REPELLENCY OF ESSENTIAL OILS EXTRACTED FROM PLANTS IN THAILAND AGAINST FOUR MOSQUITO VECTORS (DIPTERA: CULICIDAE) AND OVIPOSITION DETERRENT EFFECTS AGAINST *AEDES AEGYPTI* (DIPTERA: CULICIDAE)

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Abstract. In this study we evaluated and reported repellent effects of essential oils from Thai plants against 4 mosquito vectors: *Aedes aegypti*, *Ae. albopictus*, *Anopheles dirus* and *Culex quinquefasciatus* under laboratory conditions using human volunteers. The essential oils were extracted from 18 plant species, belonging to 11 families, and the oils were then prepared as 10% solution in absolute ethanol with additives. Two chemical repellents, deet and IR3535, were also prepared in the same formulation as the essential oil repellents and tested for repellency as controls. The essential oils were also evaluated for oviposition deterrent effects against *Ae. aegypti* under laboratory conditions. The results show night-biting mosquitoes (*An. dirus* and *Cx. quinquefasciatus*) and *Ae. albopictus* were more sensitive to all the essential oils (repellency 4.5 - 8 hours) than was *Ae. aegypti* (repellency 0.3 - 2.8 hours), whereas deet and IR3535 provided excellent repellency against all four mosquito species (repellency 6.7 - 8 hours). All essential oils exhibited oviposition deterrent activity against *Ae. aegypti* with various degrees of repellency ranging from 16.6 to 94.7%, whereas deet and IR3535 had no repellency. The present study demonstrates the potential for using essential oils as mosquito repellents and oviposition deterrents. These findings may lead to new and more effective strategies for protection from and control of mosquitoes.

INTRODUCTION

Many mosquito-borne diseases, such as malaria, dengue fever (DF), dengue hemorrhagic fever (DHF) and filariasis, are serious public health problems in tropical regions, especially in Africa and Asia. These diseases are transmitted to human beings through mosquito bite only. Since there is no effective vaccine available for the control of these diseases, prevention of mosquito bites is one of the main strategies to control or minimize incidence of these diseases. The use of insect repellents can provide a practical and economical means of preventing mosquito-borne diseases. It is important not only for local people in disease risk areas, especially in tropical countries, but also for travelers who are vulnerable to diseases

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spread by mosquito vectors when they visit and seek leisure away from their home country.

Although the most common mosquito repellents currently available on the market containing deet (*N,N*-diethyl-3-methylbenzamide) have shown excellent protection from mosquito bites (Yap, 1986; Walker *et al*, 1996; Thavara *et al*, 2001) and other biting insects (Coleman *et al*, 1993), there were reports of toxicity problems after application of deet range from mild effects, such as contact urticaria (Maibach and Johnson, 1975) and skin eruption (Reuveni and Yagupsky, 1982), to severe reactions, such as toxic encephalopathy (Zadikoff, 1979; Roland *et al*, 1985; Edwards and Johnson, 1987). To overcome these adverse effects, attempts to find and develop repellents derived from plant extracts have been made by many researchers. In Thailand, some plant extracts, such as basil (Chokechaijaroenporn *et al*, 1994), galanga (Choochote *et al*, 1999), turmeric (Tawatsin *et al*, 2001), aromatic turmeric (Pitasawat *et al*, 2003), celery (Choochote *et al*, 2004; Tuetun *et al*, 2004) and clove (Trongtokit *et al*, 2004) have been investigated for repellent activity against various mosquito species under laboratory and field conditions. The development and use of locally available plants showing repellent activity avails an alternative strategy for the control or minimization of mosquito-borne diseases, especially in developing countries. In the present study, we evaluated and report on the repellent effects of essential oils extracted from 18 species of Thai plants against four mosquito vectors: *Aedes aegypti* (L.), *Ae. albopictus* (Skuse), *Anopheles dirus* Peyton & Harrison, and *Culex quinquefasciatus* Say under laboratory conditions. Comparison of repellency over different exposure periods was also carried out to standardize repellent testing methods. In addition, we evaluated the oviposition deterrent activity of each repellent composition against *Ae. aegypti* under laboratory conditions.

MATERIALS AND METHODS

Plant species

Eighteen plant species belonging to 11 families were selected for this study because most of them are known or used traditionally as mosquito repellents by Thai people. They were *Eleutherococcus trifoliatus* (L.) (Phak paem), *Schefflera leucantha* R. Vig. (Hanuman prasankai), *Ocimum sanctum* L. (Holy basil), *Vitex trifolia* L. (Khon thi so), *Litsea cubeba* (Lour.) Pers. (Ta khrai ton), *Manglietia garrettii* Craib (Montha doi), *Aglaia odorata* Lour. (Prayong), *Myristica fragans* Houtt. (Nutmeg tree), *Melaleuca cajuputi* Powell (Cajuput tree), *Psidium guajava* L. (Guava), *Piper betle* L. (Betel pepper), *Piper nigrum* L. (Black pepper), *Murraya paniculata* (L.) Jack (Orange jasmine), *Houttuynia cordata* Thunb. (Fishwort), *Zingiber officinale* Roscoe (Ginger), *Alpinia galanga* (L.) Wild (Galanga), *Curcuma longa* L. (Turmeric), and *Hedychium coronarium* J. Konig (White ginger).

Extraction of essential oils

Essential oils were extracted from each plant by steam distillation. One to two kilograms of fresh plant material (by particular part of each plant, see Table 1) were cut into small pieces and placed in a distillation flask with approximately five times as much water, and 10 glass beads. The distillation chamber was heated in a liquid paraffin bath at about 120°C until the distillation was completed. The distillate was collected in a separate funnel in which the aqueous portion was separated from the essential oil (oily phase). The aqueous phase (lower layer) was slowly drawn off until only the oil layer remained. This procedure was repeated until at least 5 ml of essential oil was collected. Each essential oil was kept in a screwed-cap glass vial at 4°C until it was tested for mosquito repellency and ovipositional deterrent activity.

Analysis of chemical constituents

All essential oils were analyzed for chemi-

cal constituents employing the Gas Chromatography / Mass Spectroscopy (GC/MS) assay. Briefly, the essential oil (50 μl) was diluted with 1.5 ml of hexane and CH_2Cl_2 (1:1) to a final concentration of 3.33% v/v. The diluted sample (0.1 μl) was then injected into the column (DB™-1ms, 30 m x 0.25 mm x 0.25 μm, 100% dimethylpolysiloxane) for analysis with a GC-MS instrument (QP2010, Shimatzu). The operation conditions were as follows: the injection temperature was 200ºC. Helium was used as a carrier gas and the purge flow rate was 3 ml/minute. The pressure was 69.4 kPa and the split ratio was 1:100. The chemical constituents of each essential oil were obtained by searching each peak and comparing with data from the National Institute of Science and Technology (NIST) library spectra. The relative amounts of the individual chemical components of each essential oil were computed from the GC peak areas (%).

Preparation of repellents for testing

The essential oils were formulated as 10% lotion in absolute ethanol and additives (vanillin, propylene glycol and polyethylene glycol). For comparison with standard repellents, two chemical repellents, *N*,*N*-diethyl-3-methylbenzamide (deet) and ethyl butylactylamino-propionate (IR3535), were formulated as 10% lotion similar to the essential oil repellents. All formulated repellents were placed in screw-cap vials and kept at room temperature before testing.

Test mosquitoes

The mosquitoes used in this study were laboratory-reared female mosquitoes (age 4-5 days) *Aedes aegypti*, *Ae. albopictus*, *Anopheles dirus*, and *Culex quinquefasciatus*. These were reared according to the standard protocol of the National Institute of Health, Thailand, and maintained at the insectary of the institute.

Repellent test

The repellency of essential oils and standard repellents was assessed in the laboratory using a human-bait technique (Tawatsin *et al*, 2001). Ethical clearance was approved by the Ethics Committee, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand (TM-IRB004/2005). Six volunteers (age 25-61 years) participated in the laboratory tests. The testing period lasted up to eight hours, depending on the efficacy of repellent. The timing of the tests depended on whether the target mosquitoes were day- or night-biters. *Ae. aegypti* and *Ae. albopictus* were tested during the daytime from 0900 to 1700, while *An. dirus* and *Cx. quinquefasciatus* were tested during the night from 1900 to 0300. Evaluations were carried out in a 6x6x3 m room, at 25-29ºC with relative humidity of 60-80%. An area of 3x10 cm on each forearm of the six human volunteers was marked out with a permanent marker. Each test repellent formulation (0.1 ml) was applied to the marked area of one forearm of each volunteer while the other forearm was treated with 0.1 ml of solution base (without active ingredient) as a control. Before the start of each exposure period, the bare hand of the test person, used as control area for each volunteer, was exposed for up to 10 seconds in a mosquito cage (30x30x30 cm), containing 250 host-seeking female mosquitoes (4-5 days old). If at least two mosquitoes landed on or bit the hand, the repellency test was then continued. This was done to ensure that the mosquitoes were host seeking. Then each volunteer put the test forearm and hand covered by a paper sleeve with a hole corresponding to the marked area into the mosquito cage for the first three minutes of each half-hour interval. The number of mosquitoes biting the treated area of each volunteer was recorded each minute (at 1, 2 and 3 minutes) of each 3-minute exposure. To determine the duration of protection for each repellent, the exposures continued until at least two bites occurred in a given exposure period, or until a bite in the previous exposure period was followed by a confirmatory second bite in the following ex-

posure period. The time between application of the test repellent and the second successive bite was recorded as the protection time.

Ovipositional deterrent test

Ovipositional deterrent activity of essential oils and standard repellents were studied for gravid *Ae. aegypti* under laboratory conditions at room temperature. Two black plastic cups (300 ml in capacity) were filled with 200 ml de-ionized water. One cup was a control and the other cup was treated with essential oil (undiluted) or standard chemical repellent (deet or IR3535) at dosage of 20 μl/cup. The final concentration of the treated material (essential oil or chemical repellent) in each treated cup was 0.01%. Each cup was fitted inside with a white filter-paper sheet (7x28 cm) for deposition of mosquito eggs. The paper was located in each cup so as the lower half of the paper was submerged in water. The cups were placed in a mosquito cage (30x30x30 cm) containing 50 gravid female mosquitoes for 48 hours then, the eggs laid in each cup were counted after removal of the oviposition paper. Each test repellent was tested in six cages. The percentage of repellency for each essential oil and standard repellent was calculated by Xue *et al* (2001) as follows:

$$\text{Repellency (\%)} = \frac{C-T}{C} \times 100$$

where C stands for the number of mosquito eggs collected from the control cup and T denotes the number of mosquito eggs collected from the treated cup.

Data analysis

The mean protection time was used as a standard measure of repellency for the essential oils, deet and IR3535 against the four mosquito species. Comparison of repellency for each test repellent derived from the different exposure periods and oviposition deterrency against gravid *Ae. aegypti* were carried out employing the one-way analysis of variance (ANOVA) with Duncan's multiple range test. All differences were considered significant at $p \leq 0.05$.

RESULTS

Yields of the 18 essential oils distillated from different parts of each plant species and the chemical constituents identified by GC/MS are shown in Table 1. Of these, 11 oils were extracted from leaves, four oils from rhizomes (Zingiberaceae family), and the remaining oils were from seeds (*Litsea cubeba*), fruits (*Piper nigrum*) and flowers (*Houttuynia cordata*). Most of the plants in this study yielded less than 1% essential oil, except *Litsea cubeba* (3.16%). Moderate yields were obtained from *Alpinia galanga* (0.83%), *Myristica fragrans* (0.66%) *Melaleuca cajuputi* (0.43%) and *Piper betle* (0.37%), whereas the other species provided low yields of 0.20% or less. The lowest yields (less than 0.10%) were obtained from *Piper nigrum* (0.08%), *Manglietia garrettii* (0.07%), *Eleutherococcus trifoliatus* (0.05%), *Murraya paniculata* (0.05%), *Schefflera leucantha* (0.04%) and *Aglaia odorata* (0.04%).

Numerous chemical constituents, ranging from 12 to 30 peaks of different chemicals were detected in the essential oils (see Table 1). These included both common and commonly known chemicals. The commonly known chemicals were α-pinene, β-pinene, borneol, linalool, d-limonene, cymene, eucalyptol, citronellal, caryophyllene. However, a few chemical peaks found in an essential oil (*Melaleuca cajuputi*) could not be identified, since they were less than 80% similar to other compounds in the database spectra library.

Repellency (as shown in hours of protection time) obtained for the three different exposure times (1, 2 and 3 minutes) for each essential oil repellent and the two chemical repellents (deet and IR3535) assessed by mean protection time (± SE) against the four mosquito species under laboratory conditions is shown in Table 2. The blank controls (solution base without any active ingredients)

Table 1
Yields and chemical constituents of essential oils obtained from the study plants.

Plant names	Part used	Yield (%)	Chemical constituents (%)[a]
Eleutherococcus trifoliatus (L.) S.Y.Hu (ARALIACEAE)	leaves	0.05	(1S,3R,5S)-2(10)-pinen-3-ol (23.81), 2-pinen-10-ol (10.71), 1,2-epoxy-p-menth-8-ene (10.59%), 2-pinen-10-ol (10.37), (Z)-verbenol (20.49), (±)-p-menth-6-ene-2,8-diol (5.01), 2,2,3-trimethyl-3-cyclopentene-1-acetaldehyde (4.28), [1S-(1α, 2β, 4β)-1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-cyclohexane (4.22), (1R)-(-)-myrtenal (3.59), (-)-spathulenol (2.83), (±)- 2(10)-pinen-3-one (2.20), eucalyptol (1.89)
Schefflera leucantha R.Vig. (ARALIACEAE)	leaves	0.04	[1S-1α, 4α, 7α]-1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethenyl)-azulene (44.68), (-)-3,7,7-trimethyl-11-methylene-spiro[5.5]undec-2-ene (19.49), 2,3,4,4a,5,6-hexahydro-1,4a-dimethyl-7-(1-methylethyl)-naphthalene (10.12), [1S-1α, 7α, 8aβ]-1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-azulene (3.95), (-)-spathulenol (3.77), [1S-1α, 7α, 8aβ]- 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-azulene (3.49), [2R-(2α,4aα,8aβ)]- 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-naphthalene (2.50%), caryophyllene (2.26), α-caryophyllene (1.91), 8-isopropenyl-1,5-dimethyl-cyclodeca-1,5-diene(1.83), [1S-(1α,2β,4β)]-1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-cyclohexane (1.59), aristolene (1.32), copaene (1.20), (1α, 4aβ, 8aα)-1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-naphthalene (0.95), (-)-α-panasinsen (0.95)
Ocimum sanctum L. (LABIATAE)	leaves	0.16	eugenol methyl ether (66.89), caryophyllene (18.95), [3aS-(3aα, 3bβ)]-octahydro-7-methyl-3-methylene-4-(1-methylethyl)-1H-cyclopenta[1,3]cyclopropa[1,2]benzene (4.01), 8-isopropenyl-1,5-dimethyl-1,5-cyclodecadiene (3.09), [1S-(1α, 2β, 4β)]-1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-cyclohexane (1.65), α-caryophyllene (1.21), (-)-borneol (1.10), (+)-epibicyclo- sesquiphellandrene (0.45), caryophyllene oxide (0.45), β-pinene (0.29), limonene (0.27), linalool (0.27), eucalyptol (0.24%), α-pinene (0.23), camphene (0.20), eugenol (0.20), β-terpinene (0.16), cadina-1(10),4-diene (0.15), 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-cycloheptane (0.11), cycloheptane (0.08)
Vitex trifolia L. (LABIATAE)	leaves	0.16	eucalyptol (31.26), p-menth-1-en-8-ol acetate (13.48), β-phellandrene (9.99), caryophyllene (7.58), α-pinene (6.93), p-menth-1-en-8-ol (7.33), p-menth-1-en-4-ol (4.57), (-)-spathulenol (3.31), caryophyllene oxide (2.97), β-isomethyl ionone (2.80), [3R-(3α,4aβ,6aα,10aβ)]-3-ethenyldodecahydro-3,4a,7,7,10a-pentamethyl-1H-naphtho[2,1-b]pyran (2.43), β-pinene (2.28), [1R-[1α,2β]]-α,2,5,5,8a-pentamethyl-α-ethenyldecahydro-2-hydroxy-1-naphthalenepropanol (1.02)

Table 1 (continued).

Plant names	Part used	Yield (%)	Chemical constituents (%)[a]
Litsea cubeba (Lour.) Pers. (LAURACEAE)	seeds	3.16	(*E*)-3,7-dimethyl, 2,6-octadienal (75.56), 6-methyl- 5-hepten-2-one (7.92), (*R*)-(+)-citronellal (3.54), linalool (2.21), limonene (2.03), eucalyptol (1.23), 4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol (1.18), (*E*)-2-[2'-(2''-methyl-1''-propenyl)cyclopropyl]propan-2-ol (1.01), neric acid (0.87), 2,7-dimethyl-2,7-octanediol (0.85), (*E*)- 4,5-epoxy-carene (0.39), (E)-geraniol (0.37), 2,3-dimethyl-1,3-heptadiene (0.36), β-pinene (0.34), isopulegol (0.30), α-pinene (0.27), p-menth-1-en-8-ol (0.26), (Z)-p-mentha-6,8-dien-2-ol (0.22), β-myrcene (0.21), (Z)-verbenol (0.20), 1-methyl-4-(1-methylethenyl)-7-oxabicyclo[4.1.0]heptane (0.18), 2,3-dimethyl-3-buten-2-ol (0.18), 3-methyl-6-(1-methylethyl)-2-cyclohexen-1-one (0.17), caryophyllene oxide (0.17)
Manglietia garretti Craib (MAGNOLIACEAE)	leaves	0.07	±-(*E*)-nerolidol (27.04), caryophyllene (17.57), α-caryophyllene (11.14), (-)-globulol (6.38), (τ)-cadinol (9.44), α-cadinol (4.94), (1α, 4aα,8aα)-1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-naphthalene (4.13), cadina-1(10),4-diene (3.71), 1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-cyclohexane (3.29), caryophyllene oxide (2.23), cedr-9-ene (2.04), β-pinene (1.58), 2-isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene(1.38), selina-6-en-4-ol (1.33), (1S-Z)-1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-naphthalene (1.03), [1aR-(1aα, 4aα,7α,7aβ,7bα)]-decahydro-1,1,7-trimethyl-4-methylene-1H-cycloprop[e]azulene (0.97), [1aR-(1aα, 4α,4aβ,7bα)]-1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-1H-cycloprop[e]azulene (0.96), [2R-(2α,4aβ,8β)]-α,α,4a,8-tetramethyl-2,3,4,4a,5,6,7,8-octahydro-2-naphthalenemethanol (0.83)
Aglaia odorata Lour. (MELIACEAE)	leaves	0.04	[1aR-(1aα,4aβ,7α,7aβ,7bα)]-decahydro-1,1,7-trimethyl-4-methylene-1H-cycloprop[e]azulene (17.31), (1α,3aα,7α,8aβ)-2,3,6,7,8,8a-hexahydro-1,4,9,9-tetramethyl-1H-3a,7-methanoazulene (15.22%), [3R-(3α,3aβ,7β,8aα)]-octahydro-3,8,8-trimethyl-6-methylene-1H-3a,7-methanoazulene (12.74%), [1S-(1α,3aβ,4α,7α)]-octahydro-4-methyl-8-methylene-7-(1-methylethyl)-1,4-methano-1H-indenne (12.18), humulane-1,6-dien-3-ol (11.38), α-cadinol (6.10), (-)-spathulenol (4.58), cedr-8-ene (3.50), (*Z*)-β-farnesene (3.15), caryophyllene (2.96), (1α,4aα,8aα)-1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-naphthalene (2.85), ylangene (2.72), thunbergol (2.47), (τ)-cadinol (2.03), eudesma-4(14),11-diene (0.80)

Table 1 (continued).

Plant names	Part used	Yield (%)	Chemical constituents (%)[a]
Myristica fragrans Houtt. (MYRISTICACEAE)	leaves	0.66	myristicin (27.09), p-menth-1-en-4-ol (18.60), β-pinene (11.96),α-pinene (10.15), linalool (7.23), (S)-(-)-p-menth-1-en-8-ol (6.23), 1,2,3-trimethoxy-5-(2-propenyl)-benzene (4.78), β-phellandrene (2.26), isosafrole (1.46), kaur-16-ene (1.07), p-menth-1-en-8-ol acetate (1.02), bornyl acetate (0.78), 1-methyl-4-(1-methylethyl)-2-cyclohexen-1-ol (0.75), eucalyptol (0.71), 1-methyl-4-(1-methylethyl)-2-cyclohexen-1-ol (0.63), 1,2-dimethoxy-4-(2-propenyl)-benzene (0.62), d-limonene (0.54), 3-carene (0.47), terpinolene (0.45), (*E*)-p-menth-1-en-3-ol (0.64), cadina-1(10),4-diene (0.43), γ-terpinene (0.35), [S-(*E*,*E*)-1-methyl-5-methylene-8-(1-methylethyl)-1,6-cyclodecadiene (0.32), bergamol (0.27), caryophyllene (0.26), β-myrcene (0.18), camphene (0.17), neryl acetate (0.17)
Melaleuca cajuputi Powell (MYRTACEAE)	leaves	0.43	unidentified (28.37), unidentified (9.86), 1-methyl-4-(1-methylethylidene)-cyclohexene (6.93), 1-methyl-2-(1-methylethyl)-benzene (6.68), p-menth-1-en-4-ol (6.18), γ-terpinene (5.07), (-)-spathulenol (4.14), caryophyllene (4.09), α-pinene (3.77), p-menth-1-en-8-ol (2.60), selina-6-en-4-ol (2.37), caryophyllene oxide (2.28), α-caryophyllene (2.07), 2-methyl-5-(1-methylethyl)-bicyclo[3.1.0]hex-2-ene (1.64), [1S-(1α,2β,4β)]-1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-cyclohexane (1.75), 1-methyl-7-(1-methylethyl)-phenanthrene (1.25), eucalyptol (1.14), α-cadinol (1.05), 8-isopropenyl-1,5-dimethyl-1,5-cyclodecadiene (1.01), τ-cadinol (0.89), linalool (0.85), 3,4-dimethyl-3-cyclohexen-1-carboxaldehyde (0.85), α-terpinene (0.83), d-limonene (0.83), (-)-globulol (0.83), α-phellandrene (0.64), 3-cyclohexene-1-carboxaldehyde (0.62), (-)-spathulenol (0.50), β-pinene (0.47), 3,7-dimethyl-2,6-octadienal (0.45)
Psidium guajava L. (MYRTACEAE)	leaves	0.16	Caryophyllene oxide (21.97), 4,4-dimethyl-tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol (14.49), caryophyllene (11.76), ±-(*E*)-nerolidol (9.39), [1ar-(1aα,4β ,4aβ,7α, 7aβ,7bα)]-decahydro-1,1,4,7-tetramethyl-1H-Cycloprop[e]azulene-4-ol (8.26), (-)-globulol (5.96), ledol (5.53), eucalyptol (5.13), [1S-(1α,4α,4aβ,8aβ)]-1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-1-naphthalenol (4.28), α-caryophyllene (1.60), copaene (1.39), cadina,1,3,5-triene (1.36)

Table 1 (continued).

Plant names	Part used	Yield (%)	Chemical constituents (%)[a]
Piper betle L. (PIPERACEAE)	leaves	0.37	4-allyl-2-methoxy-phenol acetate (31.47), 3-allyl-6-methoxyphenol (25.96), 4-allylphenyl acetate (5.21), [3aS-(3aα,3bβ)-octahydro-7-methyl-3-methylene-4(1-methylethyl)-1H-Cyclopenta[1,3]cyclopropa[1,2]benzene (2.48%), caryophyllene (2.16), p-allylphenol (1.47), (1α,4aα,8aα)-1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-naphthalene (1.43), eucalyptol (1.03), α-caryophyllene (0.51), 3,7-dimethyl-1,6-octadien-3-ol (0.49), 1,2-dimethoxy-4-(2-propenyl)-benzene (0.44), [1S-(1α,2β,4β)]-1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-cyclohexane (0.24), (1α,4aα,8aα)-1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-naphthalene (0.19), 3,7-dimethyl-(Z)-1,3,6-octatriene (0.11), camphene (0.09)
Piper nigrum L. (PIPERACEAE)	fruits	0.08	caryophyllene (54.92), caryophyllene oxide (13.26), α-caryophyllene (3.97), copaene (2.90), cadina-1(10),4-diene (2.61), ar-tumerone (2.38), (3R,*E*)-4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-cyclohexene (1.93), caryophyllene oxide (1.80), 1-(1,5-dimethyl-4-hexenyl)-4-methyl-benzene (1.74), eudesma-4(14),11-diene (1.40), 4,4-dimethyl-tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol (1.21), isocaryophyllene (1.16), linalool (1.13), [2R-(2α,4aα,8aβ)]-1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-naphthalene (0.94), limonene (0.85), 3-carene (0.75), β-pinene (0.56), (1S-(1α,2β,4β)]-1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-cyclohexane (0.62), (R)-(-)-p-menth-1-en-4-ol (0.60)
Murraya paniculata (L.) Jack (RUTACEAE)	leaves	0.05	caryophyllene (32.44), [3aS-(3aα, 3bβ)]-octahydro-7-methyl-3-methylene-4-(1-methylethyl)-1H-cyclopenta[1,3]cyclopropa[1,2]benzene (20.94), 1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-cyclohexane (10.76), α-caryophyllene (5.03), germacrene B (4.89), [3aS-(3aα, 3bβ)]-octahydro-7-methyl-3-methylene-4-(1-methylethyl)-1H-cyclopenta[1,3] cyclopropa[1,2]benzene (4.16), (1α,4aβ,8aα)-1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-naphthalene (3.75), [1ar-(1aα,4aα,7β,7aβ,7bα)]-decahydro-1,1,7-trimethyl-4-methylene-1H-cycloprop[e]azulen-7-ol (2.88), α-cadinol (2.31), caryophyllene oxide (2.08), cadina-1(10),4-diene (1.95), (1α,4aα,8aα)-1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-naphthalene (1.53), [1R-(1α,3α,4β)]-4-ethenyl-α,α,4-trimethyl-3-(1-methylethenyl)-cyclohexanemethanol (1.41), copaene (1.03), (τ)-cadinol (0.94), phytol (0.90), ±-(*E*)-nerolidol (0.80), germacrene D-4-ol (0.79), [3R-(3α,3aβ,7β,8aα)]-2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-1H-3a,7-methanoazulene (0.77), [1ar-(1aα,4aα,7β,7aβ,7bα)]-decahydro-1,1,7-trimethyl-4-methylene-1H-cycloprop[e]azulen-7-ol (0.64)

Table 1 (continued).

Plant names	Part used	Yield (%)	Chemical constituents (%)[a]
Houttuynia cordata Thunb. (SAURURACEAE)	flowers	0.2	α-pinene (1.28), camphene (0.92), β -pinene (1.25), β-myrcene (1.17), d-limonene (0.85), (5*Z*)-2,6,10-trimethyl-1,5,9-undecatriene (0.49), 1-nonanol (0.72), bornyl acetate (8.66), n-decanoic acid (69.31), 2-dodecanone (8.42), 2-tridecanone (0.89), caryophyllene oxide (0.54), docanoic acid, ethyl ester (1.69), 2-propenoic acid, 2-ethylhexyl ester (1.28)
Zingiber officinale Roscoe (ZINGIBERACEAE)	rhizomes	0.12	(*E*)-3,7-dimethyl-2,6-octadienal (24.72), eucalyptol (20.77), (*Z*)-3,7-dimethyl-2,6-octadienal (18.27), camphene (8.71), (-)-borneol (5.46), (S)- (-)-p-menth-1-en-8-ol (3.90), linalool (2.88), 2-heptanol (2.72), 2-[2-(2-methyl-1-propenyl)cyclopropyl]-2-propanol (2.06), 1-(1,5-dimethyl-4-hexenyl)-4-methyl-benzene (2.70), α-pinene (1.74), 6-methyl-5-hepten-2-one (1.89), 2-undecanone (1.44), 2-nonanol (1.37), 13-heptadecyn-1-ol (1.36)
Alpinia galangal (L.) Willd. (ZINGIBERACEAE)	rhizomes	0.83	eucalyptol (39.50), 1,3,3-trimethyl-2-oxabicyclo[2.2.2]octan-6-ol acetate (28.61), 4-allylphenyl acetate (5.73), caryophyllene (3.35), 4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol (3.15), 2-isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,8a-octahydronaphthalene (2.22), eudesma-4(14),11-diene (2.09), (S)-1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-cyclohexene (2.05), (Z)-β-farnesene (1.72), α-caryophyllene (1.67), germacrene B (1.67), (S)-(-)-p-menth-1-en-8-ol (1.60), d-limonene (1.00), (E)-3,7-dimethyl-2,6-octadien-1-ol acetate (0.99), p-allylphenol (0.98), α-pinene (0.85), p-menth-1-en-8-ol (0.69), 1,2-dimethoxy-4-(2-propenyl)-benzene (0.68), selina-6-en-4-ol (0.66)
Curcuma longa L. (ZINGIBERACEAE)	rhizomes	0.18	tumerone (41.11), ar-tumerone (23.12), curlone(19.14), α-phellandrene (5.04), eucalyptol (3.92), 1-methyl-2-(1-methylethyl)-benzene (1.66), 2,2-dicyclohexylmalononitrile (1.28), (*Z*)-α-(*E*)-bergamotol (0.98), 1-zingiberene (0.75), 5-fluoro-2-nitrophenyl 4-methylbenzoate (0.72), 1-(1,5-dimethyl)-4-hexenyl)-4-methyl-benzene (0.63), β-sesquiphellandrene (0.62), 2-cyclohexyl-2-isobutylmalononitrile (0.52)
Hedychium coronarium J. Konig (ZINGIBERACEAE)	rhizomes	0.2	eucalyptol (56.91), β-pinene (17.21), α-terpineol (7.45), α-pinene (6.02), p-menth-1-en-4-ol (3.00), (*E*)-pinocarveol (2.58), 2-pinen-10-ol (1.96), borneol (1.28), d-limonene (0.82), 1-methyl-2-(1-methylethyl)-benzene (0.71), linalool (0.54), 2(10)pinen-3-one (0.50), 2(10)pinen-3-ol (0.34), thujol (0.34), β-thujene (0.33)

[a]Values in parentheses represent relative amounts (% area) of each chemical constituent.

showed no repellency against the four mosquito species. The average numbers of mosquitoes landing or biting the bare hand of the volunteers (within 30 seconds before the start of each exposure) were 17, 12, 5 and 10 against *Ae. aegypti*, *Ae. albopictus*, *An. dirus* and *Cx. quinquefasciatus*, respectively (data not shown). This confirms the test mosquitoes were host seeking during the test periods.

There were significant differences in repellency obtained during the three different exposure times (1, 2 and 3 minutes) for each repellent against the test mosquitoes, especially *Ae. aegypti* and *Cx. quinquefasciatus* (Table 2). The results show the repellency was inversely proportional to the exposure time. In other words, repellency of most of the essential oils declined with time of exposure. Of the 18 essential oils tested, 17, 3, 1, and 8 oils provided significantly different repellencies during the three exposure periods against *Ae. aegypti*, *Ae. albopictus*, *An. dirus* and *Cx. quinquefasciatus*, respectively. In contrast, there was no significant difference in repellency obtained for deet and IR3535 against the four mosquito species at the three exposure periods.

Table 3 shows the mean repellency (in hours) for 3 minutes of exposure with the 18 essential oils and 2 chemical repellents against the four mosquito species. This data was retrieved from Table 2 to compare repellencies of the essential oils and chemical repellents. The repellencies of the 18 essential oils against *Ae. aegypti* were between 0.3 and 2.8 hours, whereas those of deet and IR3535 were 7.5 and 6.7 hours, respectively. All the essential oils provided significantly lower repellency than deet and IR3535 ($p < 0.01$). Of the essential oils tested, a high degree of repellency was obtained from *Psidium guajava* (2.8 hours), *Curcuma longa* (2.3 hours), *Piper nigrum* (2.3 hours), *Schefflera leucantha* (1.9 hours), *Vitex trifolia* (1.8 hours), *Litsea cubeba* (1.7 hours), and *Zingiber officinale* (1.7 hours). However, there were no significant differences in repellency of *Ae. aegypti* among these essential oils ($p > 0.05$). When tested against *Ae. albopictus*, the repellency of the 18 essential oils ranged from 4.5 to 8.0 hours, while deet and IR3535 were 8.0 and 7.8 hours, respectively (Table 3). Repellency of the eight essential oils: *Eleutherococcus trifoliatus*, *Schefflera leucantha*, *Vitex trifolia*, *Melaleuca cajuputi*, *Piper nigrum*, *Alpinia galanga*, *Curcuma longa* and *Hedychium coronarium* were statistically equal to the chemical repellents, deet and IR3535 ($p > 0.01$).

Regarding the repellency against *An. dirus*, it is interesting to note that 16 out of 18 essential oils provided excellent repellency of 8 hours, equally to deet and IR3535 (Table 3). High degrees of repellency against *An. dirus* were also detected in the other two essential oils, *Piper betle* (7.6 hours) and *Hedychium coronarium* (7.1 hours). As for the repellency results against *Cx. quinquefasciatus*, the 18 essential oils demonstrated a relatively high degree of repellency, ranging from 5.0 to 8.0 hours, while those of deet and IR3535 were 8.0 hours (Table 3). Unlike the effect against *An. dirus*, excellent repellency against *Cx. quinquefasciatus* was found in only three essential oils, *Curcuma longa* (8.0 hours), *Piper nigrum* (7.8 hours), and *Schefflera leucantha* (7.5 hours), which were statistically equal to those of deet and IR3535 ($p > 0.05$).

The oviposition deterrent effects of essential oils and the two chemical repellents, deet and IR3535 (at 0.01% concentration) against *Ae. aegypti* are shown in Table 4. The average number of mosquito eggs in the control group ranged from 2,171 to 4,805, while those of the treated groups were between 232 and 2,903. As can be seen, all essential oils exhibited oviposition deterrent activity against the mosquitoes with various degrees of repellency, ranging from 16.6 to 94.7%, whereas deet and IR3535 provided no repellency. Of the essential oils tested, 12 out of 18 provided

Table 2
Repellency obtained from three different exposure times for each repellent against four mosquito species.

Repellents	Exposure time (min)	Mean* repellency in hours (± S.E.) against each mosquito species			
		Ae. aegypti	*Ae. albopictus*	*An. dirus*	*Cx. quinquefasciatus*
Eleutherococcus trifoliatus	1	2.3 (± 0.3)a	8.0 (± 0.0)a	8.0 (± 0.0)a	8.0 (± 0.0)a
	2	1.4 (± 0.4)b	8.0 (± 0.0)a	8.0 (± 0.0)a	7.8 (± 0.2)ab
	3	1.0 (± 0.2)b	8.0 (± 0.0)a	8.0 (± 0.0)a	7.4 (± 0.2)b
Schefflera leucantha	1	3.6 (± 0.5)a	8.0 (± 0.0)a	8.0 (± 0.0)a	8.0 (± 0.0)a
	2	2.8 (± 0.6)ab	8.0 (± 0.0)a	8.0 (± 0.0)a	7.7 (± 0.3)a
	3	1.9 (± 0.4)b	8.0 (± 0.0)a	8.0 (± 0.0)a	7.5 (± 0.5)a
Ocimum sanctum	1	2.0 (± 0.3)a	7.8 (± 0.1)a	8.0 (± 0.0)a	5.8 (± 0.9)a
	2	1.9 (± 0.3)a	7.6 (± 0.2)a	8.0 (± 0.0)a	5.8 (± 0.9)a
	3	1.3 (± 0.2)b	7.6 (± 0.2)a	8.0 (± 0.0)a	5.4 (± 0.9)a
Vitex trifolia	1	3.7 (± 0.5)a	8.0 (± 0.0)a	8.0 (± 0.0)a	8.0 (± 0.0)a
	2	2.3 (± 0.7)b	8.0 (± 0.0)a	8.0 (± 0.0)a	7.8 (± 0.2)ab
	3	1.8 (± 0.3)b	8.0 (± 0.0)a	8.0 (± 0.0)a	7.5 (± 0.3)b
Litsea cubeba	1	2.5 (± 0.4)a	7.7 (± 0.2)a	8.0 (± 0.0)a	7.5 (± 0.5)a
	2	1.8 (± 0.2)b	6.3 (± 0.8)b	8.0 (± 0.0)a	7.2 (± 0.8)a
	3	1.7 (± 0.3)b	6.2 (± 0.8)b	8.0 (± 0.0)a	7.0 (± 1.0)a
Manglietia garrettii	1	2.6 (± 0.5)a	7.5 (± 0.5)a	8.0 (± 0.0)a	7.7 (± 0.3)a
	2	1.5 (± 0.3)b	6.6 (± 0.7)ab	8.0 (± 0.0)a	7.4 (± 0.4)a
	3	1.4 (± 0.3)b	6.0 (± 0.9)b	8.0 (± 0.0)a	6.9 (± 0.5)a
Aglaia odorata	1	2.2 (± 0.6)a	6.8 (± 1.0)a	8.0 (± 0.0)a	8.0 (± 0.0)a
	2	1.7 (± 0.4)a	6.8 (± 1.0)a	8.0 (± 0.0)a	8.0 (± 0.0)a
	3	1.2 (± 0.3)b	5.3 (± 1.2)a	8.0 (± 0.0)a	7.2 (± 0.6)b
Myristica fragrans	1	3.5 (± 1.0)a	6.2 (± 1.1)a	8.0 (± 0.0)a	8.0 (± 0.0)a
	2	1.2 (± 0.5)b	5.8 (± 1.1)a	8.0 (± 0.0)a	7.5 (± 0.5)ab
	3	0.8 (± 0.3)b	4.5 (± 1.2)a	8.0 (± 0.0)a	6.9 (± 0.6)b
Melaleuca cajuputi	1	2.1 (± 0.6)a	8.0 (± 0.0)a	8.0 (± 0.0)a	7.9 (± 0.1)a
	2	1.3 (± 0.7)ab	8.0 (± 0.0)a	8.0 (± 0.0)a	7.6 (± 0.3)a
	3	0.7 (± 0.3)b	7.9 (± 0.1)a	8.0 (± 0.0)a	6.9 (± 0.4)b
Psidium guajava	1	4.4 (± 0.8)a	7.4 (± 0.4)a	8.0 (± 0.0)a	7.7 (± 0.3)a
	2	3.3 (± 0.8)a	6.8 (± 0.5)ab	8.0 (± 0.0)a	7.3 (± 0.4)a
	3	2.8 (± 0.9)a	5.6 (± 1.0)b	8.0 (± 0.0)a	6.9 (± 0.5)a
Piper betle	1	2.3 (± 0.4)a	7.6 (± 0.4)a	8.0 (± 0.0)a	7.8 (± 0.2)a
	2	1.8 (± 0.4)ab	7.6 (± 0.4)a	7.8 (± 0.2)a	7.3 (± 0.5)a
	3	1.3 (± 0.3)b	7.1 (± 0.6)a	7.6 (± 0.3)a	6.7 (± 1.0)a
Piper nigrum	1	4.1 (± 0.6)a	8.0 (± 0.0)a	8.0 (± 0.0)a	7.8 (± 0.2)a
	2	2.8 (± 0.5)b	8.0 (± 0.0)a	8.0 (± 0.0)a	7.8 (± 0.2)a
	3	2.3 (± 0.4)b	8.0 (± 0.0)a	8.0 (± 0.0)a	7.8 (± 0.2)a
Murraya paniculata	1	3.4 (± 0.6)a	6.8 (± 0.7)a	8.0 (± 0.0)a	6.5 (± 0.8)a
	2	2.6 (± 0.5)a	6.4 (± 0.7)a	8.0 (± 0.0)a	6.3 (± 0.8)a
	3	1.5 (± 0.3)b	5.7 (± 1.1)a	8.0 (± 0.0)a	5.0 (± 0.8)a

Table 2 (continued).

Repellents	Exposure time (min)	Mean* repellency in hours (± S.E.) against each mosquito species			
		Ae. aegypti	*Ae. albopictus*	*An. dirus*	*Cx. quinquefasciatus*
Houttuynia cordata	1	1.8 (± 0.6)a	7.5 (± 0.3)a	8.0 (± 0.0)a	8.0 (± 0.0)a
	2	0.8 (± 0.2)b	7.5 (± 0.3)a	8.0 (± 0.0)a	7.9 (± 0.1)ab
	3	0.6 (± 0.2)b	7.5 (± 0.3)a	8.0 (± 0.0)a	7.5 (± 0.4)b
Zingiber officinale	1	3.4 (± 0.4)a	7.2 (± 0.5)a	8.0 (± 0.0)a	7.7 (± 0.2)a
	2	2.3 (± 0.4)b	7.2 (± 0.5)a	8.0 (± 0.0)a	7.2 (± 0.3)a
	3	1.7 (± 0.3)b	5.9 (± 1.0)a	8.0 (± 0.0)a	5.9 (± 0.6)b
Alpinia galanga	1	2.2 (± 0.8)a	7.8 (± 0.2)a	8.0 (± 0.0)a	7.2 (± 0.6)a
	2	0.8 (± 0.3)b	7.8 (± 0.2)a	8.0 (± 0.0)a	6.9 (± 0.8)a
	3	0.6 (± 0.2)b	7.8 (± 0.2)a	8.0 (± 0.0)a	6.1 (± 0.9)a
Curcuma longa	1	3.6 (± 0.6)a	8.0 (± 0.0)a	8.0 (± 0.0)a	8.0 (± 0.0)a
	2	2.7 (± 0.5)ab	8.0 (± 0.0)a	8.0 (± 0.0)a	8.0 (± 0.0)a
	3	2.3 (± 0.4)b	7.7 (± 0.3)a	8.0 (± 0.0)a	8.0 (± 0.0)a
Hedychium coronarium	1	0.9 (± 0.3)a	8.0 (± 0.0)a	8.0 (± 0.0)a	7.5 (± 0.3)a
	2	0.4 (± 0.2)ab	7.5 (± 0.5)a	7.5 (± 0.3)b	6.8 (± 0.7)ab
	3	0.3 (± 0.1)b	7.5 (± 0.5)a	7.1 (± 0.6)b	5.8 (± 1.1)b
Deet	1	7.7 (± 0.3)a	8.0 (± 0.0)a	8.0 (± 0.0)a	8.0 (± 0.0)a
	2	7.6 (± 0.3)a	8.0 (± 0.0)a	8.0 (± 0.0)a	8.0 (± 0.0)a
	3	7.5 (± 0.2)a	8.0 (± 0.0)a	8.0 (± 0.0)a	8.0 (± 0.0)a
IR3535	1	7.5 (± 0.3)a	8.0 (± 0.0)a	8.0 (± 0.0)a	8.0 (± 0.0)a
	2	7.1 (± 0.4)a	8.0 (± 0.0)a	8.0 (± 0.0)a	8.0 (± 0.0)a
	3	6.7 (± 0.8)a	7.8 (± 0.2)a	8.0 (± 0.0)a	8.0 (± 0.0)a
Control (solution base)	1	0.0	0.0	0.0	0.0

*Means of each repellent in each column against each mosquito species followed by the same letter are not significantly different ($p > 0.05$, by one-way ANOVA with Duncant's multiple range test). Comparisons are made only among the repellencies obtained from different exposure times for each repellent against each mosquito species.

repellency of at least 80%. Relatively high oviposition deterrencies were obtained from *Curcuma longa* (94.7%), *Schefflera leucantha* (91.6%) and *Zingiber officinale* (90.1%), *Vitex trifolia* (89.1%), *Melaleuca cajuputi* (87.9%), *Hedychium coronarium* (87.5%), *Psidium guajava* (87.1%), *Manglietia garrettii* (86.1%) and *Houttuynia cordata* (85%). There were no significant differences of repellency among these essential oils. Moderate degrees of deterrency were obtained from three plant species: *Piper nigrum* (82%), *Litsea cubeba* (80.6%) and *Eleutherococcus trifoliatus* (80.2%). The remaining plants showed deterrency below 80%.

DISCUSSION

The quality of essential oils, such as yield, chemical constituents and physical properties depends on many factors. Factors affecting the quality of essential oils include plant species (variety), cultivating conditions, maturation of harvested plants, plant storage, plant preparation and methods of extraction (Tawatsin *et al*, 2001). Unfortunately, we could not describe all the factors of the plants used in this study. Data of the chemical constituents of essential oils in our study is valuable for further research regarding plant-based insect repellents. It is difficult to point out which

Table 3
Repellency obtained from a 3-minute exposure time for each repellent against four mosquito species.

Repellents	Mean* repellency in hours (± S.E.) against each mosquito species			
	Ae. aegypti	*Ae. albopictus*	*An. dirus*	*Cx. quinquefasciatus*
Eleutherococcus trifoliatus	1.0 (± 0.2)de	8.0 (± 0.0)a	8.0 (± 0.0)a	7.4 (± 0.2)b
Schefflera leucantha	1.9 (± 0.4)bc	8.0 (± 0.0)a	8.0 (± 0.0)a	7.5 (± 0.5)ab
Ocimum sanctum	1.3 (± 0.2)cd	7.6 (± 0.2)b	8.0 (± 0.0)a	5.4 (± 0.9)cd
Vitex trifolia	1.8 (± 0.3)bc	8.0 (± 0.0)a	8.0 (± 0.0)a	7.5 (± 0.3)b
Litsea cubeba	1.7 (± 0.3)bc	6.2 (± 0.8)cd	8.0 (± 0.0)a	7.0 (± 1.0)bc
Manglietia garrettii	1.4 (± 0.3)cd	6.0 (± 0.9)cd	8.0 (± 0.0)a	6.9 (± 0.5)bc
Aglaia odorata	1.2 (± 0.3)cd	5.3 (± 1.2)de	8.0 (± 0.0)a	7.2 (± 0.6)bc
Myristica fragrans	0.8 (± 0.3)de	4.5 (± 1.2)de	8.0 (± 0.0)a	6.9 (± 0.6)bc
Melaleuca cajuputi	0.7 (± 0.3)de	7.9 (± 0.1)ab	8.0 (± 0.0)a	6.9 (± 0.4)bc
Psidium guajava	2.8 (± 0.9)b	5.6 (± 1.0)d	8.0 (± 0.0)a	6.9 (± 0.5)bc
Piper betle	1.3 (± 0.3)cd	7.1 (± 0.6)bc	7.6 (± 0.3)b	6.7 (± 1.0)bc
Piper nigrum	2.3 (± 0.4)bc	8.0 (± 0.0)a	8.0 (± 0.0)a	7.8 (± 0.2)ab
Murraya paniculata	1.5 (± 0.3)cd	5.7 (± 1.1)cd	8.0 (± 0.0)a	5.0 (± 0.8)d
Houttuynia cordata	0.6 (± 0.2)e	7.5 (± 0.3)b	8.0 (± 0.0)a	7.5 (± 0.4)b
Zingiber officinale	1.7 (± 0.3)bc	5.9 (± 1.0)cd	8.0 (± 0.0)a	5.9 (± 0.6)c
Alpinia galanga	0.6 (± 0.2)e	7.8 (± 0.2)ab	8.0 (± 0.0)a	6.1 (± 0.9)c
Curcuma longa	2.3 (± 0.4)bc	7.7 (± 0.3)ab	8.0 (± 0.0)a	8.0 (± 0.0)a
Hedychium coronarium	0.3 (± 0.1)f	7.5 (± 0.5)ab	7.1 (± 0.6)b	5.8 (± 1.1)c
Deet	7.5 (± 0.2)a	8.0 (± 0.0)a	8.0 (± 0.0)a	8.0 (± 0.0)a
IR3535	6.7 (± 0.8)a	7.8 (± 0.2)ab	8.0 (± 0.0)a	8.0 (± 0.0)a
Control (solution base)	0.0	0.0	0.0	0.0

*Means in each column against each mosquito species followed by the same letter are not significantly different ($p > 0.05$, by one-way ANOVA with Duncant's multiple range test).

chemicals are responsible for the repellent effects against mosquitoes in this study, since several were uncommon or unidentified chemicals found in the essential oils. Even though there are some known chemicals found in the essential oils, they are not presented in all the essential oils that possess the same repellency against the same mosquito species. Repellent activity against particular mosquito species may be due to the synergistic effects of a combination of phytochemicals in each essential oil. Further studies would reveal more information about the relationship of phytochemicals and the repellent effects against mosquitoes.

There was inconsistency in the different exposure periods in the mosquito cage when determining the repellency of mosquito under laboratory conditions. Some earlier studies used short exposure times of one minute only. Our study clearly shows a substantial difference in repellency obtained during different exposure periods. Two hours was the minimum protection time needed against *Ae. aegypti* and *Cx. quinquefasciatus* (with a 3-minute exposure period) specified for mosquito repellents to be registered and sold in Thailand. On the basis of this regulation, there are only three repellents (*ie*, *Psidium guajava*, *Curcuma longa* and *Piper nigrum*) that meet the established criteria for registration. If the

Table 4
Oviposition deterrent effect of each repellent against *Ae. aegypti*.

Repellents	Mean no. of eggs (± S.E.)		Repellency* (%)
	Control	Treated	
Eleutherococcus trifoliatus	3,629 ± 529	717 ± 88	80.2b
Schefflera leucantha	3,950 ± 384	331 ± 73	91.6ab
Ocimum sanctum	3,992 ± 689	1,194 ± 142	70.1c
Vitex trifolia	4,805 ± 553	524 ± 167	89.1ab
Litsea cubeba	3,986 ± 338	774 ± 138	80.6b
Manglietia garrettii	3,815 ± 510	532 ± 150	86.1ab
Aglaia odorata	3,141 ± 334	1,190 ± 460	62.1c
Myristica fragrans	2,423 ± 276	2,021 ± 433	16.6d
Melaleuca cajuputi	3,797 ± 684	461 ± 151	87.9ab
Psidium guajava	3,518 ± 570	455 ± 104	87.1ab
Piper betle	3,076 ± 127	649 ± 128	78.9b
Piper nigrum	3,998 ± 660	719 ± 174	82.0b
Murraya paniculata	2,575 ± 381	1,092 ± 254	57.6c
Houttuynia cordata	4,575 ± 314	685 ± 40	85.0ab
Zingiber officinale	4,476 ± 498	443 ± 47	90.1ab
Alpinia galanga	3,569 ± 326	1,073 ± 139	69.9c
Curcuma longa	4,386 ± 438	232 ± 72	94.7a
Hedychium coronarium	3,557 ± 524	445 ± 127	87.5ab
Deet	2,171 ± 191	2,202 ± 336	0.0** e
IR3535	2,504 ± 453	2,903 ± 314	0.0** e

*Repellency followed by the same letter is not significantly different ($p > 0.05$, by one-way ANOVA with Duncant's multiple range test)

**Repellency is considered as zero when the mean number of mosquito eggs in the treated group was greater than the control group.

exposure time was one minute, the qualified repellents against *Ae. aegypti* (repellency ≥2 hours) would then be 16 out of 18 essential oils (except only *Houttuynia cordata* and *Hedychium coronarium*). As can be seen, a shorter exposure time, such as one minute, may indicate a higher repellency than a longer exposure time of two or three minutes. Similar differences in repellency among the three different exposure times were also detected in almost half of tests against *Cx. quinquefasciatus* (8 out of 18 tested essential oils). It is therefore recommended that the exposure time in mosquito cage testing should be at least three minutes in order to better reflect repellency.

The repellency of essential oils against various mosquito species obtained in our study was affected by synergism of some additives used in our formulation. However, all the essential oils and chemical repellents (deet and IR3535) were formulated in the same way for repellency comparison. We believe the essential oils without formulation would provide lower repellency than our results. Tawatsin *et al* (2001) confirmed that the repellency of volatile oils was improved dramatically when they were formulated with vanillin. Formulation technology, therefore, plays an important role for long lasting repellents.

Regarding the repellency obtained in the 3-minute exposure period, the night-biting mosquitoes (*An. dirus* and *Cx. quinquefasciatus*) and *Ae. albopictus* were more sensitive to all the essential oils (repellency 4.5 - 8 hours) than was *Ae. aegypti* (repellency 0.3 - 2.8 hours). These results indicate more aggressive biting behavior of *Ae. aegypti* over other mosquito species in this study. Different species of mosquitoes react differently to the same repellents (Rutledge *et al*, 1983). Based on the repellent results against *Ae. aegypti*, we recommend three essential oils, *Psidium guajava*, *Curcuma longa* and *Piper nigrum*, for further development as commercial repellents. These three essential oils also provided high repellency against other mosquito species. Recently, the same formulation of two essential oil repellents in this study, *Psidium guajava* and *Curcuma longa*, were evaluated for repellency in the field against mosquitoes, black flies and land leeches (Tawatsin *et al*, 2006). The results show that both *Psidium guajava* and *Curcuma longa* provided complete protection from mosquito landing and biting for up to 9 hours, and 100% protection against black flies and land leeches for 9 and at least 8 hours, respectively. These results, therefore, clearly confirm promising repellent effects against blood-sucking organisms by both *Psidium guajava* and *Curcuma longa* in the present study.

The studies on oviposition deterrent activity of chemical compounds and insect repellents have been carried out continuously against mosquito vectors, whereas those of plant extracts are scarce. Xue *et al* (2001, 2003) reported the ovipositional deterrent effects of deet and several repellent compounds, such as AI3-37220, AI3-35765, AI3-54995, AI3-55051 against *Ae. albopictus* under laboratory and field conditions. Until recently, Xue *et al* (2006) also pointed out the oviposition deterrent effectiveness (76 - 100% repellency) against *Ae. albopictus* of 21 commercial insect repellent products (at 0.1% concentration), including 12 botanical, 6 deet-based and 3 synthetic organics. As for the plant extracts, Mehra and Hiradhar (2002) revealed that the crude acetone extract of *Cuscuta hyalina* Roth. was an effective oviposition deterrent against *Cx. quinquefasciatus* at a concentration of 80 ppm. With reference to the relatively high repellency and yields, our study reveals the large potential of essential oils, such as *Curcuma longa*, *Zingiber officinale*, *Vitex trifolia*, *Melaleuca cajuputi*, *Hedychium coronarium*, *Psidium guajava*, and *Houttuynia cordata*, to be used as oviposition deterrent agents to disrupt oviposition by *Ae. aegypti* at breeding sites. These oils (at 0.01% concentration) provided 85 - 94.7% repellency, with 0.12 - 0.43% yields. In most prior oviposition deterrent studies, high levels of deterrent activity against *Ae. aegypti* have been rare. Although *Schefflera leucantha* and *Manglietia garrettii* have shown high oviposition deterrent activity (91.6 and 86.1%), both plants provided substantially low yields of essential oils (0.04 and 0.07%). These two oils, therefore, may not be appropriate for development as antioviposition agents. Further studies are needed to formulate active essential oils needed for treatment of water-storage containers, the most common breeding sites of *Ae. aegypti* in Thailand. Oviposition avoidance of insecticide-treated water-storage containers by gravid female mosquitoes can reduce levels of larval populations (Moore, 1977). Active essential oils that possess oviposition deterrent activity and include larvicidal effects against *Ae. aegypti* would be of interest as plant-based products for the control of mosquitoes.

The present study demonstrates a high potential for using essential oils as mosquito repellents against four species and ovipositional deterrent activity against *Ae. aegypti*. This may lead to new and more effective strategies to prevent and control mosquitoes.

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