Original article

Repellency screening of herbal products against the dengue fever vector, *Aedes aegypti* (Diptera: Culicidae)

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Objective To screen the repellent efficacy of herbal products, including essential oils and ethanolic and hexane extracts derived from fifteen plant species against the dengue fever vector, *Aedes aegypti*.

Methods Plant products were evaluated for repellency in a mosquito cage containing 250 blood-starved female *Ae. aegypti* under laboratory conditions, by using the human-bait technique from the standard World Health Organization (WHO) method. DEET also was tested as a standard synthetic repellent with a similar protocol to that for the plant samples. Approximately 0.1 ml of each test sample was applied evenly onto a 30 cm^2 test site on one forearm of each human volunteer. Exposure experiments continued at 30 min intervals until at least two bites occurred in a three-minute period, or when a first bite was followed by a confirming bite (second bite) in the subsequent observation period. Each test was duplicated on different days for each of two human volunteers.

Results Topical application of DEET and plant products provided effective protection against mosquitoes with varying degrees of repellency. Most hexane extracts exhibited higher repellency than that obtained from the ethanolic extracts and essential oils of the same plant. Hexane extract of *Ligusticum sinense* rhizome gave the highest repellent efficacy, with a median complete-protection time of 6.5 (5.0-8.0) h, which was comparable to that for DEET (6.25, 5.0-6.5 h). No local skin reaction such as rash, swelling, irritation, or other allergic responses was observed during the study period.

Conclusion Plant products with proven repellent efficacy, particularly hexane-extracted *L. sinense*, are potential candidates for the development of a new natural alternative to DEET, or an additional weapon used together with other chemicals/measures for integrated vector control. **Chiang Mai Medical Journal 2014;53(2):53-62.**

Keywords: plant products, essential oils, ethanolic extracts, hexane extracts, repellents, *Aedes aegypti*

Introduction

Applying repellent to the skin, clothing, and bed net is an ideal way of achieving protection from vector and nuisance arthropods, as minimal human contact leads to reduced risk of infection

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Received February 17, 2014, and in revised form March 31, 2014.

and spread of disease[1-3]. *N*,*N*-diethyl-*m*-toluamide, now called *N*,*N*-diethyl-3-methylbenzamide or DEET, is the best-known synthetic chemical used as a main active ingredient in commercial insect repellents[4]. It was formulated by the United States Department of Agriculture for the United States Army in 1946. Then, the United States Environmental Protection Agency registered DEET in 1957 for use in protecting against biting insects and controlling disease transmissions in the general population[5]. DEET has been used as a broad-spectrum repellent against mosquitoes, ticks, chiggers, flies, fleas, and other arthropods that can transmit disease[6,7]. Although effective for protection against many harmful arthropods, applying DEET gives adverse reactions, and complications deriving from accidental ingestion, exposure via inhalation, or contact with the eyes[8-11]. Furthermore, an unpleasant odor, oily feel, and damaging effects on plastic and synthetic fabric[12] are other shortcomings that resulted in refusal to use DEET-based products. Therefore, the public is seeking alternatives that are safer and more user friendly than DEET.

Products of plant origin have been used traditionally as repellents against insects in many parts of the world, primarily for avoiding nuisance biting[13]. The traditional use of plant products as natural insecticides and repellents is of great interest in the search for new active substances for vector control[14,15]. Numerous plants have been reported as potential natural sources of insect repellents, and citronella, clove, eucalyptus, and neem oils are natural substances best known for use against arthropod vectors and pests[16-19]. In Thailand, repellent tests of plant-derived products, such as essential oils and crude solvent extracts, have been carried out against both diurnal and nocturnal mosquitoes by using various procedures under laboratory and field conditions. Certain plant extracts, such as *Apium graveolens*, *Curcuma longa*, *Cu. aromatica*, *Cymbopogon winterianus*, *Kaemferia galanga*, *Ocimum americanum*, and *Syzygium aromaticum* have been documented as potential repellents against various mosquito vectors, such as *Aedes aegypti*, *Ae. albopictus*, *Anopheles dirus*, and *Culex quinquefasciatus*[20-24]. Nowadays, plant based-repellents are registered as commercially available and demand is gradually increasing, but their short-lived effectiveness and expense are critical disadvantages[25,26]. Therefore, the search for additional bioactive compounds that are expected to improve efficiency, economical feasibility, and user friendliness continues in earnest.

This study was designed to screen for the repellent activity of various natural products derived from fifteen plant species against the *Ae. aegypti* mosquito under laboratory conditions. Success of the herbal extracts selected may prove useful as potential candidates for developing new natural repellents that are effective, safer, and popular among users.

Methods

Plant materials

Fifteen plant species belonging to 11 families (Table 1) were obtained commercially from traditional herb suppliers in Chiang Mai province, Thailand. Selection of these plants was based on their abundance and data available in the literature, which included botanical information, pharmacological properties, and anti-mosquito activities[16-19,25-26]. Taxonomic identification of the plants was performed by James Franklin Maxwell, a botanist at the Herbarium, Department of Biology, Faculty of Science, Chiang Mai University (CMU), Chiang Mai province, Thailand, and Miss Wannaree Charoensup, a scientist at the Department of Pharmaceutical Science, Faculty of Pharmacy at CMU. A voucher specimen of each plant was kept for future reference at the Department of Parasitology, Faculty of Medicine at CMU. Each plant material was air-dried under shade at a prevailing temperature of about 30±5 ºC for one week prior to preparing the plant products.

Human volunteers

Four healthy volunteers (two males and two females aged 21-35 years old; weighing 44-93 kg), with no history of allergic reaction or dermatological disease to arthropod bites, stings, or repellents were recruited from graduate students at CMU. All of the volunteers were interviewed and informed on the objective and methodology of the study, probable discomforts to subjects, and remedial arrangements, before signing an informed consent form under protocol PAR-11-808-EX. This study was approved by the Research Ethics Committee of the Faculty of Medicine, CMU.

The volunteers also were advised to avoid alcohol and any fragrant products such as perfume, cologne, deodorant, and lotion during the entire study period.

Mosquitoes

The *Ae. aegypti* used in this study were laboratory colonies originating from specimens collected in Chiang Mai province, northern Thailand. They were colonized and maintained continuously for several generations under controlled insectary conditions (25-30 °C, 80-90% RH, and 14:10 h light/dark photoperiod) at the Department of Parasitology, Faculty of Medicine, CMU. Standard procedure was modified slightly for the methods of mass rearing[27]. Larvae reared in plastic trays were fed on finely ground dog biscuit. Adults kept in screened cages were provided with continuous access to 10% sucrose and 10% multivitamin syrup. Albino rats were used periodically as a source of blood meal for egg producing females. Female mosquitoes (5-7 days old) were starved prior to repellent testing, by providing them with only water for 8-12 h.

Preparation of plant extracts

Dried plant materials were powdered separately prior to extractions, which were divided into two procedures: isolating by steam distillation at 100 °C for at least 3 h to obtain volatile oils; and extracting by maceration with chemical solvents, 95% ethanol and hexane, thus yielding solvent extracts. Essential oils were extracted individually from coarsely ground plant materials and collected, dried over anhydrous sodium sulfate (Na_2SO_4) to remove traces of moisture, and stored prior to investigation of repellent activity in an amber-colored bottle under refrigeration (4 °C). For solvent extraction, half a kilogram of dried fine powder from each plant was extracted successively three times for seven days by maceration at room temperature with three liters of 95% ethanol or hexane. After vacuum filtration through a Bücher funnel, the solvent was removed from combined filtrates on a rotary evaporator at 70 °C (for 95% ethanol) or 60 °C (for hexane), until it had evaporated completely. The residues were lyophilized to yield dry plant extracts, which were then kept at -20 °C for subsequent repellent investigation. The percentage yield of each product was averaged over three experiments and calculated according to dry weight of the plant materials.

Screening for repellent activity of the plant products

Plant products, including essential oil and ethanolic and hexane extracts of each plant sample were screened for repellency against female *Ae. aegypti* under laboratory conditions (27-35 °C, 60-80% RH, 08.00 to 16.00 h) by using the human-bait technique from the standard WHO method[28]. DEET is a standard synthetic repellent that was tested by a protocol similar to that for the plant samples. Two hundred and fifty starved female mosquitoes were chosen at random, placed inside a standard mosquito cage (30 x 30 x 30 cm), and rested for 1 h before starting the experiment. The arms of the volunteers were washed and cleaned thoroughly with distilled water before applying the test samples. Each ventral part of the volunteers' forearm was covered by a plastic sleeve with an open window of 3 x 10 cm, thus exposing the treated area only. The hands were protected by rubber gloves. The test samples were dissolved in proper solvent (e.g., absolute ethanol, isopropanol), which served as a control. Approximately 0.1 ml of undiluted essential oil, 25 g% solvent (ethanol/hexane) extracts, or 25 g% DEET solution was applied evenly onto a 30 cm^2 test site on one forearm of each volunteer. The other forearm, which acted as a control, was treated with solvent by a similar procedure to that for the tested arm. Tests were conducted by exposing the repellent-treated forearm to 250 caged female mosquitoes at 30-min intervals, until at least two bites occurred in a three-minute period, or a first bite was followed by a confirming bite (second bite) in the subsequent observation period. During the experiment, the control arms were introduced successfully to the mosquitoes, in the same manner, before each treated arm was exposed. This confirmed and standardized the mosquitoes' readiness to bite. The time between application of the test sample and the first two consecutive bites was considered as the complete-protection time, which is the usual criterion used to determine the repellent efficacy of a sample. Each test was duplicated on different days for each of two human volunteers (1 adult female, 1 adult male). No one tested more than one sample per day. Randomization was used to assign the order of tests and treatment of volunteers, who were blinded to the repellent applied.

Data analysis

The median complete-protection time was used as a standard criterion for the repellent efficacy of the tested substances against *Ae. aegypti* in the laboratory. The Kruskal-Wallis one-way ANOVA was used to determine the significance of difference among the test samples at the critical level of 0.05, using the SPSS version 16.0 program.

Results

Plant products derived from extraction

Seventeen dried materials that derived from different parts of 15 plant species were selected for extraction by steam distillation and solvent (ethanol/hexane) maceration. In most cases, only one part of each plant was used, whereas two parts of *Aegle marmelos* (leaf, fruit) and *Zingiber zerumbet* (rhizome, flower) were extracted separately. Several herbal products with different appearance, color, and odor were obtained in the

extraction process. The ethnobotanical data and percentage yields of the herbal products, including essential oils and ethanolic and hexane extracts are summarized in Table 1. For the isolation of essential oils, only two plant materials, including *Saussurea lappa* root and *A. marmelos* leaf provided liquid oils, with a yield of 0.32% and 1.50% (v/w), respectively. The distillate oil of *S. lappa* root was pale yellow with a pungent odor. While the leaf of *A. marmelos* provided light yellow oil with an aromatic odor, its fruit yielded no oil. None of the remaining plant samples offered any essential oils. Solvent extraction provided the ethanolic and hexane plant extracts with varying yields ranging from 5.12-65.00% (w/w) and 0.66-15.98% (w/w), respectively. The highest yield of ethanolic extracts was obtained from *Rheum palmatum* root

Table 1. Ethnobotanical data and yields (%Yield) of products derived from fifteen plants

Family/Species	English name	Voucher specimen	Part used	% Yield		
				Essential oil	Ethanolic extract	Hexane extract
Apiaceae						
Ocimum basilicum L.	Sweet basil	PARA-OC-001-Le/1	Leaf	0.00	7.42	3.34
Ocimum americanum L.	Hairy basil	PARA-OC-002-Se/1	Seed	0.00	12.72	15.98
Asteraceae						
Chromolaena odoratum (L.) R.M. King & H. Rob.	Bitter bush	PARA-CH-001-St-Le/1	Stem & Leaf	0.00	9.35	3.75
Saussurea lappa Clark	Costus	PARA-SA-001-Ro/1	Root	0.32	20.47	3.48
Bixaceae						
Bixa orellana L.	Annatto	PARA-BI-001-Se/1	Seed	0.00	9.07	2.25
Fabaceae						
Clitoria ternatea L.	Blue pea	PARA-CL-001-Se/1	Seed	0.00	15.00	9.05
Acacia concinna (Willd.) DC.	Soap pod	PARA-AC-001-Po/1	Pods	0.00	27.16	0.73
Poaceae						
Vetiveria zizanioides (L.) Nash	Vetiver	PARA-VE-001-Rh-Ro/1	Rhizome & Root	0.00	14.98	0.83
Polygonaceae						
Rheum palmatum L.	Rhubarb	PARA-RH-001-Ro/1	Root	0.00	65.00	0.66
Rutaceae						
Aegle marmelos (L.) Correa ex Roxb.	Bale	PARA-AE-001-Le/1	Leaf	1.50	15.36	2.09
		PARA-AE-001-Fr/1	Fruit	0.00	9.97	1.25
Saururaceae						
Houttuynia cordata Thunb.	Fish mint	PARA-HO-001-Le/1	Leaf	0.00	15.77	4.74
Umbelliferae						
Ligusticum sinense Oliv. cv. Chuanxiong Hort	Chuanxiong rhizoma	PARA-LI-001-Rh/1	Rhizome	0.00	25.10	5.20
Angelica dahurica Fisch.ex Hoffm	Chinese angelica	PARA-AN-001-Ro/1	Root	0.00	7.70	1.35
Verbebaceae						
Lantana camara L.	Common lantana	PARA-LA-001-Fl/1	Flower	0.00	22.80	3.74
Zingiberacae						
Zingiber zerumbet (L.) Smith	Shampoo ginger	PARA-ZI-001-Rh/2	Rhizome	0.00	26.74	3.26
		PARA-ZI-001-Fl/1	Flower	0.00	5.12	2.57

(65.00%, w/w), whereas that of hexane extract was acquired from *Ocimum americanum* seed (15.98%, w/w).

Potential repellency of plant products against the *Ae. aegypti* mosquito

Topical application of 25% DEET and plant products was effective in protecting against mosquitoes, with varying degrees of repellency. The repellency of plant samples against *Ae. aegypti* mosquitoes under laboratory conditions are demonstrated in Table 2. While the root oil of *S. lappa* exerted repellent potential against *Ae. aegypti*, with the median complete-protection time of 2.75 h, no repellency was observed from *A. marmelos* leaf oil. Repellency determination revealed that both ethanolic and hexane extracts from the *O. americanum* seed, *Chromolaena odoratum* (stem & leaf), *Acacia concinna* pods, *R. palmatum* root, *A. marmelos* fruit, *Lantana camara* flower, and *Z. zerumbet* flower were ineffective in repelling mosquitoes. Only five

Table 2. Repellency of 25% DEET and plant products, including essential oils and ethanolic and hexane extracts against female *Ae. aegypti* mosquitoes under laboratory conditions

Plant/Chemical	Part used	Median complete-protection time (Range, h)		
		Essential oil	Ethanolic extract	Hexane extract
Apiaceae				
Ocimum basilicum	Leaf	ND	0.25 (0.0-0.5)	0.0 (0.0-0.5)
Ocimum americanum	Seed	ND	0.0 (0.0)	0.0 (0.0)
Asteraceae				
Chromolaena odoratum	Stem & Leaf	ND	0.0 (0.0)	0.0 (0.0-0.5)
Saussurea lappa	Root	2.75 (2.5-3.0)	0.0 (0.0)	2.0 (1.0-3.5)
Bixaceae				
Bixa orellana	Seed	ND	0.0 (0.0)	0.25 (0.0-0.5)
Fabaceae				
Clitoria ternatea	Seed	ND	0.0 (0.0)	0.5 (0.0-1.0)
Acacia concinna	Pods	ND	0.0 (0.0)	0.0 (0.0)
Poaceae				
Vetiveria zizanioides	Rhizome & Root	ND	0.0 (0.0)	0.5 (0.0-1.5)
Polygonaceae				
Rheum palmatum	Root	ND	0.0 (0.0)	0.0 (0.0)
Rutaceae				
Aegle marmelos	Leaf	0.0 (0.0)	0.75 (0.5-1.5)	2.25 (1.5-3.0)
	Fruit	ND	0.0 (0.0)	0.0 (0.0)
Saururaceae				
Houttuynia cordata	Leaf	ND	0.5 (0.5-1.0)	0.75 (0.0-1.0)
Umbelliferae				
Ligusticum sinense	Rhizome	ND	5.0 (4.0-5.5)	6.5 (5.0-8.0)
Angelica dahurica	Root	ND	0.5 (0.0-0.5)	0.0 (0.0)
Verbebaceae				
Lantana camara	Flower	ND	0.0 (0.0)	0.0 (0.0)
Zingiberacae				
Zingiber zerumbet	Rhizome	ND	0.0 (0.0)	1.5 (0.5-2.0)
	Flower	ND	0.0 (0.0)	0.0 (0.0-0.5)
25% DEET	-		6.25 (5.0-6.5)	

ND: not determined; as no essential oil was obtained from this plant species.

herbal ethanolic extracts, including *Ocimum basilicum* leaf, *Houttuynia cordata* leaf, *Angelica dahurica* root, *A. marmelos* leaf, and *Ligusticum sinense* rhizome possessed repellency, with median complete-protection times of 0.25, 0.5, 0.5, 0.75, and 5.0 h, respectively. The other twelve ethanolic extracts appeared to be ineffective. Repellent activities were observed in most herbal hexane extracts, including *S. lappa* root, *Bixa orellana* seed, *Clitoria ternatea* seed, *Vetiveria zizanioides* (rhizome & root), *A. marmelos* leaf, *H. cordata* leaf, *L. sinense* rhizome, and *Z. zerumbet* rhizome, with median complete-protection times ranging from 0.25-6.5 h. The hexane extract of *L. sinense* rhizome provided the greatest repellent efficacy, with a median complete-protection time of 6.5 (5.0-8.0) h, which was comparable to that of DEET (6.25, 5.0-6.5 h). No local skin reaction such as rash, swelling, irritation, or other allergic responses was observed during the study period.

Discussion

The preparations of plant products in this study were performed by two procedures; steam distillation that generated volatile oils, and solvent maceration, which provided ethanolic and hexane extracts. Steam distillation is a common and economical technique used for separating volatile compounds from nonvolatile contaminants, and has been employed extensively in the isolation of natural products. From 17 plant samples, only two plant materials, *S. lappa* root and *A. marmelos* leaf, provided essential oils that yielded 0.32% and 1.50% (v/w), respectively. These are quite large yields when compared to those obtained by other essential oil extractions. In earlier studies, the oil yields obtained from hydro-distillation for 3-6 h varied considerably when using Clevenger apparatus for *S. lappa* root and *A. marmelos* leaf derived from different locations. While the yields of *S. lappa* root oil varied from 0.020% to 0.89% (v/w)[29-31], those of *A. marmelos* leaf oil were 0.30% to 1.50% (v/w)[32-34]. The remaining plant samples, such as *O. basilicum* leaf, *O. americanum* seed, and ; *L. camara* flower were reported previously with yields of essential oils ranging from 0.05-0.3%, when extracted by hydro-distillation in conventional Clevenger-type apparatus[35-37]. However, in current extraction steps they provide no essential oil when extracted by steam distillation. In addition to plant species and extraction procedures, parts of plants also affected the yield of extracted oils. Examples of these were afforded by the fact that while the leaf of *A. marmelos* provided light yellow oil with an aromatic odor, its fruit yielded no oil. Although extracted by a similar method of steam distillation, the previously reported oil yield of *H. cordata* flower was 0.20%[38], whereas that of its leaf was zero when presented in this study.

All plants, except *O. americanum*, were found to provide higher yields when extracted by ethanol (5.12-65.0%, w/w) than those obtained by hexane extraction (0.66-15.98%, w/w). Greater yields from ethanolic extraction could be attributed to its intermediate polarity, which leads to a large number of chemical constituents, with extraction of both polar and non-polar compounds [39]. In addition to this advantage, ethanol is often the first choice use for phytochemical extraction because it is non-toxic, economical, and easy to evaporate at a low temperature[40]. Quantity of the resulting products is not the only key to successful extraction, but quality also is, and this should be taken into consideration. Therefore, this study used hexane as extracting solvent, due to reports of its products with strong repellency against many species of mosquito vectors[20,41-44], and apparent findings supported this information.

In screening test samples, the effectiveness of different plant products, including essential oils and ethanolic and hexane extracts, for protection against *Ae. aegypti* was documented at varying degrees. Most hexane extracts, including *S. lappa* root, *B. orellana* seed, *C. ternatea* seed, *V. zizanioides* (rhizome & root), *A. marmelos* leaf, *H. cordata* leaf, *L. sinense* rhizome, and *Z. zerumbet* rhizome exhibited higher repellent activity than the other products of the same plant. These findings indicate that the active com-

pounds are more soluble in hexane. The chemical ingredients of hexane-extracted products, which demonstrate greater repellency, are principally non-polar substances, due to hexane being a non-polar solvent that usually dissolves non-polar molecules[40]. Various products derived from the same plant species, which were extracted by distinct chemicals and processes, demonstrated differences in repellency. Therefore the nature of the solvent and extraction technique are critical factors, which affect the chemical principles that influence the bioactivity of plant products[40,45].

The initial success of this study is the protection time of up the 2.0 h from three herbal products, including *S. lappa*, *A. marmelos*, and *L. sinense*. This meets the requirement of the Food and Drug Administration (FDA), which needs a minimum protection time of 2 h before allowing sales of repellents in Thailand. Furthermore, no local skin reaction such as rash, swelling, irritation, or other allergic responses was observed during the study period. Plant products with proven repellency and no irritation to the skin, particularly the hexane-extracted *L. sinense*, demonstrated the greatest repellent efficacy, with a median complete-protection time of 6.5 (5.0-8.0) h, which was comparable to that of DEET (6.25, 5.0-6.5 h). These plant products are considered as satisfactory potential candidates for developing new and more effective natural repellents. Although sensitivity to tested substances of mosquitoes such as *Ae. aegypti* laboratory strain can be an indicator of repellent activity [46]. The protective effect of repellent against this mosquito species may not ensure success against other species under similar or different circumstances, particular in the field, with ambient temperature and humidity. For practical use of these plant products, as an alternative approach to personal protection, further research on their potential as repellent against a wide range of mosquito species should be carried out under laboratory and field conditions. Isolation and identification of active principles responsible for repellent activity, as well as formulating preparations for enhancing potency and stability, and safety in administration, are needed.

Acknowledgments

Financial support from the Diamond Research Grant of the Faculty of Medicine, Chiang Mai University, Chiang Mai province, Thailand is gratefully acknowledged. The authors would like to thank the individuals who served as subject volunteers and also staff members of the Department of Parasitology, Faculty of Medicine, Chiang Mai University for their cooperation.

Conflict of interest statement

There is no conflict of interest.

References

1. **Curtis CF**. Personal protection methods against vectors of disease. Rev Med Vet Entomol 1992;80:543-53.
2. **Gupta RK, Rutledge LC**. Role of repellents in vector control and disease prevention. Am J Trop Med Hyg 1994;50:82-6.
3. **Fradin MS**. Protection from blood-feeding arthropods. In: Auerbach PS, editor. Wilderness medicine. 4th ed. St. Louis: Mosby; 2001. p. 754-68.
4. **Fradin MS, Day JF**. Comparative efficacy of insect repellents against mosquito bites. N Engl J Med 2002; 347:13-8.
5. **Sax NI, Lewis RJ**. Dangerous properties of industrial materials. 7th ed. New York: van Nostrand Reinhold Company; 1989.
6. **Davis EE**. Insect repellents: concepts of their mode of action relative to potential sensory mechanisms in mosquitoes (Diptera: Culicidae). J Med Entomol 1985; 22:237-43.
7. **Jensenius M, Pretorius AM, Clarke F, Myrvang B**. Repellent efficacy of four commercial DEET lotions against *Amblyomma hebraeum* (Acari: Ixodidae), the principal vector of *Rickettsia africae* in southern Africa. Trans R Soc Trop Med Hyg 2005;99:708-11.
8. **Petrucci N, Sardini S**. Severe neurotoxic reaction associated with oral ingestion of low-dose diethyltoluamide-containing insect repellent in a child. Pediatr Emerg Care 2000;16:341-2.
9. **Briassoulis G, Narlioglou M, Hatzis T**. Toxic encephalopathy associated with use of DEET insect repellents: a case analysis of its toxicity in children. Hum Exp Toxicol 2001;20:8-14.
10. **Bell JW, Veltri JC, Page BC**. Human exposures to *N,N*-diethyl-m-toluamide insect repellents reported to the American Association of Poison Control Centers

1993-1997. Int J Toxicol 2002;21:341-52.

11. **Agency for Toxic Substances and Disease Registry (ATSDR).** Health effects in humans: DEET (*N,N*-diethyl-meta-toluamide) chemical technical summary for public health and public safety professionals [online]. Available from: URL:http://www.atsdr.cdc.gov/consultations/deet/health-effects.html. [Accessed August 9, 2012.]
12. **Qui H, Jun HW, John WM**. Pharmacokinetics, formulation, and safety of insect repellent *N,N*-diethyl-3-methylbenzamide (DEET): a review. J Am Mosq Control Assoc 1998;14:12-27.
13. **Curtis CF, Lines JD, Lu B, Renz A**. Natural and synthetic repellent. In: Curtis CF, editor. Control of disease vectors in the community. London: Wolf Publishers Ltd.; 1991.
14. **World Health Organization**. International travel and health guide: vaccination requirements and health advice. Geneva: WHO; 1995.
15. **Pålsson K, Jaenson TG**. Comparison of plant products and pyrethroid-treated bed nets for protection against mosquitoes (Diptera: Culicidae) in Guinea Bissau, West Africa. J Med Entomol 1999;36:144-8.
16. **Sukumar K, Perich MJ, Boobar LR**. Botanical derivatives in mosquito control: a review. J Am Mosq Control Assoc 1991;7:210-36.
17. **Shaalan E, Canyon D, Faried MW, et al**. A review of botanical phytochemicals with mosquitocidal potential. Environ Int 2005;31:1149-66.
18. **Amer A, Mehlhorn H**. Repellency effect of forty-one essential oils against *Aedes, Anopheles*, and *Culex* mosquitoes. Parasitol Res 2006;99:478-90.
19. **Lupi E, Hatz C, Schlagenhauf P**. The efficacy of repellents against *Aedes*, *Anopheles*, *Culex* and *Ixodes* spp. A literature review. Travel Med Infect Dis 2013; 11:374-411.
20. **Choochote W, Kanjanapothi D, Panthong A, et al**. Larvicidal, adulticidal and repellent effects of *Kaempferia galanga*. Southeast Asian J Trop Med Public Health 1999;30:470-6.
21. **Tawatsin A, Wratten SD, Scott RR, et al**. Repellency of volatile oils from plants against three mosquito vectors. J Vector Ecol 2001;26:76-82.
22. **Pitasawat B, Choochote W, Tuetuen B, et al**. Repellency of aromatic turmeric *Curcuma aromatica* under laboratory and field conditions. J Vector Ecol 2003;28:234-40.
23 **Tuetun B, Choochote W, Kanjanapothi D, et al**. Repellent properties of celery, *Apium graveolens* L., compared with commercial repellents, against mosquitoes under laboratory and field conditions. Trop Med Int Health 2005;10:1190-8.
24 **Trongtokit Y, Rongsriyam Y, Komalamisra N, Apiwathnasorn C**. Comparative repellency of 38 essential oils against mosquito bites. Phytother Res 2005;19:303-9.
25. **Maia MF, Moore SJ**. Plant-based insect repellents: a review of their efficacy, development and testing. Malar J 2011;10:1-14.
26. **Patel PK, Prajapati NK, Dubey BK**. *Madhuca indica*: A review of its medicinal properties. IJPSR 2012; 3:1285-93.
27. **Limsuwan S, Rongsriyam Y, Kerdpibule V, et al**. Rearing techniques for mosquitoes. In: Supavej S, editor. Entomology malaria and filariasis practical entomology malaria and filariasis. Bangkok: Museum and Reference Center, Faculty of Tropical Medicine, Mahidol University; 1987.
28. **World Health Organization**. Report of the WHO informal consultation on the evaluation and testing of insecticides. CTD/WHOPES/IC/96.1, Control of Tropical Diseases Division. Geneva: WHO; 1996.
29. **Liu ZL, He Q, Chu SS, et al**. Essential oil composition and larvicidal activity of *Saussurea lappa* roots against the mosquito *Aedes albopictus* (Diptera: Culicidae). Parasitol Res 2012;110:2125-30.
30. **Gwari G, Bhandari U, Andola HC, et al**. Volatile constituents of *Saussurea costus* roots cultivated in Uttarakhand Himalayas, India. Pharmacognosy Res 2013;5:179-82.
31. **Negi JS, Bisht VK, Bhandari AK, et al**. Antidiarrheal activity of methanol extract and major essential oil contents of *Saussurea lappa* Clarke. Afr J Pharm Pharmacol 2013;7: 474-7.
32. **Kumar R, Kumar A, Prasal CS, et al**. Insecticidal activity *Aegle marmelos* (L.) Correa essential oil against four stored grain insect pests. Internet J Food Safety 2008;10:39-49.
33. **Satyal P, Woods KE, Dosoky NS, et al**. Biological activities and volatile constituents of *Aegle marmelos* (L.) Correa from Nepal. J Med Active Plants 2012;1:114-22.
34. **Verma RS, Padalia RC, Chauhan A**. Essential oil composition of *Aegle marmelos* (L.) Correa: chemotypic and seasonal variations. J Sci Food Agric 2013. doi: 10.1002/jsfa.6510.
35. **Khan M, Srivastava SK, Syamasundar KV, et al**. Chemical composition of leaf and flower essential oil of *Lantana camara* from India. Flavour Fragr J 2002; 17:75-7.
36. **Shadia E, el-Aziz A, Omer EA, Sabra AS**. Chemical composition of *Ocimum americanum* essential oil and its biological effects against, *Agrotis ipsilon*, (Lepidoptera: Noctuidae). Res J Agric & Biol Sci 2007;3:740-7.
37. **al-Maskri AY, Hanif MA, al-Maskari MY, et al**. Essential oil from *Ocimum basilicum* (Omani Basil): a desert crop. Nat Prod Commun 2011;6:1487-90.
38. **Tawatsin A, Asavadachanukorn P, Thavara U, et al**. Repellency of essential oils extracted from plants in

Thailand against four mosquito vectors (Diptera: Culicidae) and oviposition deterrent effects against *Aedes aegypti* (Diptera: Culicidae). Southeast Asian J Trop Med Public Health 2006;37:915-31.

39. **Harborne JB**. Phytochemical methods: A guide to modern technique of plant analysis. London: Chapman and Hall; 1984.
40. **Mehta RM**. Pharmaceutics-I. Delhi: Vallabh Prakashan; 2002.
41. **Tuetun B, Choochote W, Rattanachanpichai E, et al**. Mosquito repellency of the seeds of celery (*Apium graveolens* L.). Ann Trop Med Parasitol 2004;98:407-17.
42. **Tuetun B, Choochote W, Pongpaibul Y, et al**. Field evaluation of G10, a celery (*Apium graveolens*)-based topical repellent, against mosquitoes (Diptera: Culicidae) in Chiang Mai province, northern Thailand. Parasitol Res 2009;104:515-21.
43. **Panneerselvam C, Murugan K**. Adulticidal, repellent, and ovicidal properties of indigenous plant extracts against the malarial vector, *Anopheles stephensi* (Diptera: Culicidae). Parasitol Res 2013;112:679-92.
44. **Singh SP, Mittal PK**. Mosquito repellent and oviposition deterrent activities of *Solanum nigrum* seed extract against malaria vector *Anopheles stephensi*. OIIRJ 2013;3:326-33.
45. **Wandscheer CB, Duque JE, da Silva MAN, et al**. Larvicidal action of ethanolic extracts from fruit endocarps of *Melia azedarach* and *Azadirachta indica* against the dengue mosquito *Aedes aegypti*. Toxicon 2004;44:829-35.
46. **Badolo A, Ilboudo-Sanogo E, Ouédraogo AP, Costantini C**. Evaluation of the sensitivity of *Aedes aegypti* and *Anopheles gambiae* complex mosquitoes to two insect repellents: DEET and KBR 3023. Trop Med Int Health 2004;9:330–4.

การคัดกรองประสิทธิภาพในการป้องกันยุงกัดของผลิตภัณฑ์สมุนไพรต่อยุงลายบ้าน *Aedes aegypti* (Diptera: Culicidae) พาหะนำโรคไข้เลือดออก

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วัตถุประสงค์ เพื่อคัดกรองประสิทธิภาพในการป้องกันยุงกัดของผลิตภัณฑ์สมุนไพร ได้แก่ น้ำมันหอมระเหยและสารสกัดเอทานอลและเฮกเซนที่เตรียมจากพืชจำนวน 15 ชนิด โดยทดสอบกับยุงลายบ้าน *Aedes aegypti* พาหะนำโรคไข้เลือดออก

วิธีการ การประเมินประสิทธิภาพในการป้องกันยุงกัดของผลิตภัณฑ์สมุนไพรภายใต้ห้องปฏิบัติการจะทำการทดสอบกับอาสาสมัครชายและหญิง ตามวิธีมาตรฐานขององค์การอนามัยโลก (WHO) ในกรงบรรจุยุงลายบ้าน *Ae. aegypti* ตัวเต็มวัยเพศเมียที่ผ่านการอดอาหารจำนวน 250 ตัว โดยศึกษาเปรียบเทียบกับ สารเคมีไล่ยุงมาตรฐานดีท (DEET) ภายใต้สภาวะเดียวกัน ในการทดลองจะทาสารทดสอบปริมาตร 0.1 มิลลิลิตร ลงบนผิวหนังด้านหน้าแขนบริเวณระหว่างข้อมือถึงข้อศอกในพื้นที่ขนาด 30 ตารางเซนติเมตร จากนั้นยื่นแขนเข้าไปในกรงยุงเป็นเวลานาน 3 นาที โดยทำทุก ๆ 30 นาที การทดสอบจะสิ้นสุดลงหากมียุงมากัดในพื้นที่ทดสอบอย่างน้อย 2 ตัว ภายในเวลา 3 นาที ของแต่ละช่วง หรือกัดครั้งแรกและตามด้วยครั้งที่สองในช่วงเวลาต่อมา ในการทดสอบสารแต่ละชนิดจะใช้อาสาสมัคร 2 คน ทำซ้ำคนละ 2 ครั้ง โดยแต่ละครั้งจะทำคนละวัน

ผลการทดลอง DEET และผลิตภัณฑ์สมุนไพรมีประสิทธิภาพสามารถป้องกันยุงกัดได้ในระดับที่แตกต่างกัน ส่วนใหญ่สารสกัดเฮกเซนจะมีประสิทธิภาพสูงกว่าน้ำมันหอมระเหยและสารสกัดเอทานอลที่เตรียมได้จากพืชชนิดเดียวกัน โดยสารสกัดที่มีประสิทธิภาพสูงสุดคือ สารสกัดเฮกเซนจากเหง้าโกฐหัวบัว (*Ligusticum sinense*) ที่มีค่ามัธยฐานของระยะเวลาป้องกันยุงกัดเท่ากับ 6.5 (5.0-8.0) ชั่วโมง ซึ่งถือว่ามีประสิทธิภาพเทียบเท่ากับ DEET ที่มีค่ามัธยฐานของระยะเวลาป้องกันยุงกัดเท่ากับ 6.25 (5.0-6.5) ชั่วโมง ตลอดระยะเวลาที่ทำการศึกษาไม่พบอาการผิดปกติของผิวหนัง เช่น ผื่นแดง บวม ระคายเคือง หรืออาการแพ้อื่น ๆ ในอาสาสมัครที่ทำการทดสอบ

สรุปผลการทดลอง ผลิตภัณฑ์สมุนไพรที่พิสูจน์แล้วว่ามีประสิทธิภาพในการป้องกันยุงกัด โดยเฉพาะสารสกัดเฮกเซนจากเหง้าโกฐหัวบัวถือเป็นตัวเลือกที่มีศักยภาพสามารถนำไปพัฒนาเป็นสารไล่ยุงธรรมชาติชนิดใหม่ เพื่อใช้ทดแทน DEET หรือนำไปใช้ร่วมกับสารเคมีหรือวิธีการอื่น ๆ ในการควบคุมแมลงพาหะแบบบูรณาการ
เชียงใหม่เวชสาร 2557;53(2):53-62.

คำสำคัญ: plant products, essential oils, ethanolic extracts, hexane extracts, repellents, *Aedes aegypti*