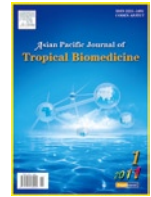




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Evaluation of herbal essential oil as repellents against *Aedes aegypti* (L.) and *Anopheles dirus* Peyton & Harrion

Duangkamon Sritabutra*, Mayura Soonwera, Sirirat Waltanachanobon, Supaporn Pongjai

Entomology and Environmental Programme, Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, 10520 Thailand

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ABSTRACT

Objective: To investigate the repellent activity of herbal essential oils from garlic (*Allium sativum*), clove (*Syzygium aromaticum*), lemon grass (*Cymbopogon citratus*), citronella grass (*Cymbopogon nardus*), peppermint (*Mentha piperita*), eucalyptus (*Eucalyptus globulus*), orange (*Citrus sinensis*) and sweet basil (*Ocimum basilicum*) and their combinations against *Aedes aegypti* (*Ae. aegypti*) (L.) and *Anopheles dirus* (*An. dirus*) Peyton & Harrion under laboratory conditions. **Methods:** In laboratory condition, 0.1 mL of each essential oil was applied to 3–10 cm of exposed area on a volunteer's forearm. The test was carried out every 30 min until fewer than two mosquitoes bit or land during the 3 min study period and then the repellency test was stopped. **Results:** Essential oil from lemon grass exhibited protection against biting from two mosquito species, for *Ae. aegypti* [98.66 ± 11.56 min protection time and 0.97% biting rate] and for *An. dirus* [98.00 ± 15.28 min protection time and 0.80% biting rate]. The combinations from eucalyptus oil and sweet basil oil were effective as repellents and feeding deterrents against *Ae. aegypti* [98.87 ± 10.28 min protection time and 0.90% biting rate] and *An. dirus* [210 ± 10.70 min protection time and 0.93% biting rate]. All herbal repellents exhibited the period of protection time against *Ae. aegypti* which was lower than 120 min. **Conclusions:** It can be concluded that oils of lemon grass and combination from eucalyptus–sweet basil are the most effective in repellent activity.

1. Introduction

Aedes aegypti (*Ae. aegypti*) (L.) and *Anopheles dirus* (*An. dirus*) Peyton & Harrion are the major vectors for dengue fever, yellow fever, chikungunya and malaria diseases responsible for a number of morbidity and mortality around the world, especially in tropical and sub-tropical regions[1,2].

However, the only efficient way to control these diseases is to control mosquito vector populations and prevent mosquito bites. Insect repellents are known to play an important role in preventing the mosquito vector, deterring an insect from flying to, landing on or biting human and animal skin. Widely used compounds as insect repellents are synthetic chemical repellents which are not safe for humans, especially children, domestic animals because they may cause skin irritation, hot sensation, rashes or

allergy[3]. Many people prefer to use a repellent from natural origin, natural product or herbal product and the demand for natural repellent is gradually increasing. The natural repellents, especially repellents from herbal essential oils are safe to human and environment and herbal essential oils are reported to have repellency against mosquito adults. Strong repellent actions of *Azadirachta indica*, *Cymbopogon martini* var *sofia*, *Cymbopogon citratus* (*C. citratus*), *Cymbopogon nardus* (*C. nardus*) and *Ocimum* sp. have been reported against some mosquitoes[4–11].

Many researchers pointed that essential oils from *Acantholippia salsoloides*, *Aloysia catamarcensis*, *Aloysia polytachya*, *Lippia integrifolia*, *Lippia junelliana*, *Baccharis salicifolia*, *Eupatorium buniifolium*, *Tagetes filifolia*, *Eucalyptus* spp., *Olea europaea*, *Ostostegia integrifolia*, *Silene macroserene*, *Eugenia caryophyllus*, *Litsea cubeba*, *Melaleuca leucadendron*, *Melaleuca quinquenervia*, *Viola odorata*, *Nepeta cataria*, *Cinnamomum osmophloeum*, *Cymbopogon winterianus*, *Syzygium aromaticum* (*S. aromaticum*), *Ianthoxylum limonelia* show strong repellency against *Ae. aegypti*, *Aedes albopitus* (*Ae. albopitus*), *Culex quinquefasciatus* (*Cx. quinquefasciatus*), *An. dirus* and *Anopheles minimus* (*An. minimus*)[3,4,12–22].

*Corresponding author: Duangkamon Sritabutra, Entomology and Environment Program, Plant Production Technology Section, King Mongkut's Institute of Technology Ladkrabang, Chalong Krung Road, Ladkrabang, Bangkok 10520, Thailand.

E-mail: i_zanaa_ploy@hotmail.com

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In Thailand, Tawatsin *et al*[23] reported the volatile oils from *Curcuma longa*, *Cymbopogon winterianus* and *Ocimum americanum* with the addition of 5% vanillin repelled *An. dirus*, *Ae. aegypti* and *Cx. quinquefasciatus* under mosquito cage conditions for up to 8 h. Trongtokit *et al*[24] reported that *C. nardus*, *Posgostrmon cablin*, *S. aromaticum* and *Zanthoxylum limonella* were the most effective repellents against *Ae. aegypti*, *Cx. quinquefasciatus* and *An. dirus* and could prevent mosquito bites for 2–4 h. The essential oils from citronella and eucalyptus with the addition 5% vanillin repelled *Ae. albopitus* with the protection time up to 5 h[25]. The essential oils from *Psidium guajava*, *Piper nigrum* and *Curcuma longa* are reported to have repellency against *Ae. aegypti*, *Ae. albopitus*, *An. dirus* and *Cx. quinquefasciatus*[26]. Choochote *et al*[27] reported the essential oil of *Zanthoxylum piperitum* fruit may prove useful in the development of mosquito repellents as an effective personal protection measure against *Ae. aegypti* mosquito bites. Phasomkusolsil and Soonwera[28] reported the plant oils from *Zingiber cassumunar* and *Ocimum basilicum* (*O. basilicum*) are effective against *An. minimus*, *Cx. quinquefasciatus* and *Ae. aegypti*. Oyedele *et al* reported the formulation of mosquito-repellent product from lemongrass oil (*Cymbopogon citrates*) found that 15% v/w hydrophilic ointment formulation of the oil exhibited more than 50% repellency lasting 2–3 hours against mosquito bite-deterrent[29]. Ansari *et al* reported the essential oil of peppermint oil (*Mentha piperita*) showed strong repellent action against *An. annularis*, *An. culicifacies* and *Cx. quinquefasciatus* was 100%, 92.3% and 84.5%, respectively[30]. Thavara *et al* reported product of mosquito repellents that contain synthetic chemicals that are commercially available, such as deet, KBR3023 and IR3535 are very toxic to humans and animals. In study, observed 44 formulations of mosquito repellents containing plant extracts such as citronella oil, eucalyptus oil, tea tree oil, turmeric oil, bergamot oil, lavender extract, tobacco-leaves extract, clove extract and neem-leaves extract. These agents can prevent up to 6.3 hours, but there are only 12 species that have been registered to be sold in the market and must be protected at no less than 2 hours by a variety of formats such as citronella oil, eucalyptus oil and tea tree oil were the main active ingredients. However, National Institute of Public Health has developed a substance that consists turmeric oil and eucalyptus oil found that this repellent provide protection time for 7 hours against *Ae. aegypti* and at least 8 hours against *Cx. quinquefasciatus* and *An. dirus*[31].

In the present study, an attempt has been made to evaluate the repellent efficacy of herbal essential oils and their combinations against *Ae. aegypti* and *An. dirus* under laboratory conditions.

2. Materials and methods

2.1. Herbal essential oils

The herbal essential oils used as mosquito repellents were extracted from each plant by steam distillation of the leaf *Eucalyptus globules* (*E. globules*), leaf of *Mentha piperita* (*M. piperita*), bulb of *Allium sativum* (*A. sativum*), fruit of *Citrus sinensis* (*C. sinensis*), stem of *C. nardus*, stem of *C. citratus*, flower of *S. aromaticum* and leaf of *O. basilicum*. Soybean oil was obtained from the market and used as bases of the

repellents. The repellents were formulated into 2 groups *i.e.* herbal essential oils and the combination of herbal essential oils.

2.2. Mosquitoes

Ae. aegypti and *An. dirus* were reared and maintained in the Laboratory of Entomology and Environment, Plant Production Technology Section, Faculty of Agricultural Technology Ladkrabang, Bangkok, Thailand. Adults of two mosquito species were fed on 10% glucose under (28±2) °C and (78±2)% relative humidity. 5 days old of 250 female mosquitoes per insect cage (30 cm × 30 cm × 30 cm) were starved for 8 h before testing.

2.3. Repellent test

Herbal essential oils and their combinations were screened for repellency against *Ae. aegypti* and *An. dirus* under laboratory conditions [(28±2) °C and (78±2)% relative humidity] by using human-bait method and TISI guidelines. The *Ae. aegypti* was tested during the daytime from 8.00 am to 4.00 pm, while *An. dirus* was tested during night time from 4.00 pm to 12.00 pm[28,32].

Before application of the repellents, the arms of three human volunteers were washed and cleaned thoroughly with distilled water. Both arms were covered with rubber sleeve with a window area of (3 cm × 10 cm) on the ventral part of forearm. The left arm was used for treatment and the right arm for control. 0.1 mL of test repellent was applied to the treatment area of left forearm of each volunteer. After applying the test repellent, the volunteer was instructed not to rub, touch or wet the treated forearm. The right forearm, which acted as a control was not treated and was exposed for up to 30 sec to mosquito cage (30 cm × 30 cm × 30 cm) contained 250 nulliparous female mosquitoes (5–7 days old). If at least two mosquitoes landed on or bit the arm the repellency test was then continued. The test continued until at least two bites occurred in a 3-min period. If no mosquitoes bit or landed during the 3-min period the arm was withdrawn from the cage. The repellency test period was carried out every 30 min until fewer than 2 mosquitoes bit or landed during the 3-min study period and then the repellency test was stopped. The time between application of the repellents was recorded as the protection time.

2.4. Data analysis

The median protection time was used to compare the tested repellents. Differences in significance were analyzed by one-way analysis of variance (ANOVA) and Duncan's new multiple range test (DMRT). Percentage of mosquito biting or landing was calculated for each test using the following formula[26,28].

$$\% \text{ Biting} = B/250 \times 100$$

Where B is the total number of biting or landing by the end of the test. The test was carried out 3 times per sample.

3. Results

Table 1List of herbal essential oil repellents and combination of herbal essential oil repellents tested against *Ae. aegypti* and *An. dirus*.

Category	Code No.	Common name / Scientific name	Plant used	Formulation
Essential oils	E1	Eucalyptus (<i>E. globules</i>)	Leaf	10% Eucalyptus oil in soybean oil
	E2	Peppermint (<i>M. piperita</i>)	Leaf	10% Peppermint oil in soybean oil
	E3	Garlic (<i>A. sativum</i>)	Bulb	10% Garlic oil in soybean oil
	E4	Orange (<i>C. sinensis</i>)	Fruit	10% Orange oil in soybean oil
	E5	Citronella grass (<i>C. nardus</i>)	Stem	10% Citronella grass oil in soybean oil
	E6	Lemon grass (<i>C. citratus</i>)	Stem	10% Lemon grass oil in soybean oil
	E7	Clove (<i>S. aromaticum</i>)	Flower	10% Clove oil in soybean oil
	E8	Sweet basil (<i>O. basilicum</i>)	Leaf	10% Sweet basil oil in soybean oil
Combination of essential oils	M1	Citronella grass + Orange	Stem, fruit	5% Citronella grass oil + 5% orange oil in soybean oil
	M2	Citronella grass + Eucalyptus	Stem, leaf	5% Citronella grass oil + 5% eucalyptus oil in soybean oil
	M3	Lemon grass + Orange	Stem, fruit	5% Lemon grass oil + 5% orange oil in soybean oil
	M4	Clove + Eucalyptus	Flower, leaf	5% Clove oil + 5% eucalyptus oil in soybean
	M5	Peppermint + Eucalyptus	Leaf, leaf	5% Peppermint oil + 5% eucalyptus oil in soybean oil
	M6	Citronella grass + Lemon grass	Leaf, fruit	5% Citronella grass oil + 5% lemongrass oil in soybean oil
	M7	Eucalyptus + Sweet basil	Leaf, leaf	5% Eucalyptus oil + 5% sweet basil oil in soybean oil
	M8	Peppermint + Orange	Leaf, leaf	5% Peppermint oil + 5% orange oil in soybean oil

Table 2Efficacy of herbal essential oil formulation E1–E8 and M1–M8, as repellents against *Ae. aegypti* and *An. dirus* (mean±SD).

Repellents	Protection time		Biting (%)		
	<i>Ae. aegypti</i>	<i>An. dirus</i>	<i>Ae. aegypti</i>	<i>An. dirus</i>	
Essential oils	E1	81.67±11.55 ^{ab}	95.00±10.00 ^a	0.67 ^a	0.80 ^a
	E2	98.33±15.28 ^a	65.00±10.00 ^b	0.93 ^a	0.80 ^a
	E3	31.67±5.77 ^a	41.67±5.77 ^c	0.93 ^a	0.80 ^a
	E4	30.65±2.33 ^d	50.33±11.55 ^{bc}	0.80 ^a	0.80 ^a
	E5	88.33±15.28 ^{ab}	48.33±5.77 ^c	0.97 ^a	0.80 ^a
	E6	98.66±11.56 ^a	98.00±15.28 ^a	0.97 ^a	0.80 ^a
	E7	80.33±10.56 ^b	60.00±10.00 ^b	0.80 ^a	0.97 ^a
	E8	65.00±10.00 ^c	45.00±10.00 ^c	0.97 ^a	0.80 ^a
	Soybean oil (positive control)	5.00±0.00 ^d	5.00±0.00 ^d	4.80 ^b	4.00 ^b
Untreated (negative control)	0.00±0.00	0.00±0.00	8.80 ^c	10.00 ^c	
Combination of essential oils	M1	98.67±10.58 ^a	100.00±10.00 ^{bc}	0.93 ^a	0.80 ^a
	M2	92.73±12.28 ^a	80.56±11.35 ^c	0.80 ^a	0.93 ^a
	M3	60.53±10.54 ^c	55.33±6.74 ^{de}	1.10 ^a	0.80 ^a
	M4	60.33±15.28 ^c	48.37±5.76 ^c	1.10 ^a	0.80 ^a
	M5	80.35±15.28 ^b	60.67±10.53 ^d	0.90 ^a	0.80 ^a
	M6	60.31±5.78 ^c	100.34±10.56 ^{bc}	0.90 ^a	0.93 ^a
	M7	98.87±10.28 ^a	210.00±10.70 ^a	0.80 ^a	0.93 ^a
	M8	70.33±15.30 ^b	125.33±5.77 ^b	0.80 ^a	0.93 ^a
	Soybean oil (positive control)	5.00±0.00 ^d	5.00±0.00 ^f	6.00 ^b	4.80 ^b
Untreated (negative control)	0.00±0.00 ^d	0.00±0.00 ^f	10.00 ^c	12.00 ^c	

Mean in each column against each species followed by the same letter are not significantly different ($P>0.05$) by one-way ANOVA with DMRT.

Table 1 listed herbal essential oils and combinations of essential oil repellents tested against *Ae. aegypti* and *An. dirus*. The protection time of herbal essential oils formulation E1–E8, soybean oil (positive control) and untreated (negative control) against *Ae. aegypti* and *An. dirus* was shown in Table 2. The lemon grass oil repellent (E6) had the best efficiency against *Ae. aegypti* and *An. dirus* in which the protection times were (98.66±11.56) and (98.00±15.28) min, respectively. All herbal essential oil repellents exhibited lower protection against *Ae. aegypti* and *An. dirus* than Thai Industrial Standards Institute (TISI) determines whose protection time against mosquitoes should be more than 120 min. The protection times of soybean oil (positive control) and untreated (negative control) against two mosquito species

were (5.00±0.00) and (0.00±0.00) min, respectively.

The percentage of *Ae. aegypti* and *An. dirus* biting or landing on soybean oil (positive control), untreated (negative control) and treated areas was shown in Table 2. Eucalyptus oil (E1) had the best efficiency against two mosquitoes species in which the biting percentage was 0.67% and 0.80%, respectively. All tested repellents exhibited the biting percentage against two mosquitoes species ranged from 0.67% to 0.97% while soybean oil (positive control) and untreated (negative control) gave a range of 4.00% to 10.00%.

Comparison of protection time and biting percentage for each repellent essential oil group *i.e.* E1–E8 and M1–M8 against *Ae. aegypti* and *An. dirus* was shown in Figure 1 and Figure 2.

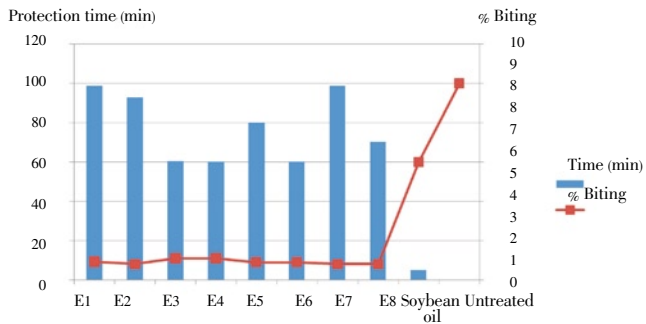
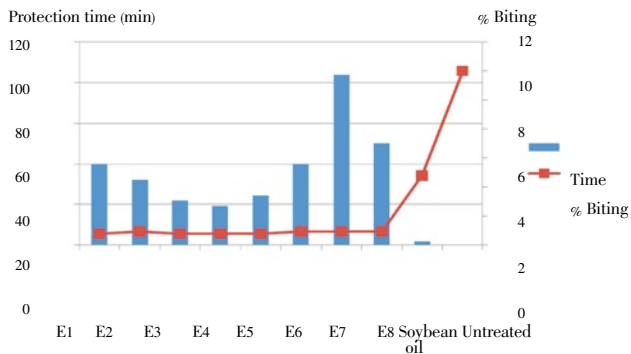
a. *Ae. aegypti*b. *An. dirus*

Figure 1. Comparison of protection time (min) and biting percentage for each repellent essential oil group against two mosquito species. E1: eucalyptus; E2: peppermint; E3: garlic; E4: orange; E5: citronella grass; E6: lemon grass; E7: clove; E8: sweet basil.

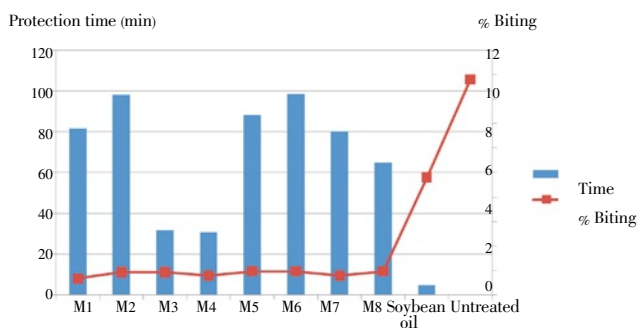
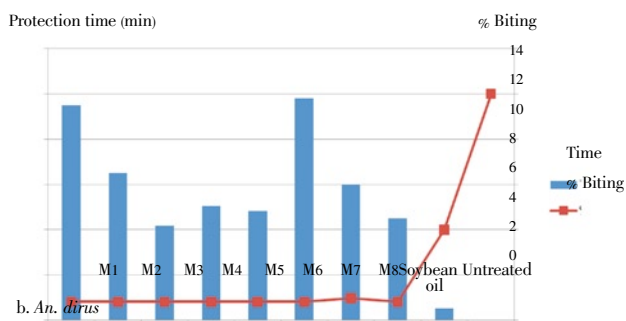
a. *Ae. aegypti*b. *An. dirus*

Figure 2. Comparison of protection time (min) and biting percentage for each repellent essential oil group against two mosquito species. M1: citronella + orange; M2: citronella grass + eucalyptus; M3: lemon grass + orange; M4: clove + eucalyptus; M5: peppermint + eucalyptus; M6: citronella grass + lemon grass; M7: eucalyptus + sweet basil; M8: peppermint + orange.

The result of repellency for herbal essential oil formulation M1–M8 soybean oil (positive control) and untreated (negative control) against two mosquitoes species were shown in Table 2. The protection time of herbal essential oil formulation (M1–M8) against *Ae. aegypti* was 60–98 min and against *An. dirus* was 48–210 min and biting percentage gave a range of (0.80–1.10)% and (0.80–0.93)%, respectively. The repellents showing the best protection time for *Ae. aegypti* were citronella grass oil + orange oil [M1 (98.67±10.58) min] and eucalyptus oil + sweet basil oil [M7 (98.87±10.28) min] with biting percentage of 0.80% and 0.93%, respectively. For *An. dirus*, the protection time of eucalyptus oil + sweet basil (M7) was (210.00±10.70) min and peppermint oil + orange oil (M3) was (125.33±5.77) min with biting percentage of 0.93% and 0.93%, respectively.

While the protection time and biting percentage of soybean oil and untreated against two mosquitoes species were (5.00±0.00) and (0.00±0.00) min and (4.80–6.00)% and (10.00–12.00)%, respectively.

4. Discussion

The essential oil derived from lemon grass, peppermint, eucalyptus, citronella grass, and clove were effective against the two mosquito species and that from garlic, orange and sweet basil showed the least protection time. The combination of essential oil showed better protection time against two mosquito species than each essential oil. The combinations of eucalyptus oil + sweet basil oil and peppermint oil + orange oil were effective and the protection time was more than 120 min and biting rate was less than 1.0%. Therefore, the two combinations are efficient in repellency and can be used as biting deterrent. Treatment with them makes no skin irritation on forearm area of the test volunteers.

However, the protection time of this study is short in some essential oils and some formulations can improve the protection time. Many researchers pointed that the volatile oil from plants should be formulated with 5%–10% vanillin in order to improve the repellent efficacy[23,27]. Although, the repellent effects of herbal essential oils do not usually last as long as synthetic chemical which can protect from mosquito bite for up to 6 h[33], and none of herbal essential oils tested up to now provide the wide effectiveness and duration of protection time, herbal essential oil repellents are safe for human life, human and domestic animal skin with no side effect and no feedback of environmental ill effect.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

- [1] Silva WL, Doria GAA, Maia RT, Nunes RS, Carvalho GA, Blank AF, et al. Effects of essential oils on *Aedes aegypti* larvae: alternatives to environmentally safe insecticides. *Bioresour Technol* 2008; **99**: 3251–3255.
- [2] Wikipedia. *Anopheles dirus*. [Online] Available from: http://en.wikipedia.org/wiki/Anopheles_dirus. [Accessed on 27 December, 2010]
- [3] Das NG, Baruah I, Talukdar PK, Das SC. Evaluation of botanicals as repellents against mosquitoes. *J Vector Borne Dis* 2003; **40**: 49–53.
- [4] Makhaik M, Naik SN, Tewary DK. Evaluation of anti-mosquito properties of essential oils. *J Sci Ind Res* 2005; **64**: 129–133.
- [5] Govindarajan M. Evaluation of *Andrographis paniculata* Burm. f. (Family: Acanthaceae) extracts against *Culex quinquefasciatus* (Say.) and *Aedes aegypti* (Linn.) (Diptera: Culicidae). *Asian Pac J Trop Med* 2011; **4**(3): 169–252.
- [6] Govindarajan M, Karuppanan P. Mosquito larvicidal and ovicidal properties of *Eclipta alba* (L.) Hassk (Asteraceae) against chikungunya vector, *Aedes aegypti* (Linn.) (Diptera: Culicidae). *Asian Pac J Trop Med* 2011; **4**(1): 24–28.
- [7] Govindarajan M. Larvicidal and repellent properties of some essential oils against *Culex tritaeniorhynchus* Giles and *Anopheles subpictus* Grassi (Diptera: Culicidae). *Asian Pac J Trop Med* 2011; **4**(2): 106–111.
- [8] Sedaghat MM, Sanei AR, Khnavi M, Abai MR, Hadjiakhoondi A, Mohtarami F, et al. Phytochemistry and larvicidal activity of *Eucalyptus camaldulensis* against malaria vector, *Anopheles stephensi*. *Asian Pac J Trop Med* 2011; **3**(11): 841–845.
- [9] Govindarajan M, Sivakumar R, Amsath A, Niraimathi S. Mosquito larvicidal properties of *Ficus benghalensis* L. (Family: Moraceae) against *Culex tritaeniorhynchus* Giles and *Anopheles subpictus* Grassi (Diptera: Culicidae). *Asian Pac J Trop Med* 2011; **4**(7): 505–509.
- [10] Hossain E, Rawani A, Chandra G, Mandal SC, Gupta JK. Larvicidal activity of *Dregea volubilis* and *Bombax malabaricum* leaf extracts against the filarial vector *Culex quinquefasciatus*. *Asian Pac J Trop Med* 2011; **4**(6): 463–441.
- [11] Zhu L, Tian YJ. Chemical composition and larvicidal effects of essential oil of *Blumea martiniana* against *Anopheles anthropophagus*. *Asian Pac J Trop Med* 2011; **4**(5): 371–374.
- [12] Gleiser RM, Bonino MA, Zygadlo JA. Repellence of essential oils of aromatic plants growing in Argentina against *Aedes aegypti* (Diptera: Culicidae). *Parasitol Res* 2011; **108**: 69–78.
- [13] Lucia A, Licastro S, Ierba E, Audino PG, Masuth H. Sensitivity of *Aedes aegypti* adult (Diptera: Culicidae) to the vapors of *Eucalyptus* essential oils. *Bioresour Technol* 2009; **100**: 6083–6087.
- [14] Karunamoorthi K, Mulelam A, Wassie F. Assessment of knowledge and usage custom of traditional insect mosquito repellent plants in Addis Zemen Town, South Gonder, North Western Ethiopia. *J Ethnopharmacol* 2009; **121**: 49–53.
- [15] Tjahjani S. *Efficacy of several essential oils as Culex and Aedes repellents*. Proceedings of the Third ASEAN Congress of Tropical Medicine and Parasitology (ACTMP3). Bangkok: The Windsor Suites Hotel; 2009, p. 33–37.
- [16] Amer A, Mehlhorn H. Repellency effect of forty one essential oils against *Aedes*, *Anopheles* and *Culex* mosquitoes. *Parasitol Res* 2006; **99**: 478–490.
- [17] Cheng SS, Liu JY, Tsai KH, Chen WJ, Chano ST. Chemical composition and mosquito larvicidal activity of essential oils from leaves of different *Cinnamomum osmophloeum* provenances. *J Agric Food Chem* 2004; **52**: 4395–4400.
- [18] Choochote W, Chaayasit D, Kanjanapothi D, Rattanachanpichai E, Jitpakdi A, Tuetun B, et al. Chemical composition and anti-mosquito potential of rhizome extract and volatile oil derived from *Curcuma aromatica* against *Aedes aegypti* (Diptera: Culicidae). *J Vector Ecol* 2005; **30**(2): 302–309.
- [19] Choochote W, Tuetun B, Kanjanapothi D, Rattanachanpichai E, Chaithong U, Chaiwong P, et al. Potential of crude seed extract of celery, *Apium graveolens* L., against the mosquito *Aedes aegypti* (L.) (Diptera: Culicidae). *J Vector Ecol* 2004; **29**(2): 340–346.
- [20] Prajapati V, Tripathi AK, Aggarwal KK, Khanuja SP. Insecticidal, repellent and oviposition-deterrent activity of selected essential oils against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. *Bioresour Technol* 2005; **96**: 1749–1757.
- [21] Sophai N, Pandian RS. Screening of the efficacy of phytochemical repellents against the filarial vector mosquito, *Culex quinquefasciatus* Say. *Curr Biot* 2009; **3**(1): 14–31.
- [22] Trongtokit Y, Rongsriyam Y, Komalamisra N, Krisadaphong P, Apiwathnasorn C. Laboratory and field trial of developing medicinal local Thai plant products against four species of mosquito vectors. *Southeast Asian J Trop Med Public Health* 2004; **35**(2): 325–334.
- [23] Tawatsin A, Wratten SD, Scott RR, Thavara U, Techadamrongsin Y. Repellency of volatile oils from plants against three mosquito vectors. *J Vector Ecol* 2001; **26**: 76–82.
- [24] Trongtokit Y, Rongsriyam Y, Komalamisra N, Krisadaphong P, Apiwathnasorn C. Comparative repellency of 38 essential oils against mosquito bites. *Phytother Res* 2005; **19**: 303–309.
- [25] Yang P, Ma Y. Repellent effect of plant essential oils against *Aedes albopictus*. *J Vector Ecol* 2005; **30**(2): 231–234.
- [26] Tawatsin S, Asavadachanukorn P, Thavara U, Wongsinkongman P, Bansighi J, Boonruad T, et al. Repellency of essential oils extracted from plants in Thailand against four mosquito vectors (Diptera: Culicidae) and oviposition deterrent effect against *Aedes aegypti* (Diptera: Culicidae). *Southeast Asian J Trop Med Public Health* 2006; **37**: 915–931.
- [27] Choochote W, Chaithong U, Kamsuk K, Jitpakdi A, Tippawangkosol P, Tuetun B, et al. Repellent activity of selected essential oils against *Aedes aegypti*. *Fitoterapia* 2007; **78**: 359–364.
- [28] Phasomkusolsil S, Soonwera M. Insect repellent activity of medicinal plant oils against *Aedes aegypti* (Linn.) *Anopheles minimus* (Theobald) and *Culex quinquefasciatus* Say based on protection time and biting rate. *Southeast Asian J Trop Med Public Health* 2010; **47**: 831–840.
- [29] Oyedele AO, Gbolade AA, Sosan MB, Adewoyin FB, Soyelu OL, Orafidiya OO. Formulation of an effective mosquito-repellent topical product from lemongrass oil. *Phytomedicine* 2002; **9**: 259–262.
- [30] Ansari MA, Vasudevan P, Tandon M, Razdan RK. Larvicidal and mosquito repellent action of peppermint (*Mentha piperita*) oil. *Bioresour Technol* 2000; **71**(3): 267–271.
- [31] Thavara U, Tawatsin A, Chompoonsri J. *Phytochemicals as repellents against mosquitoes in Thailand*. Proceedings International Conference on Biopesticide; 2002, p. 244–250.
- [32] Thai Industrial Standards Institute (TISI). *Mosquito repellents*. Bangkok: Ministry of Industry; 1986, p. 15.
- [33] Debboun M, Strickman D, Solberg VB. Field evaluation of DEET and piperidine repellent against *Aedes communis* (Diptera: Culicidae) and *Simulium venustum* (Diptera: Simuliidae) in the Adirondack Mountains of New York. *J Med Entomol* 2000; **37**: 919–923.