EXCITO-REPELLENCY PROPERTIES OF ESSENTIAL OIL FROM MICROMELUM MINUTUM WIGHT & ARM AGAINST LABOTORY POPULATIONS OF *AEDES AEGYPTI* AND AEDES ALBOPICTUS (DIPTERA: CULICIDAE)

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ABSTRACT

The essential oil extracted from *Micromelum minutum* leaves was investigated at three different concentrations (0.5%, 1.5% and 2.5% v/v) for their repellency properties against laboratory strains of *Aedes aegypti* and *Ae. albopictus* by using an excito-repellency test system. The results showed that both *Ae. aegypti* and *Ae. albopictus* exhibited varied escape rates after being affected by different concentrations of the oil. The best escape rate for *Ae. aegypti* was observed in both contact and non-contact chambers at the lowest concentration (50% escape), whereas the other two concentrations provided low-level escape rates. Meanwhile, a high escape rate was found in *Ae. albopictus* in all of the tested oil concentrations (41.7-66.7% contact, 60-70% non-contact chambers). The greatest knockdown rate in *Ae. aegypti* and *Ae. albopictus* was found after exposure to an oil concentration of 2.5% (36.7% contact, 25% non-contact chambers) and 1.5% (20% contact, 23.3%non-contact chambers), respectively. Moreover, the mortality rate of non-escaped *Ae. aegypti* was observed at 2.5% oil concentration (8.3-25%) after a 24-h holding period in both contact and non-contact chambers. Gas chromatography–mass spectrometry (GC-MS) analysis revealed that the components of *M. minutum* leaf oil were caryophyllene (26.6%), alpha-selinene (14.4%) and 1,4,7,-cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z- (8.1%). This was the first study on the irritant and repellent activities of the essential oil of *M. minutum* leaves against *Ae. aegypti* and *Ae. albopictus*. Further studies will perform the protection time assay to develop an appropriate formulation of a new herbal-based repellent**.**

Keyword: Excito-repellency/ Essential oil/ *Aedes aegypti*/ *Ae. albopictus*/ GC-MS analysis

INTRODUCTION

Aedes aegypti and *Ae. albopictus* are known generally as vectors of several arboviruses responsible for dengue fever, chikungunya and Zika. In Thailand, dengue and dengue hemorrhagic fever are important public health concerns, with more than 20,000 cases and 38 deaths being reported in 2016. Chikungunya has been found in areas of eastern and southern Thailand, and Zika has become a new threat to Thai people, due to more than 500 confirmed cases (Bureau of Vector-Borne Disease, 2016). *Ae. aegypti* is the main vector of the dengue and Zika virus, whereas *Ae. albopictus* is a main vector of chikungunya and Zika, and a secondary vector for dengue (Thavara *et al*, 2009).

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One of the most successful methods for mosquito control is the use of insecticides, such as deltamethrin and permethrin. However, long term use of insecticides can induce resistance in mosquito populations. At present, they are used commonly in households, with insecticide resistance against deltamethrin and permethrin being reported (Hemingway and Ranson, 2000; Chareonviriyaphap *et al,* 2013). Therefore, current control of mosquitoes is difficult. Mosquito repellents against mosquito bites, such as DEET, Picaridin and IR3535, are alternative methods for controlling a disease outbreak. However, many synthetic repellents are toxic when used for long periods of time and have several side effects such as skin irritation, damage to the nervous system and inhibition of motor skills, due to their strong chemical odor that penetrates through human skin and into the bloodstream. In addition, Stanczyk et al. (2013) found that long-term use of synthetic repellents could create resistance in *Anopheles* and *Aedes* mosquitoes. In order to avoid toxicity and resistance in mosquitoes, it is necessary to evaluate new bioactive repellents that are more effective, environment friendly, less toxic and more cost effective than chemical products.

Many plants have been studied regarding their repellency properties against mosquitoes, for example, lemon (*Corymbia citriodora*, Myrtaceae), lemon bush (*Lippia javanica*, Verbenaceae), thyme (*Thymus vulgaris*, Lamiaceae), lemongrass (*Cymbopogon citratus*, Poaceae) and neem (*Azadirachta indica*, Meliaceae) (Maia and Moore, 2011). *Micromelum minutum* or lime berry belong to the family of Rutaceae; mainly shrubs and trees. The oil of *M. minutum* has been used in Thai traditional medicine as a carminative, an antitussive and anthelmintic medicine, and also an expectorant (Pongboonrod, 1950). The characteristic of this family is an aromatic tree that presents oil glands in its leaves and other parts, and its constituents include a broad variety of alkaloids, volatile oils, coumarins and terpenoids (Trease and Evans, 1996). The essential oil from many plants in this family such as kaffir lime (*Citrus hystrix*), orange (*C. sinensis*), mandarin orange (*C. reticulata*), elephant-apple (*Ferronia elephantum*) and makaen *(Zanthoxylum limonella*) (Venkatachalam and Jebanesan, 2001; Tawatsin *et al,* 2001; Trongtokit *et al,* 2005 and Phasomkusolsil and Soonwera, 2010) are reported as having insect repellent efficacy. Therefore, the objective of this study was to investigate the repellent efficacy of essential oil extracted from *M. minutum* leaves against *Ae. aegypti* and *Ae. albopictus* by using the excito-repellency assay.

MATERIALS AND METHODS

Mosquitoes used

The mosquitoes used for testing were female *Ae. aegypti*, MCM-S strain, and *Ae. albopictus*, NS strain, which both originated from Chiang Mai and Nakhon Si Thammarat province, respectively. All of the mosquitoes were maintained continuously without insecticide or pathogen exposure. The mosquito colonies were reared by following the methods of Kongmee et al. (2004) with minor modifications in standard conditions (27 ± 2°C, 70-80 RH, 14:10 h light/dark photoperiod cycle) in the insectary of the Faculty of Veterinary Science, Prince of Songkla University. Three- to four-day-old female mosquitoes were fasted for 8-12 h before testing.

Essential oil extraction

Fresh leaves of *M. minutum* were collected from Songkhla province, Thailand, in March 2016. The voucher plant specimens (#SKP166030501) were deposited at the Department of Pharmacognosy and Pharmaceutical Botany Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla, Thailand.

The essential oil was extracted by the water distillation technique at 130°C for 6-12 h, dried over anhydrous sodium sulfate, and then collected and kept at 4°C in an amber-colored container.

Gas chromatography–mass spectrometry (GC-MS) analysis of essential oil

Micromelum minutum leaf oil was diluted to 1:100 in absolute ethanol and injected into GC-MS apparatus by the single quadrupole acquisition method in conditions following Kamkaen and Ruangrangsi (2003). The spectra were recorded and compared with the library program (Adam, 1995).

Behavioral tests

Evaluation of repellent activity was conducted by using the excito-repellency test chambers (Polsomboon *et al*, 2008) between 08:00 and 17:00 h under laboratory controlled conditions (Chareonviriyaphap *et al*, 2001; Boonyuan *et al*, 2014) to compare the behavioral response (contact irritant and non-contact repellent) of Ae. aegypti and Ae. albopictus. Three concentrations (0.5%, 1.5% and 2.5% v/v) of essential oil were diluted in absolute ethanol and applied on filter paper (15×17.5 cm, Whatman® No. 1). All of the treated papers were tested at the rate of 2.8 ml of test solution per 180 cm2. Control papers were treated with absolute ethanol only. All of the tested papers were allowed to air dry for 1 h before being used. Four chambers were set up in each trial: i.e. two contact chambers (treatment & control), in which the mosquitoes had direct contact with the paper, and two non-contact chambers (treatment & control), with the paper being protected by a fine mesh barrier screen. Fifteen fasted female mosquitoes were introduced into the excito-chamber and allowed to adjust to environmental conditions for 3 min before the escape funnel was opened. The number of escaped mosquitoes was recorded at 1-min intervals for a period of 30 min. All knockdown and live mosquitoes left in the chamber and receiving cage were transferred separately to holding cups and provided with 10% sugar solution for 24 h, before mortality (toxicity) was observed.

Data analysis

The Kaplan-Meier survival analysis method was used to analyze and interpret mosquito behavioral response to each of the three concentrations of essential oil (Kleinbaum, 1995; Roberts *et al*, 1997), and the log-rank test was used to compare the patterns of escape behavior between different designs. The discriminating level of statistical significance for all of the tests was set at 95% (Mantel and Haenzel, 1959).

RESULTS

The escape response and mortality data after exposure to various concentrations (0.5%, 1.5% and 2.5% v/v) of *M. minutum* leaf oil in a 30-min and 24-h holding period, respectively, of *Ae. aegypti* and *Ae. albopictus* were determined in contact (irritancy response) and non-contact (repellency response) chambers. (Table 1 and Figure 1)

The highest escape rate of *Ae. aegypti* was observed at the lowest concentration (50% escape) in both contact and non-contact chambers, whereas the other two concentrations provided low escape rates. Meanwhile, many escape rates were found in *Ae. albopictus* in all of the tested oil concentrations (41.7-66.7% contact, 60-70% non-contact chambers). The knockdown rate of *Ae. aegypti* in both contact (36.7%) and non-contact (25%) chambers treated with 2.5 % *M. minutum* leaf oil was higher than that of *Ae. albopictus*, while the knockdown rate of *Ae. albopictus* in both contact and non-contact chambers treated with 1.5% concentration (20-23.33%) was higher than that in those treated with 2.5 % concentration (6.7-8.3%). Low mortality of *Ae. aegypti* was observed in contact chamber with 1.5% and 2.5% oil concentration (1.67-8.33%), whereas that of *Ae. albopictus* was found in both contact and non-contact chambers with 1.5% oil concentration (3.33-5.00).

Multiple log-rank comparisons were used to compare the escape behavior between different conditions in contact, non-contact and control chambers for each concentration of *M. minutum* leaf oil (Table 2). A marked difference in escape patterns by *Ae. albopictus* was observed in comparisons between contact and non-contact chambers with 0.5% and 1.5% *M. minutum* leaf oil ($P<0.05$), whereas, no significant difference was observed in all concentrations for *Ae. aegypti* ($P>0.05$). Statistical difference in the escape response of *Ae. albopictus* was found when contact and non-contact chambers were compared to all paired controls at all

Table1 Escape response and mortality of *Ae. aegypti* and *Ae. albopictus* after a 24-h holding period following exposure to *M. minutum* leaf oil at three different concentrations (0.5%, 1.5% and 2.5%v/v) in contact and non-contact chamber.

Mosquitoes	Conditions	Dose %(v/v)	% Esc.	% Knockdown		% Mortality	
				Esc.	NEsc.	Esc.	NEsc.
Ae. aegypti	Contact	0.5	50.0	0	0	0	0
		1.5	6.7	0	1.67	0	1.67
		2.5	10.0	0	36.67	0	8.33
	N-contact	0.5	50.0	0	0	0	0
		1.5	6.7	0	0	0	0
		2.5	1.7	0	25.00	0	0
Ae. albopictus	Contact	0.5	46.67	0	0	0	0
		1.5	41.67	0	20.00	0	5.00
		2.5	66.67	0	6.67	0	0
	N-contact	0.5	70.00	0	0	0	0
		1.5	60.00	0	23.33	0	3.33
		2.5	70.00	0	8.33	0	0

Esc.: Escaped mosquitoes, NEsc.: Non- Escaped mosquitoes, N-contact: Non-contact
*Number Tested was sixty mosquito females in each condition

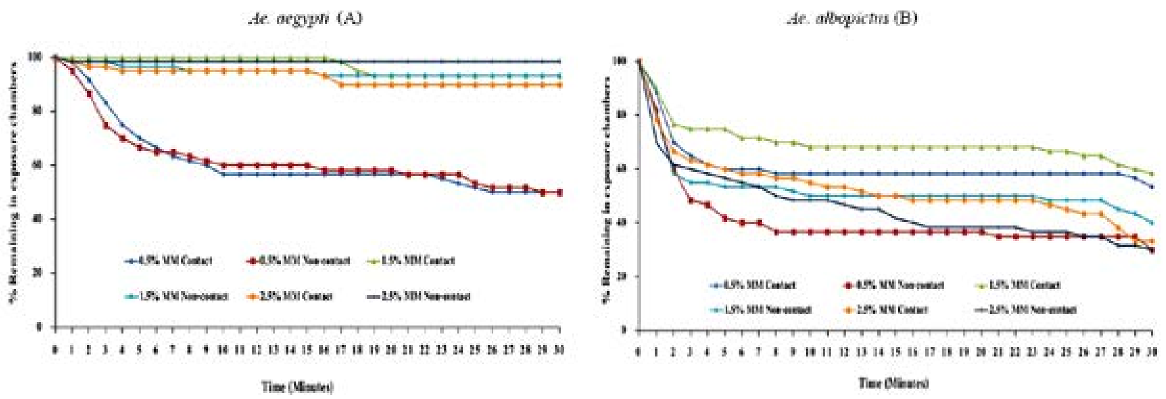

Fig 1- Survival analysis curves showing cumulative percent of *Ae. aegypti* (A) and *Ae. albopictus* (B) escaping treated test contact and non-contact chamber at three different concentrations (0.5%, 1.5% and 2.5%v/v) of *M. minutum* leaf oil during a 30-min.

concentrations ($P<0.05$). The comparisons of 1.5 % and 2.5% *M. minutum* leaf oil between contact vs control and non-contact vs control were found to differ significantly in *Ae. aegypti* ($P<0.05$).

Log-rank statistical comparisons between each percentage concentration of *M. minutum* leaf oil were analyzed in both contact and non-contact chambers, and a marked difference in

Table 2 Log-rank comparisons of the escape response between paired contact chamber and control, non-contact chamber and control, and paired contact and non-contact chambers for *Ae. aegypti* and *Ae. albopictus* exposed to *M. minutum* leaf oil at three different concentrations (0.5%, 1.5% and 2.5%v/v).

Mosquito	Dose %(v/v)	Contact vs Control	Non-contact vs Control	Contact vs Non-contact
Ae. aegypti	0.5	<0.0001*	<0.0001*	0.9057
	1.5	0.1777	0.0428*	0.9586
	2.5	0.0123*	0.3173	0.0543
Ae. albopictus	0.5	<0.0001*	<0.0001*	0.0129*
	1.5	<0.0001*	<0.0001*	0.0329*
	2.5	<0.0001*	<0.0001*	0.5244

* Log-rank tests with statistically significant (P<0.05) difference in escape response between pairs.

Table 3 Multiple paired log-rank comparisons of the escape response between three different concentrations of *M. minutum* leave oil in *Ae. aegypti* and *Ae. albopictus* exposed to contact and non-contact chambers.

Mosquito	Dose %(v/v)	Contact	Non-contact
Ae. aegypti	0.5 vs 1.5	<0.0001*	<0.0001*
	0.5 vs 2.5	<0.0001*	<0.0001*
	1.5 vs 2.5	0.4711	0.1753
Ae. albopictus	0.5 vs 1.5	0.5174	0.3166
	0.5 vs 2.5	0.0447*	0.9030
	1.5 vs 2.5	0.0064*	0.2622

* Log-rank tests with statistically significant (P<0.05) difference in escape response between pairs.

behavioral response among each concentration of oil was observed. A significant difference in escape response was found in *Ae. aegypti* at 0.5% concentration of oil when compared to 1.5% and 2.5% in both contact and non-contact chambers (P<0.05); while the escape response in *Ae. albopictus* differed significantly with the highest concentration of oil (2.5%), when compared to lower concentrations (0.5&1.5%) found only in the contact chamber (P<0.05) (Table 3).

Statistical comparisons of escape responses between mosquito species to difference conditions are presented in Table 4. There was a significant difference in escape responses between *Ae. aegypti* and *Ae. albopictus*, (P<0.05) in both contact and non-contact chambers, except for 0.5%oil concentration in only the contact chamber (P>0.05).

Twenty chemical compounds were derived from *M. minutum* leaf oil, of which the main constituents were caryophyllene (26.6%), alpha -selinene (14.4%) and 1,4,7,-cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z- (8.1%) (Table 5).

Table 4 Log-rank comparisons of the escape response between *Ae. aegypti* and *Ae. albopictus* exposed to *M. minutum* leaf oil at three different concentrations (0.5%, 1.5% and 2.5%v/v) in contact and non-contact chambers.

Conditions	Dose %(v/v)	Ae. aegypti vs Ae. albopictus
Contact	0.5	0.8757
	1.5	<0.0001*
	2.5	<0.0001*
Non-contact	0.5	0.0066*
	1.5	<0.0001*
	2.5	<0.0001*

* Log-rank tests with statistically significant ($P<0.05$) difference in escape response between pairs.

Table 5 The chemical compositions of *M. minutum* leaf oil derived from GC-MS analysis.

Number of peak	Retention times*	Component	%Area
1	5.98	δ-3-Carene	5.05
2	7.62	β-Pinene	0.81
3	9.01	4(10)-Thujene	3.43
4	9.72	3-Carene	1.81
5	24.15	β-elemene	1.92
6	25.78	Caryophyllene	26.64
7	26.06	Cis-α-Bergamotene	1.84
8	26.15	1β,4βH,10βH-Guaia-5,11-diene	0.88
9	26.87	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	8.13
10	27.29	(+)-Eremophilene	0.61
11	27.58	γ-Selinene	1.99
12	28.16	β-Selinene	7.22
13	28.66	α-Selinene	14.44
14	28.85	Valencen	1.08
15	29.46	δ-Cadinene	0.89
16	31.08	Nerolidol	0.76
17	31.70	Caryophylene oxide	1.21
18	32.89	Neointermedeol	1.63
19	33.32	Selin-6-en-α-ol	0.62
20	49.59	Phytol	0.69

* Retention times (min) and all component identified by retention indices and library of mass spectra.

DISCUSSION

The excito-repellency test system has been demonstrated on numerous occasions to measure the behavioral response of adult mosquitoes to insecticides (Roberts *et al*, 1994; Chareonviriyaphap *et al*, 1997). Robert et al. (2000) reported that two different types of behavioral response; irritancy and repellency, were due to exposure to chemicals. It is possible to observe both contact irritant and non-contact repellent responses and quantitatively measure behavioral responses of mosquitoes by following exposure to test compounds (Chareonviriyaphap *et al*, 2002).

In screening the repellency properties of essential oil from various plant sources, several essential oils have been studied for the behavioral response of mosquitoes. For example, Suwansirisilp et al. (2012) reported the behavioral effects of four essential oils extracted from orange peel (*C. aurantium*), cinnamon leaf (*Cinnamomum verum*), citronella grass (*C. winteriianus*) and clove flower (*Syzygiuma aromaticum*), which resulted in *Ae. aegypti* and *Culex quinquefacciatus* demonstrating a varied level of escape response to different oils. Later, Boonyuan et al. (2014) investigated the behavioral response of *Ae. aegypti* to the essential oil extracted from hairy basil (*Ocimum americanum*), ginger (*Zingiber officinale*), lemongrass (*C. citratus*), citronella grass (*C. nardus*), and plai (*Z. cassumunar*), all of which produced a stronger irritant and repellent response.

The essential oil extracted from *M. minutum* leaves showed excito-repellent action against *Ae. aegypti* and *Ae. albopictus*. The result of *M. minutum* leaf oil elicited strong repellent and irritant action in *Ae. aegypti* (50% escape in both contact and non-contact chambers) and stronger repellency than irritancy response in *Ae. albopictus* (41.7-66.7% contact, 60-70% non-contact) at 0.5% concentration. From these results, *Ae. albopictus* showed a better escape response than *Ae. aegypti*, as Sathantriphop et al. (2014) also suggested that *Ae. albopictus* exhibited more sensitivity to several essential oils than *Ae. aegypti*, and mosquito species showed different escape patterns to various test compounds. Moreover, a high concentration (2.5%) of *M. minutum* leaf oil caused a knockdown effect on *Ae. aegypti* and *Ae. albopictus* (6.67-36.67%), but a low mortality rate was observed, suggesting that high concentrations of essential oil can cause toxicity in mosquitoes. Likewise, Polsomboon et al. (2008) reported that a high concentration (10%) of essential oil from catnip exhibited a high knockdown rate (6.67-40%), however, less than a 10% mortality rate was observed. Similarly, Boonyuan et al. (2014) demonstrated that 10% citronella showed a high knockdown rate in *Ae. aegypti*, and Sathantriphop et al. (2014) found that hairy basil and citronella achieved more than a 50% knockdown rate in *Ae. albopictus*.

This study showed that the main constituents of *M. minutum* leaf oil were caryophyllene (26.6%), alpha-selinene (14.4%) and 1,4,7,-cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z- (8.1%). Likewise, its main components in oil from Nakhon Ratchasima province were bicyclogermacrene (19.79), 9-epi-(E)-caryophyllene (15.65%) and tricyclene (8.74%), which were in the terpenoid group (Kamkaen and Ruangrangsi, 2003). Nerio et al. (2010) reported that the monoterpenes group (cineole, eugenol, limonene, citronellol, terpinolene, etc.) and sesquiterpeenes group (β-caryophyllene) are active components in essential oil that act as a repellent. This study provided preliminary results on the repellent and irritant efficacy of essential oil from *M. minutum* against *Aedes* mosquitoes, and they could be used to develop appropriate repellent formulations, which would increase efficacy and cost effectiveness.

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REFERENCES

Adam RP. Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy. *Allured Publishing Corporation* Carol Stream 2001; 455.

Boonyuan W, Grieco JP, Bangs MJ, Prabaripai A, Tantakom S, Chareonviriyaphap T. Excito-repellency of essential oils against an *Aedes aegypti* **(**L.**)** field population in Thailand**.** *J Vector Ecol* 2014; 39: 112-22.

Bureau of Vector-Borne Disease, Department of Disease Control, Ministry of Public Health. 2016. http://www.thaivbd.org.

Chareonviriyaphap T, Roberts DR, Andre RG, Harlan H, Bangs MJ. Pesticide Avoidance Behavior in *Anopheles albimanus* Wiedemann. *J Am Mosq Control Assoc* 1997; 13(2): 171-83.

Chareonviriyaphap T, Sungvornyothin S, Ratanatham S, Prabaripai A. Pesticide-Induced Behavioral Responses of *Anopheles minimus*, a Malaria Vector in Thailand. *J Am Mosq Control Assoc* 2001; 17(1): 13-22.

Chareonviriyaphap T, Prabaripai A, Sungvornyothin S. An improved excito-repellency test chamber for mosquito behavioral tests. *J Vector Ecol* 2002; 27: 250-2.

Chareonviriyaphap T, Bangs MJ, Suwonkerd W, Kongmee M, Corbel V, Ngoen-Klan R. Review of insecticide resistance and behavioral avoidance of vectors of human diseases in Thailand. *Parasites & Vectors* 2013; 6: 280.

Hemingway J and Ranson H. Insecticide resistance in insect vectors of human disease. *Annu Rev Entomol* 2000; 45: 371–91.

Kamkaen N and Ruangrangsi N. Chemical Compositions and Antimicrobial Activities of Essential Oil from *Micromelum minutum*. *Thai J Health Res* 2003; 17(2): 147-53.

Kleinbaum DG. Survival Analysis. *Springer* New York 1995: 324

Kongmee M, Prabaripai A, Akaratanakul P, Bangs MJ, Chareonviriyaphap T. Behavioral responses of *Aedes aegypti* (Diptera: Culicidae) exposed to deltamethrin and possible implications for disease control. *J Med Entomol* 2004; 41: 1055-63

Maia MF, Moore SJ. Plant-based insect repellents: a review of their efficacy, development and testing. *Malar J* 2011; 10(Suppl 1): S11.

Mantel N, Haenzel W. Statistic aspects of the analysis of data from retrospective studies of diseases. *J Natl Cancer Inst* 1959; 22: 719-48.

Nerio LS, Olivero-Verbel L, Stashenko E. Repellent activity of essential oil: a review, *Bioresour. Technol* 2010; 101(1); 372-8.

Phasomkusolsil S, Soonwera M. Insect repellent activity of medicinal plant oils against *Aedes aegypti* (Linn.), *Anopheles minimus* (Theobald) and *Culex quinquefasciatus* Say based on protection time and biting rate. *Southeast Asian J Trop Med Public Health* 2010; 41(4): 831-40.

Polsomboon S, Poolprasert E, Bangs MJ, *et al*. Effects of physiological conditioning on behavioral avoidance by using a single age group of *Aedes aegypti* exposed to deltamethrin and DDT. *J Pest Sci* 2008; 86: 309–20.

Pongboonrod S. Mai-tat-muang-thai. *Kasem Bannakich* Bangkok 1950; 624-5.

Roberts DR, Andre RG. Insecticide Resistance Issues in Vector Borne Disease Control. *Am J Trop Med Hyg* 1994; 50(6): 21-34.

Roberts DR, Chareonviriyaphap T, Harlan HH, Hshieh, P. Methods for Testing and Analyzing Excito-Repellency Responses of Malaria Vectors to Insecticides. *J Am Mosq Control Assoc* 1997; 13(1): 13-7.

Roberts DR, Alecrim WD, Hshieh P *et al*. A Probability Model of Vector Behavior: Effects of DDT Repellency, Irritability, and Toxicity in Malaria Control. *J Vector Ecol* 2000;25(1):48-61.

Sathantriphop S, Thanispong K, Sanguanpong U, Achee NL, Bangs MJ, Chareonviriyaphap T. Comparative behavioral responses of pyrethroid–susceptible and resistant *Aedes* *aegypti* (Diptera: Culicidae) populations to citronella and eucalyptus oils. *J Med Entomol* 2014;51:1182-1191.

Stanczyk NM, Brookfield J FY, Field LM, Logan JG. *Aedes aegypti* Mosquitoes Exhibit Decreased Repellency by DEET following Previous Exposure. *PLoS ONE* 2013;8(2): e54438.

Suwansirisilp K, Visetson S, Prabaripai A, *et al.* Behavioral responses of *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae) to four essential oils in Thailand. *J Pest Sci* 2012; 86: 309-20.

Tawatsin A, Wratten SD, Scott RD, Thavara1 U, Techadamrongsin Y. Repellency of Volatile Oils from Plants against Three Mosquito Vectors. *J Vector Ecol* 2001; 26(1): 76-82.

Thavara U, Tawatsin A, Pengsakul T, *et al*. Outbreak of chikungunya fever in Thailand and virus detection in field population of vector mosquitoes, *Aedes aegypti* (L.) and *Aedes albopictus* Skuse (Ditera: Culicidae). *Southeast Asian J Trop Med Public Health* 2009; 40: 951-62.

Trease GE and Evans WC. Pharmacognosy, *University Press, London* 1966; 42.

Trongtokit Y, Rongsriyam Y, Komalamisra N, Apiwathnasorn A. Comparative Repellency of 38 Essential Oils gainst Mosquito Bites. *Phytother Res* 2005; 19: 303–9.

Venkatachalam MR, Jebanesan A. Repellent activity of *Ferronia elephantum* Corr. (Rutaceae) leaf extract against *Aedes aegypti* (L.). *Bioresour Technol* 2001; 76: 287-8.