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

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ABSTRACT

Objective: To investigate the repellent activity of herbal essential oils from garlic (*Allium sativum*), clove (*Syzygium aromaticum*), lemon grass (*Cybopogon 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. **Conlussions:** 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, *Cybopogon 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*, *Lippa junelliana*, *Baccharis salicifolia*, *Euphatorium 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].

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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 nigum* 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 *Ocinum 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 (*Menta 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) ℃ 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) ℃ 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 unitl as 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