4.51	4.86	7.43	3	0.05	1.04
3.06	43 ± 1	(3.10-3.42)	(4.27-4.87)	(4.56-5.31)				
3.57	59 ± 2							
4.08	77 ± 2							
4.59	94 ± 2							
Cinnamaldehyde (µg/mg female)								
MCM-S								
2.62	22 ± 2	3.49	5.22	5.71	8.51	2	0.06	0.74
3.15	45 ± 4	(3.01-4.08)	(4.44-8.99)	(4.74-10.48)				
3.68	56 ± 4							
4.20	68 ± 3							
PMD-R								
3.15	26 ± 2	3.73	4.89	5.22	0.3	2	0.06	1.11
3.68	47 ± 2	(3.67-3.80)	(4.78-5.03)	(5.08-5.40)				
4.20	70 ± 3							
4.72	86 ± 1							
Permethrin (ng/mg female)								
MCM-S								
0.3	23 ± 1	0.43	0.68	0.75	3.2	2	0.31	5.22
0.4	46 ± 1	(0.42-0.45)	(0.65-0.71)	(0.72-0.79)				
0.5	60 ± 1							
0.6	82 ± 1							
PMD-R								
2	33 ± 1	3.72	8.16	9.42	7	2	0.02	0.29
4	52 ± 3	(2.28-4.68)	(6.78-11.43)	(7.71-13.69)				
6	71 ± 1							
8	92 ± 1							

[a]400 female mosquitoes (4 replicates) exposed to 0.1 µl of the test sample for 24 hours.

from different localities at different times to these conventional pyrethroids emphasizes the development and widespread of insecticide resistance in natural mosquito populations in Thailand. Consequently, application of an alternative choice of insecticides or other control measures is required for implementing effective mosquito control management.

Topical bioassay of adult mosquitoes using EOs conducted by various investigators revealed promising results against many vectors, particularly *Ae. aegypti*. For instance, a study of Chaiyasit *et al* (2006) reported excellent adulticidal effects against laboratory *Ae. aegypti* of EOs extracted from five plant species, namely, *Apium graveolens*, *Carum carvi*, *Curcuma zedoaria*, *Illicium verum*, and *Piper longum*, with LD_{50} value of 5.44, 5.94, 5.96, 8.52, and 6.21 μg/mg female, respectively. The organic extract of *Nerine sarniensis* bulb and its alkaloid sarniensine showed notable adulticidal activity against female *Ae. aegypti*, with LD_{50} values of 4.6 and 1.38 μg/mosquito, respectively (Masi *et al*, 2017). These results correspond to those of Norris *et al* (2015), who determined the susceptibility of *Ae. aegypti* and *An. gambiae* to a variety of commercially available EOs and reported a wide variation of toxicity, with patchouli oil exhibiting the highest adulticidal activity against both *Ae. aegypti* and *An. gambiae*, LD_{50} value of 1,500 and 500 μg/g mosquito, respectively; cinnamon bark and leaf oils also are efficacious, with LD_{50} value of 3,500 and 3,700 μg/g mosquito, respectively, against *Ae. aegypti* and 2,900 and 2,100 μg/g mosquito, respectively, against *An. gambiae*.

Bark and leaf oils of *C. verum*, also known as *Cinnamomum zeylanicum* Blume, are among the most important *Cinnamomum* (cinnamon) oils in world trade (Keller *et al*, 1992; Coppen, 1995). GC-MS analysis carried out by several researchers revealed some similarities in the chemical composition of *C. verum* (*C. zeylanicum*) oil isolated from cinnamon plants of different geographical regions using various extraction methods. Superheated water extraction of *C. zeylanicum* bark and leaf collected from plantations in Sri Lanka yielded EOs containing over 80% cinnamaldehyde and 98% eugenol, respectively, as the predominant constituent (Jayawardena and Smith, 2010); however, the percent cinnamaldehyde and eugenol in the bark and leaf EO, respectively, are lower than 65% and 88%, respectively, when extracted using the conventional steam distillation. In Turkey, Unlu *et al* (2010) extracted EO from *C. zeylanicum* bark by water distillation, obtaining nine constituents representing 99.24% of the isolated oil, with the major compounds being (E)-cinnamaldehyde (68.9%), benzaldehyde (9.9%) and (E)-cinnamyl acetate (7.4%). Eight components were identified in *C. zeylanicum* oil obtained by hydrodistillation of cinnamon bark in India (Pooja *et al*, 2013). *C. zeylanicum* bark EO from Iran prepared by hydrodistillation yielded 21 compounds, the major constituent being cinnamaldehyde (52.3%), followed by α-copaene (11.4%), δ-cadinene (6.2%), styrene benzebe,ethenyle-(CAS) (5.5%), and cis-calamanene (3.61%) (Kazemi and Mokhtariniya, 2016). In Fiji, Patel *et al* (2007) reported that steam distilled *C. verum* leaf oil contained 31components, consisting primarily of eugenol (86.0%), (E)-caryophyllene (5.7%) and linalool (2.3%). EOs of Chinese *C. verum* leaves at different growth stages obtained by hydrodistillation and subsequent extraction with methylene dichloride contained mainly eugenol (89.98-93.69%), primarily from leaf oil, which gradually decreased with development of the leaves (Li *et al*,

2016). Thus it is clear that although the number and percent identified substances in the isolated EOs were different depending on the source of plant material (bark or leaf) and technique of distillation, the principal constituents were cinnamaldehyde and eugenol. In our study, we used bark of *C. verum* and steam distillation, the latter being a simple and low-cost extraction technique.

In general, yield of isolated cinnamaldehyde and eugenol are important because cinnamon oil is graded based on the percent active chemicals responsible for the beneficial biological effects, particularly in medicinal and pharmacological application (Lis-Balchin *et al*, 1998; Simić *et al*, 2004; Parthasarathy *et al*, 2008). In addition to the antibacterial, antifungal and antioxidant properties (Ranasinghe *et al*, 2002; Saleem *et al*, 2015; Vazirian *et al*, 2015), cinnamon oil and its main constituents such as cinnamaldehyde and eugenol that are grouped as aromatic monoterpenoids (phenylpropanoids) are efficacious insecticides against a variety of mosquito species (Cheng *et al*, 2004, 2009; Samarasekera and Kalhari, 2005). The mosquitocidal activity of bark and leaf oil lies mainly with cinnamaldehyde and eugenol, respectively. Thus, it is highly recommended that further studies on EO standardization for yield of bioactive compounds, specifically cinnamaldehyde and eugenol, need to be carried out to have information regarding the insecticidal potency of different *C. verum* EO preparations.

Our findings regarding the equal efficacy of *C. verum* EO and its constituent cinnamaldehyde against female *Ae. aegypti* were slightly different from those of Samarasekera and Kalhari (2005), investigating the mosquitocidal property of *C. zeylanicum* oils and their eight compounds using WHO insecticide susceptibility test-kits (WHO, 1981), reported cinnamaldehyde a major constituent of bark, having greater adulticidal activity against *Ae. aegypti* and *Culex quinquefasciatus* (LD_{50} = 0.28 and 0.27 μg/ml, respectively), than bark (LD_{50} = 2.25 and 0.66 μg/ml respectively) and leaf (LD_{50} = 1.60 and 2.10 μg/ml, respectively) oils as well as eugenol (LD_{50} = 2.03 and 0.74 μg/ml, respectively), the main component of leaf oil; however, the mosquitocidal activity of cinnamaldehyde (LD_{50} = 0.32 μg/ml) against *Anopheles tessellatus* is comparable to that of bark oil (LD_{50} = 0.33 μg/ml), but higher than those of leaf oil (LD_{50} = 1.03 μg/ml) and eugenol (LD_{50} = 0.45 μg/ml). Except for cinnamyl acetate and eugenyl acetate, the other compounds, linalool, β-caryophyllene, 1,8-cineole, and safrole show less or no activity against the mosquitoes tested. It was surprising to note that the mosquitocidal potential of *C. zeylanicum* bark oil that comprises various bioactive compounds, particularly cinnamaldehyde, cinnamyl acetate and eugenyl acetate, is comparable to or less than that of cinnamaldehyde. It is possible that there is antagonism or no synergism among the adulticidal activities of these bioactive substances.

The permethrin was 1,000 times more potent than *C. verum* EO and its constituent cinnamaldehyde against female *Ae. aegypti* is in agreement with the findings of Norris *et al* (2015), who reported the lower efficacy of 33 commercially available EOs, as compared with a variety of synthetic pyrethroids, such as bifenthrin, β-cyfluthrin, λ-cyhalothrin, deltamethrin, and permethrin. The most toxic oil, patchouli oil, cinnamon leaf and bark oil was ≃3,600, 8,500 and 9,000 times, respectively, less effective than permethrin and ≃1,700, 4,000 and 4,200 times, respectively, than bifenthrin (the least toxic pyrethroid),

against adult female *Ae. aegypti*. Similarly, lower adulticidal potency of *Petroselinum crispum* fruit oil compared to those of permethrin and deltamethrin was documented against both pyrethroid resistant (PMD-R and UPK-R) and susceptible (MCM-S) local strains of *Ae. aegypti* (Intirach *et al*, 2016). Also, adulticidal efficacy against *Ae. aegypti* of *N. sarniensis* extract and sarniensine, with LD_{50} value of 4.6 and 1.38 μg/mosquito, respectively, is significantly less than that of permethrin, manifesting100% mortality at 2.27 ng/mosquito (Masi *et al*, 2017).

As demonstrated in this study and in other publications, it is evident that most plant EOs and their isolated principles generally display considerable lower potent insecticidal effects than synthetic compounds currently used in mosquito control program. However, as biologically active natural products, with widespread pesticidal property through different modes of action (Okumu *et al*, 2007; Ghosh *et al*, 2012; Norris *et al*, 2015), EOs may still be potential substitutes or supplements when incorporated with conventional insecticides. Enhancement of EO effectiveness could be achieved by increasing the dose applied, optimizing proper application rate and designing formulation technology for more effective delivery of the active agents. Plant EOs have advantages in terms of their availability, biodegradable nature and environmental safety (Ghosh *et al*, 2012; Norris *et al*, 2015; Intirach *et al*, 2016). The implementation of plant-derived products such as EOs in mosquito management programs not only is beneficial in eradication of mosquitoes that have already developed resistance to a large variety of synthetic insecticides, but also lessens the chance of resistance in the target populations.

In conclusion, the results obtained from this study reveal the potential usefulness of *C. verum* essential oil and its main constituent, cinnamaldehyde, as adulticide against the pyrethroid-resistant and -susceptible strains of *Ae. aegypti*. The findings also provide the possibility for improvement of synthetic insecticide chemicals with a history of inducing rapid development of resistance by combining with effective plant essential oils or by rotation between synthetic insecticides and plant-derived products, the latter strategy having an added value of reducing chemical contamination of the environment.

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