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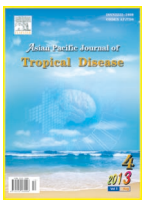

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Repellent activity of herbal essential oils against *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* (Say.)

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PEER REVIEW

Peer reviewer

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Comments

This work evaluated the repellency effect of several herbal oils against medically important mosquitoes. The study benefits from a good and standard design and seems to give reliable and confident results.
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ABSTRACT

Objective: To determine the mosquito repellent activity of herbal essential oils against female *Aedes aegypti* and *Culex quinquefasciatus*.
Methods: On a volunteer's forearm, 0.1 mL of each essential oil was applied to 3 cm×10 cm of exposed skin. The protection time was recorded for 3 min after every 30 min.
Results: Essential oil from clove oil in olive oil and in coconut oil gave the longest lasting period of 76.50 min and 96.00 min respectively against *Aedes aegypti*. The citronella grass oil in olive oil, citronella grass oil in coconut oil and lemongrass oil in coconut oil exhibited protection against *Culex quinquefasciatus* at 165.00, 105.00, and 112.50 min respectively.
Conclusions: The results clearly indicated that clove, citronella and lemongrass oil were the most promising for repellency against mosquito species. These oils could be used to develop a new formulation to control mosquitoes.

KEYWORDS

Herbal essential oils, Repellent, *Aedes aegypti*, *Culex quinquefasciatus*, Protection time, Olive oil, Coconut oil

1. Introduction

Mosquitoes are the most important of insects in terms of public health importance which transmit a number of diseases such as dengue, chikungunya, Japanese encephalitis, filariasis and malaria, causing millions of deaths every year. *Aedes aegypti* (*Ae. aegypti*) and *Culex quinquefasciatus* (*Cx. quinquefasciatus*) are major urban vectors of dengue fever, dengue haemorrhagic fever, chikungunya and Japanese encephalitis. Mosquito bites may also cause allergic responses including local skin reactions and systemic reactions such as urticarial. Personal protection is one approach to prevent mosquito bites[1-3]. Most common mosquito repellents available contain N,N-diethyl-3-methylbenzamide or also called DEET that has shown strong protection from mosquitoes. However, it may exert toxic reaction under some circumstances and age groups and damage plastic, synthetic materials, thus the alternative new products need to be explored[4-8].

Plant essential oils in general have been recognized as important natural resources of insecticides because some are selective, biodegrade to non-toxic products and have few effects on non-target organisms and environment[9]. Many research insect repellents derived from plant extract, such as *Eucalyptus citriodara* (*E. citriodara*),

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Syzygium aromaticum, *Cymbopogon nardus*, *Cymbopogon citratus* (*C. citratus*), *Curcuma longa*, *Zingiber officinale* (*Z. officinale*), *Azadirachta indica*, *Ageratum houstonianum*, *Pogostemon cablin*, *Albizzia amara*, *Ocimum basilicum*, *Zanthoxylum piperitum*, *Anethum graveolens*, *Kaempferia galangal*, *Aristolochia bracteata*, *Cardiospermum halicacabum*, *Clausena anisata and Vetiveria zizanioides*, have been studied as possible mosquito repellents and have demonstrated good efficacy against *Aedes* spp., *Culex* spp. and *Anopheles* spp[4,10-22].

In the present, most insecticides are non-selective and can be harmful to other organisms and environment. There is a need to develop new formulations for controlling mosquitoes in an environmentally safer way, using biodegradable and target-specific insecticides against them[23-24]. Therefore, the present study aimed to investigate the mosquito repellent of eight essential oils: *Cananga odorata* (*C. odorata*), *Syzygium aromaticum*, *Z. officinale*, *C. citratus*, *Cymbopogon nardus*, *E. citriodara*, *Citrus reticulate* and *Ocimum basilicum* against *Ae. aegypti* and *Cx. quinquefasciatus*.

2. Materials and methods

2.1. Plant materials

The plant materials were collected from *C. odorata* Hook. f. & Th. (ylang-ylang flowers), *Syzygium aromaticum* (L.) Merr. & L.M.Perry (clove flowers), *Z. officinale* Roscoe. (*Ginger rhizome*), *C. citratus* (DC.) Staph. (lemongrass stems), *Cymbopogon nardus* (Linn.)Rendle (citronella grass stems), *E. citriodara* Hook. (eucalyptus leaves), *Citrus reticulate* Blanco. (orange peels) and *Ocimum basilicum* Linn. (sweet basil leaves). Each plant material was extracted for essential oils by steam distillation. All formulations were kept at room temperature before testing (Table 1).

2.2. Mosquitoes

All two species of mosquitoes (*Ae. aegypti* and *Cx. quinquefasciatus*) were reared in the laboratory of the Plant Production Technology Section, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok. Collecting eggs of *Ae. aegypti* and *Cx. quinquefasciatus* were hatched in plastic trays (24 cm×33 cm×5.5 cm), which contained tap water. The hatched larvae were held in plastic trays and larval diet with fish food was added to each tray. Newly emerged pupae were transferred to screen cage (size 30 cm×30 cm×30 cm) and emerged as adults. Adults continuously provided with 5% glucose solution in water soaked on cotton pads. In this study, 5 to 7 day-old female mosquitoes were starved only by providing them water for 8 h.

2.3. Repellent activity

Volunteers for testing are students of the Plant Production Technology Section, Faculty of Agricultural Technology, KMITL, and test times was determined by normal feeding times for each mosquito species. The *Ae. aegypti* testing time was between 8.00 am to 4.00 pm, while the *Cx. quinquefasciatus* testing time was 6.00 pm to 12.00 pm.

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

Table 1
List of repellents tested in this study.

Common name/Scientific name	Plant used	Formulation (10%)
Ylang-ylang (*C. odorata*)	Flower	ylang-ylang oil in olive oil
Clove (*Syzygium aromaticum*)	Flower	clove oil in olive oil
Ginger (*Z. officinale*)	Rhizome	ginger oil in olive oil
Lemongrass (*C. citratus*)	Stem	lemongrass oil in olive oil
Citronella grass (*Cymbopogon nardus*)	Stem	citronella grass oil in olive oil
Eucalyptus (*E. citriodara*)	Leave	eucalyptus oil in olive oil
Orange (*Citrus reticulate*)	Peel	orange oil in olive oil
Sweet basil (*Ocimum basilicum*)	Leave	sweet basil oil in olive oil
Ylang-ylang (*C. odorata*)	Flower	ylang-ylang oil in coconut oil
Clove (*Syzygium aromaticum*)	Flower	clove oil in coconut oil
Ginger (*Z. officinale*)	Rhizome	ginger oil in coconut oil
Lemongrass (*C. citratus*)	Stem	lemongrass oil in coconut oil
Citronella grass (*Cymbopogon nardus*)	Stem	citronella grass oil in coconut oil
Eucalyptus (*E. citriodara*)	Leave	eucalyptus oil in coconut oil
Orange (Citrus reticulate)	Peel	orange oil in coconut oil
Sweet basil (*Ocimum basilicum*)	Leave	sweet basil oil in coconut oil

part of forearm, the left arm for treatment and the right arm for control. A total of 0.1 mL of test repellent was applied to the treatment area of left forearm of each volunteer and used the olive oil and coconut oil as a negative control and Kor Yor 15® insect repellent lotion (containing DEET 25.63%) as a positive control. 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 seconds to mosquito cage (30 cm×30 cm×30 cm) contained 250 nulliparous female mosquitoes (5 to 7 day-old). If at least two mosquitoes landed on or bit the arm, the repellency test was then continued. The test continued unit as least two bites occurred in a three-minute period. If no mosquitoes bit or landed during the three-minute 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 land during the three-minute study period at which time the repellency test was stopped. The time between application of the repellents was recorded as the protection time.

For comparison, a percentage of mosquito biting was calculated for each test using the following formula[25-26]:

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

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

2.4. Statistical analysis

The mean protection time was used to compare the eight essential oils. Differences in significance were analyzed by one-way analysis of variance (ANOVA) and Duncan's multiple comparisons by SPSS for Windows.

3. Results

The results of the protection time and the biting percentage of essential oils in olive oil against *Ae. aegypti* and *Cx. quinquefasciatus* are shown in Table 2. There were

Table 2
Protection time for each repellent in the olive oil group against *Ae. aegypti* and *Cx. quinquefasciatus* and biting percentage.

Repellents	Protection time (min)		Biting percentage (%)	
	Ae. aegypti	*Cx. quinquefasciatus*	*Ae. aegypti*	*Cx. quinquefasciatus*
Ylang-ylang oil	48.00±18.00^{bcd}	84.00±18.00^{cd}	1.40±0.90	1.40±0.69
Clove	76.50±3.00^{b}	57.00±25.46^{cd}	1.25±1.48	1.25±0.25
Ginger	51.75±7.97^{bcd}	102.00±23.24^{c}	0.80±0.88	3.60±0.20
Lemongrass	59.25±27.86^{bc}	97.50±34.07^{c}	1.00±0.70	1.00±0.23
Citronella grass	54.75±21.27^{bc}	165.00±103.92^{b}	1.20±0.21	1.20±0.56
Eucalyptus	51.75±25.50^{bcd}	67.50±42.53^{cd}	1.50±0.57	1.50±0.60
Orange	30.75±19.50^{cd}	81.75±30.63^{cd}	1.75±1.29	1.75±1.32
Sweet basil	41.25±23.67^{cd}	97.50±9.00^{c}	1.40±1.07	1.40±0.51
Olive oil (negative control)	21.00±0.00^{d}	25.50±9.00^{d}	0.90±0.69	0.90±0.23
Kor Yor 15® (positive control)	433.00±19.80^{a}	421.00±12.65^{a}	0.80±0.20	4.80±0.80

Data are expressed as mean±SD. Mean in each column against each mosquito species followed by the difference letters are significantly different (P<0.05) by one-way ANOVA with Duncan's multiple range test (DMRT).

Table 3
Protection time for each repellent in the coconut oil group against *Ae. aegypti* and *Cx. quinquefasciatus* and biting percentage.

Repellents	Protection time (min)		Biting percentage (%)	
	Ae. aegypti	*Cx. quinquefasciatus*	*Ae. aegypti*	*Cx. quinquefasciatus*
Ylang-ylang oil	63.00±8.49^{de}	67.50±9.00^{cde}	0.93±0.18	0.90±0.20
Clove	96.00±15.87^{b}	85.50±19.21^{c}	0.97±0.20	0.97±0.20
Ginger	54.00±10.39^{e}	66.00±10.39^{de}	0.97±0.20	1.07±0.18
Lemongrass	87.00±8.50b^{c}	112.50±12.37^{b}	1.03±0.20	0.93±0.18
Citronella grass	82.50±17.23^{bcd}	105.00±8.49^{b}	1.03±0.20	1.17±0.33
Eucalyptus	82.50±19.21^{bcd}	70.50±9.00^{cde}	0.93±0.18	0.90±0.20
Orange	54.00±10.39^{e}	54.00±6.00^{e}	0.97±0.20	1.03±0.20
Sweet basil	73.50±12.37^{cde}	84.00±10.39^{cd}	1.13±0.35	1.03±0.20
Coconut oil (negative control)	33.00±8.49^{f}	25.50±9.00^{f}	1.17±0.33	0.90±0.20
Kor Yor 15® (positive control)	421.50±13.30^{a}	425.50±19.00^{a}	0.90±0.20	0.97±0.20

Data are expressed as mean±SD. Mean in each column against each mosquito species followed by the difference letters are significantly different (P<0.05) by one-way ANOVA with Duncan's multiple range test (DMRT).

significant differences in repellency among the repellents by mosquito species ($P<0.05$). The clove oil repellent and citronella grass oil had the best efficiency against *Ae. aegypti* and *Cx. quinquefasciatus*, respectively in which the protection time were (76.50±3.00) and (165.00±103.92) min respectively. The essential oil of *Z. officinale* (ginger oil) and *C. citratus* (lemongrass oil) had the best efficiency against *Ae. aegypti* and *Cx. quinquefasciatus*, respectively in which the biting percentage were 0.80% and 1.00%, respectively.

Table 3 shows the repellency for the essential oils in coconut oil against *Ae. aegypti* and *Cx. quinquefasciatus*. There were significant differences in repellency among the mosquito species ($P<0.05$). The clove oil had the best efficiency against *Ae. aegypti* [(96.00±15.87) min protection time]. The lemongrass oil and citronella grass oil had the best efficiency against *Cx. quinquefasciatus* [(112.50±37.00 and (105.00±8.49) min protection time, respectively]. The protection time of eight herbal essential oils in coconut oil against *Ae. aegypti* was 54 to 96 min (0.90%-1.13% biting rate) and against *Cx. quinquefasciatus* was 54.00 to 112.50 min (0.90%-1.17% biting rate). The essential oil of *C. odorata* (ylang-ylang oil) exhibited good efficiency against *Ae. aegypti* in which the biting percentage was 0.93%. And essential oils of *C. odorata* (ylang-ylang oil) and *E. citriodara* (eucalyptus oil) exhibited good efficiency against *Cx. quinquefasciatus* in which both of the biting percentages were 0.90%.

The Thai Industrial Standards Institute (TISI) standard determines the repellency time against mosquitoes should be >2 h[27]. Some repellents provided nearly 2 h protection against *Ae. aegypti* and *Cx. quinquefasciatus*.

4. Discussion

The results of our study showed that the clove oil was effective against *Ae. aegypti*. The citronella grass oil and lemongrass oil were effective against *Cx. quinquefasciatus*. The results are similar with report by Tjahjani reported that the clove oil was the most effective against *Aedes* species (131 min protection time) and the citronella oil and clove oil were effective against *Culex* species (287 and 287 min protection time, respectively)[4]. Phasomkusulsil and Soonwera have reported that the lemongrass oil was effective against *Ae. aegypti*, *Anopheles dirus* and *Cx. quinquefasciatus* in which the protection time were 72, 132 and 84 min, respectively[28]. Sritabutra and Soonwera have reported that the lemongrass oil exhibited a high protection time of 98.66 and 98.00 min against *Ae. aegypti* and *An. dirus*, respectively[29]. The phytochemical constituent of clove and citronella oil are appreciable such as eugenol, citronellal, citronellol, geraniol, citral, α pinene and limonene. These constituents have properties to repellent activity of mosquitoes[30-32].

From the results, it showed that the herbal essential oils have a protection time less than Kor Yor 15® insect repellent lotion (containing DEET 25.63%). Therefore, it may be improved by developing a formulation that would prolong the time of constituents of the oil on the skin. Many researchers have demonstrated improved repellency of repellent products after formulation with a base or fixative materials such as report from Songkro *et al*., who also reported that effect of glucam P-20, vanillin and fixolide on the mosquito repellent property of citronella oil lotions and found that the lotion containing emulwax and 5% vanillin was the most effective repellent[33]. It provided the longest protection time of 4.8 h, while the lotion containing emulwax and 2.5% glucam P-20 had the shortest protection time of 1 h. Kongkaew *et al*. have reported that the combination of citronella oil and vanillin provided complete repellency at least 3 h in *Anopheles* and *Culex* mosquitoes, a combination of citronella oil and vanillin product demonstrated a comparable protection time against DEET[34]. Kim *et al*. have reported that the combination of lemongrass oil, xanthoxylum oil and vanillin (1:3:1, v:vo:w) provided 270 min of complete protection time compared with 15% N,N-diethyl-3-methylbenzamide (247.5 min of complete protection time)[35].

The results of this study clearly demonstrated that clove oil, citronella oil and lemongrass oil had high potency to control two species of vector mosquitoes. Hence, the results may contribute to a reduction in the application of chemical in mosquito repellents, which in turn increases the opportunity for natural product for control of vector-borne disease. Further studies on identification of active compounds, toxicity and field trials are needed to recommend the active fraction of these plant extracts for development of eco-friendly for control insect vectors.

Conflict of interest statement

We declare that we have no conflict of interest.

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Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand and the Department of Medicinal Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand for providing the eggs of *Ae. aegypti* and *Cx. quinquefasciatus*.

Comments

Background

Mosquitoes (especially members of the genus *Anopheles*, *Culex*, *Aedes*, *etc.*) play a notable role in the transmission of several diseases and lead to more than one million death annually. Several control measures have been developed against them to reduce their contact with their blood donors.

Using natural plant-derived extracts as repellent agents against vector species of mosquitoes will be useful as a complementary control method.

Research frontiers

This study has evaluated the repellency effect of several plant-derived extracts against two most important disease vectors (*Ae. aegypti* and *Cx. quinquefasciatus*).

Related reports

Several studies focused on the repellency effect of plant oils against insects especially mosquitoes (Culicidae). The aims of all studies are to find suitable plant extracts and develop their using as a part of mosquitoes and their transmitted diseases.

Innovations & breakthroughs

This work has used a standard and reliable method to determine the repellency effect and lasting period of several natural plant-derived oils against two mosquito species. Also the authors tried to give a comprehensive literature review and comparison among different studies and their results.

Applications

The results of the present study and related works will help to improve our knowledge in the field of repellency of natural oils against medically important mosquitoes. Also one-step toward studies which define the chemical properties of these herbal extracts and their effective parts, will lead to gather good understanding of their mode of action. The final desired step will be the application of herbal oils as a complementary part of vector control programs which reduce the contact between host and the blood-feeding vectors.

Peer review

This work evaluated the repellency effect of several herbal oils against medically important mosquitoes. The study benefits from a good and standard design and seems to give reliable and confident results. Using insecticides to control the mosquitoes leads to various raising environmental problems and on the other hand, resistance of mosquitoes to different classes of synthetic chemicals, has complicated the problematic situation. Efforts like present study are valuable and in a step by step process, will lead to complete the finding, evaluation and application of natural plant-derived compounds puzzle as a complementary and effective part of vector control programs.

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