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Short Communication

Efficacy of Thai herbal essential oils as green repellent against mosquito vectors

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ABSTRACT

Repellency activity of Thai essential oils derived from ylang ylang (*Cananga odorata* (Lamk.) Hook.f. & Thomson: Annonaceae) and lemongrass (*Cymbopogon citratus* (DC.) Stapf: Poaceae) were tested against two mosquito vectors, *Aedes aegypti* (L.) and *Culex quinquefasciatus* (Say). There were compared with two chemical repellents (DEET 20% w/w; Sketolene Shield® and IR3535, ethyl butylacetylaminopropionate 12.5% w/w; Johnson's Baby Clear Lotion Anti-Mosquito®). Each herbal repellent was applied in three diluents; coconut oil, soybean oil and olive oil at 0.33 μl/cm^2 on the forearm of volunteers. All herbal repellent exhibited higher repellent activity than IR3535 12.5% w/w, but lower repellent activity than DEET 20% w/w. The *C. odorata* oil in coconut oil exhibited excellent activity with 98.9% protection from bites of *A. aegypti* for 88.7 ± 10.4 min. In addition, *C. citratus* in olive oil showed excellent activity with 98.8% protection from bites of *C. quinquefasciatus* for 170.0 ± 9.0 min. While, DEET 20% w/w gave protection for 155.0 ± 7.1–182.0 ± 12.2 min and 98.5% protection from bites of two mosquito species. However, all herbal repellent provided lower repellency activity (97.4–98.9% protection for 10.5–88.7 min) against *A. aegypti* than *C. quinquefasciatus* (98.3–99.2% protection for 60–170 min). Our data exhibited that *C. odorata* oil and *C. citratus* oil are suitable to be used as green repellents for mosquito control, which are safe for humans, domestic animals and environmental friendly.

1. Introduction

Repellents, clothing, netting and other personal protection measures can help prevent mosquito bites and the diseases mosquitoes carry. Especially, insect repellents are effective and safe when properly used. Repellent repels mosquitoes, it reduces the chances of being bitten. In this light, repellent use offers individuals added protection against mosquito-borne diseases. In the community, it reduces the transmission of mosquito-borne diseases (Gul et al., 2013). N,N-diethyl-m-toluamide (DEET) seems to be most effective and is the best studied insect repellent currently available to the general public. DEET is designed for direct application to human skin to repel insects, rather than kill them. Currently, DEET is formulated in aerosols, pump sprays, lotions, creams, liquids, sticks, roll-ons and impregnated towelettes, with concentrations ranging from 5% to 100%. However, there are concerns about the potential toxic effects of DEET, especially when used by children. Children who absorb high amounts of DEET through insect repellents have developed seizures, slurred speech, hypotension and bradycardia (Clem et al., 1993). Mosquito repellents based on chemicals has a remarkable safety profile, but they are toxic against the skin and nervous system like rashes, swelling, eye irritation, and worse problems, though unusual including brain swelling in children, anaphylactic shock, and low blood pressure (Shasany et al., 2000; Phal et al., 2012). To overcome these problems, it is necessary to search for alternative methods of vector control. The failure of chemical insecticides to control the insect and growing public concern for safe food and a healthy environment have catalyzed the search for more environmentally benign control methods for the management of the vectors (Amerasan et al., 2012).

Essential oil has been the active principle of most important herbal remedies since ancient times. Insecticides of plant origin have been extensively used on agricultural pests, and to a very limited extent, against insect's vectors of public health importance, which deserve careful and thorough screening. The use of plant extracts for insect control has several appealing features, as these are generally more biodegradable, less hazardous, and rich storehouse of chemicals of diverse biological activity (Nath et al., 2006). In Thailand, several Thai herbs act as natural insect repellent, including ylang ylang (*Cananga odorata*) and lemongrass

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(*Cymbopogon citratus*) that expressed high degree of repellency against insect (Caballero-Gallardo et al., 2011; Adeniran and Fabiyi, 2012).

The present investigation aims to assess the repellent properties of the essential oils of ylang ylang and lemongrass which mixed in the natural oils by six formulations against medically important mosquitoes of dengue vector (*Aedes aegypti*) and filarial vector (*Culex quinquefasciatus*). Additionally, the study utilized natural oils; soybean oil, coconut oil and olive oil used locally for cooking and cosmetic purposes to formulate the volatile oil into lotions to improve its acceptability and preclude any adverse effect that could emanate from use of synthetic bases.

2. Materials and methods

2.1. Mosquito cultures and rearing conditions

A. aegypti and *C. quinquefasciatus* lab bred mosquitoes were used in this study. They were maintained in the laboratory of the Entomology and Environment Program, Plant Production Technology Section, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok. Adult mosquitoes were reared at 25 ± 2 °C, and a relative humidity of 80 ± 10% with a photoperiod of 12 h light followed by 12 h dark (12L:12D); adults were fed with 5% multivitamin solution. Prior to testing, 5- to 7-day-old female mosquitoes were starved by providing them with only water for 12 h.

2.2. Preparation of herbal essential oils

In this paper, the efficacy of essential oils of ylang ylang (*C. odorata* (Lamk.) Hook.f. & Thomson) and lemongrass (*C. citratus* DC. Stapf) were compared. Flowers of ylang ylang (*C. odorata*) and stems of lemongrass (*C. citratus*) were collected and essential oils from plant parts were extracted by water distillation method (Charles and Simo, 1990). These essential oils were then prepared at 0.33 μl/cm^2 in three diluents; coconut oil, soybean oil and olive oil.

2.3. Chemical repellent

(1) DEET, 20% w/w; Sketolene Shield® is a commercial chemical repellent in Thailand.
(2) IR3535, 12.5% w/w Ethyl butylacetylaminopropionate; Johnson's Baby Clear Lotion Anti-Mosquito®, is a commercial chemical repellent in Thailand.

2.4. Human volunteers

Five adult volunteers of both sexes, 25–45 years old, weight 50–70 kg, who had no history of allergic reaction to arthropod bites were recruited. Before signing an informed consent form, the volunteers were interviewed and instructed on the methodology, probable discomforts to subjects and remedial arrangements.

2.5. Laboratory repellent bioassay

Repellency of *C. odorata* and *C. citratus* oils and their formulations were evaluated against *A. aegypti* and *C. quinquefasciatus* under laboratory conditions using the methods which followed the guidelines by World Health Organization (2009). To compare the repellency of essential oils with that of the standard repellent, DEET 20% w/w and IR3535 12.5% w/w were tested. Due to different biting behavior, the tests against *A. aegypti* was carried out from 0800 am to 0400 pm, whereas those against *C. quinquefasciatus* was conducted between 0400 pm and 1200 pm because *A. aegypti* is a day-biter but *C. quinquefasciatus* is a night-biter (Govindarajan, 2011). Two hundred and fifty nonblood-fed starved female mosquitoes were randomly selected and placed in an experimental cage (30 cm × 30 cm × 30 cm) and left to acclimatize for 1 h. After cleaning with distilled water, each forearm of the volunteer was wrapped in a plastic sleeve attached with double-sided tape, and a cutout was aligned with a 3 cm × 10 cm area on the ventral portion of the forearm. Therefore, only a restricted zone of the skin was exposed to the mosquitoes. However, before the start of each exposure, the bare hand, used as control area (no treatment) of each volunteer, was exposed for up to 30 s. If at least two mosquitoes landed on the test area of the control arm, the arm was shaken off before imbibing any blood and withdrawn from the cage and the repellency test was then continued. This was done to ensure that the mosquitoes were still active. An amount of 0.1 ml of test repellent was applied to the marked area of one forearm of each volunteer. Subsequently, the test arm was introduced into the cage for 3 min. The total number of mosquitoes biting on the treatment was recorded. If no mosquito bite occurred within 3 min, the forearm was then taken out and the test was repeated every 30-min interval. The experiment was completed after two mosquitoes had bitten. The study period was carried out every 30 min until at least 2 mosquitoes bit during the 3 min study period, at which time the study was stopped. The protection time was the time from repellent application until the study was stopped. On each day, only one repellent preparation was tested to assure that residual material has disappeared from the skin before the next test (Curtis and Hill, 1988).

Table 1
Repellency activities of two herbal essential oils which diluted in three diluents at 0.33 μl/cm^2 (coconut oil, soybean oil and olive oil) against *Aedes aegypti*.

Herbal essential oils	Protection time (min)[a]	% Biting	% Protection
Ylang ylang oil + coconut oil	88.7 ± 10.4^b	1.1	98.9
Ylang ylang oil + soybean oil	10.50 ± 2.1^c	2.4	97.6
Ylang ylang oil + olive oil	85.5 ± 12.0^b	1.4	98.6
Lemongrass oil + coconut oil	85.5 ± 10.5^b	1.1	98.9
Lemongrass oil + soybean oil	72.0 ± 12.4^b	2.6	97.4
Lemongrass oil + olive oil	60.0 ± 12.5^b	1.2	98.8
DEET 20% (w/w) (Sketolene Shield®)	155.0 ± 7.1^a	1.5	98.5
IR3535 12.5% (w/w) (Johnson's Baby Clear Lotion®)	3.0 ± 0^c	21.0	79.0

[a] Means in each column against each mosquito species followed by the difference letters are significantly different ($P < 0.05$, by one-way ANOVA and Duncan's Multiple Range Test).

2.6. Statistical analysis

The mean protection time was used as a standard repellency measure of the test samples against *A. aegypti* and *C. quinquefasciatus* in the laboratory. Differences in significance were analyzed by one-way analysis of variance (ANOVA) and Duncan's multiple comparisons by SPSS for Windows (version 16.0).

3. Results

Each of plant products was applied onto the forearm and they were evaluated. The results for plant oils repellency against *A. aegypti* were summarized in Table 1. Ylang ylang oil containing coconut oil provided longer lasting complete repellency (88.7 min) while the ylang ylang oil containing olive oil and lemongrass oil containing coconut oil which gave complete protection for