

This file has been cleaned of potential threats.

If you confirm that the file is coming from a trusted source, you can send the following SHA-256 hash value to your admin for the original file.

9c95d2f1e37a0ef69b41f54eb9c86036eb6c808d69793e74f91212409ac5c00b

To view the reconstructed contents, please SCROLL DOWN to next page.

Evaluation of *Litsea petiolata* Hook.f. (Lauraceae) as a repellent against *Aedes aegypti* (Diptera: Culicidae) in Chiang Rai Province, Thailand

Roongtawan Muangmoon^{1*}, Orawan Sinpaiboonlert²,
³Saowalak Kaewmee⁴ and Phukijj Laojerunkul

^{1,2,3,4}College of Allied Health Sciences, Suan Sunandha Rajabhat University,
Samut Songkram Province, Thailand.

E-mail: ¹roongtawan.mu@ssru.ac.th, ²orawan.si@ssru.ac.th, ³saowalak.ka@ssru.ac.th, ⁴phukijj.la@ssru.ac.th

*Corresponding Author Email: roongtawan.mu@ssru.ac.th

Abstract

Products of plant origin, with deterrents and repellents effects are now recognized as attractive alternatives to conventional personal protection for the management of mosquito vectors. This study was, therefore, carried out to evaluate the repellents mosquito-borne diseases potential of plant essential oils against the dengue vector, *Aedes aegypti*. Plant samples used for extraction of essential oils (EO), including stem (SEO), bark (BEO) and flower (FEO) of *Litsea petiolata* were collected from their natural environment in Pong Thewi village, Ban Pong sub-district, Wiang Pa Pao district, Chiang Rai province, Thailand. The percentage yields of SEO, BEO and FEO oils obtained by steam distillation were 1.85, 0.65 and 1.75 %, respectively, according to dry weight (v/w). The repellent efficacy against the laboratory strain of *Aedes aegypti* by using the human-bait technique of the WHO (1996) standard method, with slight modifications. In the repellent bioassays, FEO offered no repellency against *Ae. aegypti*, weak repellent activity was obtained from EO treatment, SEO complete protection of 0.5 hour. and BEO show complete protection of 1.0 hour. In conclusion, this study clearly demonstrated the promising potential of plant essential oils, particularly BEO of, which has the possibility of developing as into new natural repellents against mosquito vectors.

Keywords: repellent, *Aedes aegypti*, essential oil

1. Introduction

Aedes aegypti mosquito is the most important vectors of Dengue Virus, Chikungunya virus, Zika virus, yellow fever, Japanese encephalitis, and West Nile virus in many parts of the world. Especially, Dengue fever has become an important public health problem, as the number of reported cases continues to increase with more severe forms of the disease such as dengue hemorrhagic fever, dengue shock syndrome, or unusual manifestations like central nervous system involvement. Dengue continued to affect several South American countries, mostly Honduras, Costa Rica, and Mexico. In Asia, Singapore reported an increase in cases after a lapse of several years, and outbreaks have also been reported in Laos, Cambodia, China, Malaysia, Philippines and Vietnam. (WHO, 2023). In Thailand, dengue infection is a high-

burden disease, with the highest morbidity found in the Northeast region. According to the Department of Disease Control, Ministry of Public Health, the year 2023 reported that dengue cases are 160,841 and 180 deaths throughout Thailand (Bureau of Epidemiology, 2023). Over 20 years, synthetic insecticides; namely organochlorines, organophosphates, carbamates, and pyrethroids have been used over in vector control programs (Chareonviriyaphap et al., 2013). Although, synthetic substances that dramatically reduce the risk of vector-borne diseases have been documented, the overuse and misuse of conventional chemicals, for example, pyrethroids and other insecticides, have led to mosquito resistance, which threatens the potentiality of vector control. Moreover, environmental changes, including climate and habitat shifts, can increase mosquito populations. Studies on mosquitoes in Thailand show high genetic diversity and phenotypic variation, indicating rapid adaptation. (Chaiphongpachara et al., 2024) Products of plant origin that have low toxicity to living organisms and environments are attractive as replacements for synthetics. Phytochemicals that possess anti-mosquito effect would be of interest for applying to mosquito control. Application of pesticides to control larvae (larvicides) is an important process in mosquito management. (Chaiphongpachara & Laojun, 2020)

Moreover, repellents are used commonly as personal protective measures to avoid mosquito bites, and if used properly, can reduce the risk of mosquito-transmitted diseases (Noguera-Gahona, 2025). Although certain botanical-based repellents provide short-lived efficacy compared with synthetics, increasing awareness of quality of life and the environment is stimulating further investigations into plants. In view of the increasing interest in natural plant origin as an alternative anti-mosquito product to synthetic chemicals, a vital point of this study is to develop and produce new botanical-derived repellents for personal protection against mosquito bites and reducing mosquito-transmitted diseases.

1.1 Research Objective

To screen under laboratory conditions the essential oils of *Litsea petiolata* (stem, bark and flower) for potential repellent activity against *Ae. aegypti*, the vector of dengue fever.

2. Materials and Methods

2.1 Plant materials

Litsea petiolata plant were selected on their abundance and data available in the literature, which includes botanical information, pharmacological properties and anti-mosquito potential (Lupi et al., 2013). The plant materials were collected from Chiang Rai provinces of Thailand. Taxonomic identification of these plants was performed by a scientist at Department of Pharmaceutical Science, Faculty of Pharmacy, Chiang Mai University (CMU), Chiang Mai province, Thailand. A voucher specimen of each plant was deposited for further reference at the Department of Parasitology, Faculty of Medicine, CMU. After air-drying under shade at environmental temperatures ($30 \pm 5^{\circ}\text{C}$ daytime) for 1-2 weeks, each dried plant material was ground mechanically by electrical blender. The coarse and fine powders of each plant thus obtained were used to prepare essential oil.

2.2 Experimental animals

2.2.1 Mosquitoes

Mosquito populations used in this study were the free-mating laboratory *Ae. aegypti* were maintained and colonized separately in a temperature-controlled insectary ($25 \pm 2^{\circ}\text{C}$, $80 \pm 10\%$ RH and 14:10 h light/dark photoperiod cycle) at the Department of Parasitology, Faculty of Medicine, CMU.

2.2.2 Albino rats (*Ratus ratus*)

Male adult rats, age 6-8 weeks and weight 250-300 g, were obtained from the laboratory animal house, Faculty of Medicine, CMU. The animals were kept in an animal room where the temperature was maintained at $25-30^{\circ}\text{C}$ in a 14:10 h light: dark period. Rats were used as a source of blood meal for female mosquitoes during egg production. The blood feeding protocol has been approved by the Animal Ethics committee, the Faculty of Medicine, CMU.

2.3 Chemicals

- Dimethylsulphoxide (AR1054-G2.5L: RCI Labscan Limited, Thailand)
- Silicone grease (ACC Silicones Limited, England)
- Sodium sulphate anhydrous (CAS No. 7757-82-6: Fisher scientific, UK)
- Absolute ethanol (CAS No. 64-17-5, UN No.1170: RCI-Labscan, Thailand)
- 95% Ethanol

2.4 Human volunteers

Six healthy human volunteers of either sex without a history of dermatological disease or allergic reaction to arthropod bites, stings or repellents were selected for repellent assessment of plant-derived products. The volunteers who take part in mosquito repellency tests were interviewed and advised fully on the purpose and methodology of the study, probable discomforts from exposure to test substances and mosquito bites, and remedial arrangements, before signing an informed consent form under protocol PAR-2557-02535. This study was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, CMU.

2.5 Mosquito rearing

Ae. aegypti were maintained and colonized separately in an insectary (6.2 x 7.3 x 3 m) without exposure to any insecticides or pathogens at $25 \pm 2^{\circ}\text{C}$ and $80 \pm 10\%$ RH under 14:10 light-and-dark cycles, by following the standard operating procedures for mosquito maintenance (Limsuwan et al., 1987). Approximately 300 larvae of each strain reared in a white plastic pan containing 2 liters of tap water were fed on finely ground dog-biscuit. The water was changed two or three times weekly in order to avoid scum forming. Rearing trays containing aquatic stages of mosquitoes were covered tightly with a nylon screen at all times in order to keep all of the colonies strictly isolated from each other. After pupation, the pupae were transferred from the rearing pans to plastic cups containing distilled water. Each cup containing approximately 300 pupae was placed into a mosquito cage (30 x 30 x 30 cm). After emerging, the adults were fed with 10% sucrose plus 10% multivitamin syrup. Female mosquitoes were

periodically blood-fed on rats for egg production. The eggs were laid on filter paper soaked with water in an egg collecting cup. The collected eggs were kept to air-dry for 3-4 days before being used in the next rearing. The eggs on filter paper were placed into trays filled with tap water for hatching. The larvae were hatched within 24 h after immersion and then be transferred to a new rearing pan. Various stages of mosquitoes, including larvae, pupae, and adults were selected randomly for investigating anti-mosquito activity of plant-based products.

2.6 Preparation of crude plant extracts

Dried materials of *L. petiolata* were subjected to extraction by steam distillation methods for producing essential oil (EO). The percentage yield of each product was averaged over three experiments and calculated according to dry weight of the plant materials.

Steam distillation

Coarsely ground material of each part of *L. petiolata* was extracted individually for EO by steam distillation. The distillation apparatus comprised an electromantle, a round-bottomed distillation flask, an extraction column, a condenser and a cool ace. Dried plant material (250 g) of each plant was placed in the extraction column connected to a distillation flask containing approximately 1,600 ml of distilled water and 10-15 glass beads. The distillation flask was heated to approximately 100°C and allowed to boil until the distillation is complete. The water vapor generated in the flask was ventilated to the extraction column containing the plant material. After passing through the extraction column, steam was condensed by cool water passing from the cool ace through the coil in the condenser. The liquid formed, together with volatile oil, was collected in a separating funnel. The mixture was allowed to settle for 3-5 days, after which, the water (lower) layer was drawn off slowly until only the oil layer remains. The collected EO was dried over anhydrous sodium sulfate (Na₂SO₄) and then kept in a brown bottle at 4°C until required for investigating repellent activity.

2.7 Investigation of anti-mosquito activity of plant products

Repellent bioassay

Repellency of botanical extract was investigated against female *Ae. aegypti*, by using the human-treated procedure of the WHO (1996) standard method, with slight modifications. As the target mosquito is a day-biting *Ae. aegypti*, the experiments were performed between 0800 h and 1600 h in a 10 × 10 × 3 m room, at 27-30°C and 60-80% RH. Two hundred and fifty non-blood-fed starved females were chosen randomly and placed inside a mosquito test cage (30 × 30 × 30 cm), and rested for 1 h before starting the experiment. The forearm of each volunteer was covered by a plastic sleeve, with a rectangular hole (3 × 10 cm) cut to match the ventral part, thus exposing the treated area only as a test site. The hand of the forearm was gloved. A volume of 0.1 ml of 25% plant solution or undiluted essential oil was applied evenly onto the 30 cm² test site of each volunteer. The other forearm was act as a control, and be treated with solvent by a similar protocol as that for the test repellent. Prior to each treated arm insertion into the cage, the control arm was placed into it for 3 min in order to make comparative checks and determine the readiness of the mosquitoes to bite. If at least 2 mosquitoes land on the

control arm, the repellency test was carried out by exposing the treated forearm in a similar manner; otherwise, the experiment was not being conducted. Care was taken between testing periods to ensure that the treated surface does not rub off or make contact with any other influence. The complete protection time was recorded after exposing the treated forearm for 3 min at 30 min-intervals until either two bites occur in a single exposure period or one bite occurs in each of two consecutive exposure periods. After each experiment, the tested mosquitoes were discarded. Each test was repeated on each of the six volunteers (3 adult females, 3 adult males) with a new batch of mosquitoes on different days, and no volunteer tested more than 1 sample per day. Therefore, each sample could be tested twice on each subject and there were 12 replicates for each sample test. Any skin irritation, hot sensation, or unpleasant odors from the plant extract were recorded.

2.8 Research location

Pong Thewi village, Ban Pong sub-district, Wiang Pa Pao district, Chiang Rai province

2.9 Statistical Analysis

For repellent bioassay, the median complete-protection times were used as a standard repellency measure of the test samples. Differences in significance were determined by comparing the range of protection time in each sample.

2.10 Human Research Ethics

This study was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, CMU.

3. Research Results

Table 1: Percentage yield (% Yield) and physical characteristics (color, appearance, and density) of plant products, essential oils (EOs) against *Ae. aegypti*

Plant product	% Yield	Physical characteristics		
		Color	Appearance	Density (g/ml)
Essential oil				
SEO	1.85	Colorless	Liquid	0.82
BEO	0.65	Colorless	Liquid	0.76
FEO	1.75	Colorless	Liquid	0.86

The percentage yields of SEO, BEO and FEO obtained by steam distillation were 0.82, 0.76 and 0.86 %, respectively, according to dry weight (v/w).

Table 2: Repellent activity of different part of *L. petiolata* against *Ae. aegypti* mosquito.

Plant part	Median complete-protection time (Range, h)
SEO	0.5
BEO	1.0
FEO	0.0

Repellent activity of *L. petiolata* EO against *Ae. aegypti* is presented in Table 2. It was found that *L. petiolata*-BEO exerted an effective biting protection against *Ae. aegypti*, with the median complete protection times of 1.0 hour and FEO offered no repellency against *Ae. aegypti*, weak repellent activity was obtained from EO treatment, SEO complete protection of 0.5 hour.

4. Discussion

In the present study, botanical products with different yields and physical characteristics evaluated the repellent activity of essential oils extracted from the stem bark and flower of *Litsea petiolata* against *Aedes aegypti*, a major dengue vector. The results demonstrated that the BEO provided complete protection for 1.0 hour, while the FEO exhibited no repellency. The previous studies have indicated that Lauraceae family rich in aromatic and medicinal plants. Likewise, essential oils derived from members of this family have demonstrated a myriad of biological activities. It is hypothesized that members of the Lauraceae from Vietnam will yield essential oils that may be useful in controlling mosquito populations and treating microbial infections. In this work, the leaf essential oils of eleven species of Lauraceae (*Beilschmiedia erythrophloia*, *B. robusta*, *B. yunnanensis*, *Cryptocarya concinna*, *C. impressa*, *C. infectoria*, *Litsea viridis*, *Machilus balansae*, *M. grandifolia*, *Neolitsea ellipsoidea*, and *Phoebe angustifolia*) have been obtained by hydrodistillation and the chemical compositions analyzed by gas chromatography - mass spectrometry (GC-MS) (Dao Thi Minh Chau, 2020).

5. Conclusion

Essential oil of *L. petiolata* bark (BEO) repellent products with proven repellent efficacy, no side effects on the skin, and relatively stable physical and biological performance could qualify for developing and registering a new natural alternative product.

Acknowledgment

I am deeply indebted and grateful to Mr. Tong Kantawong and his family, Pong Thewi village, Ban Pong sub-district, Wiang Pa Pao district, Chiang Rai province, for their kindness and support in plant sampling during my research work.

References

- Bureau of Epidemiology. (2023). *Annual epidemiological surveillance report 2023*. Department of Disease Control, Ministry of Public Health.
- Chaiphongpachara, T., & Laojun, S. (2020). Comparative efficacy of commercial ylang-ylang (*Cananga odorata*) essential oils from India and Thailand against larval *Aedes aegypti* (L.) (Diptera: Culicidae). *Journal of Advanced Veterinary and Animal Research*, 7(3), 391-396.
- Chaiphongpachara, T., Laojun, S., Changbunjong, T., Wichit, S., & Saba Villarreal, P. M. (2024). Demographic inference from the mt-DNA COI gene and wing geometry of *Culex gelidus* (Diptera: Culicidae), an important vector of Japanese encephalitis in Thailand. *Acta Tropica*, 256, 107276.

- Chareonviriyaphap, T., Bangs, M. J., Suwonkerd, W., Kongmee, M., Corbel, V., & Ngoen-Klan, R. (2013). Review of insecticide resistance and behavioral avoidance of vectors of human diseases in Thailand. *Parasites & Vectors*, 6, Article 280.
- Chau, D. T. M., Chung, N. T., Huong, L. T., Hung, N. H., Ogunwande, I. A., Dai, D. N., & Setzer, W. N. (2020). Chemical compositions, mosquito larvicidal and antimicrobial activities of leaf essential oils of eleven species of Lauraceae from Vietnam. *Plants*, 9(5), 606.
- Limsuwan, S., Rongsriyam, Y., Kerdpibule, V., Apiwathnasorn, C., Chiang, G. L., & Cheong, W. H. (1987). Rearing techniques for mosquitoes. In S. Sucharit & S. Supavej (Eds.), *Practical entomology: Malaria and filariasis* (1st ed., pp. xx-xx). Museum and Reference Centre, Faculty of Tropical Medicine, Mahidol University.
- Lupi, E., Hatz, C., & Schlagenhauf, P. (2013). The efficacy of repellents against *Aedes*, *Anopheles*, *Culex* and *Ixodes* spp.: A literature review. *Travel Medicine and Infectious Disease*, 11, 374-411.
- Noguera-Gahona, M., Peña-Moreno, C., Quiñones-Sobarzo, N., Weinstein-Oppenheimer, C., Guerra-Zúñiga, M., & Collao-Ferrada, X. (2025). Repellents against *Aedes aegypti* bites: Synthetic and natural origins. *Frontiers in Insect Science*, 4, 1510857.
- World Health Organization. (1996). *Report of the WHO informal consultation on the evaluation and testing of insecticides* (CTD/WHOPES/IC/96.1). Control of Tropical Diseases Division, World Health Organization.
- World Health Organization. (2013). *TDR global alert and response: Dengue/dengue haemorrhagic fever* [Internet]. World Health Organization.