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Acaricidal Effects of Thai Herbal Essential Oils against *Dermatophagoides pteronyssinus*

C. Veeraphant[1], V. Mahakittikun[2] and N. Soonthornchareonnon[1],*

[1]Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand
[2]Siriraj Dust Mite Center for Service and Research (SDMC), Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Abstract

Twenty four herbal essential oils and reference standards were examined for acaricidal activity against *Dermatophagoides pteronyssinus* (*Dp*) house dust mites at 24/48 h. The results show that at a 0.1% concentration level of clove bud oil, cinnamon leaf oil, jasmine ABS, and hairy basil oil can kill 100% of *Dp* within 10 min. The active essential oils of clove bud, cinnamon leaf, turmeric, jasmine, betel vine and lemongrass showed LC_{50} values (24 h) of 0.0026, 0.0041, 0.0069, 0.0079, 0.0091 and 0.0091 ml/ml, respectively. The chemical compositions of the active essential oils were analyzed using Gas Chromatography-Mass Spectroscopy (GC-MS), and the major components were also tested for acaricidal activity. In addition, the active essential oils were examined for preliminary toxicity using the brine shrimp (*Artemia salina* Leach) lethality test (BST). However, the LD_{50} values of the toxicity of these oils were not relative to acaricidal activities.

Key words: Acaricidal activity, House dust mite, Thai herbal essential oils

***Corresponding author:** Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, 447 Sri-Ayuthaya Road, Rajthevi, Bangkok 10400, Thailand.
E-mail: pynsk@mahidol.ac.th Tel. +66 (0) 2644-8677-91 Ext. 5527 Fax +66 (0) 2644-8701

INTRODUCTION

Allergic diseases such as asthma, allergic rhinitis, allergic conjunctivitis and atopic eczema have affected millions of people worldwide. These diseases are essentially caused by the House Dust Mites (HDMs), *Dermatophagoides pteronyssinus* (*Dp*), which is the dominant species in the household. Nowadays, allergic diseases have recently become a serious health problem[1].

The establishment to control HDMs can be divided into the following: 1) reduce mite population which is the source of new allergen, 2) denature mite allergen, and 3) allergen avoidant. However, there has not been a well-qualified way to control HDMs, whereas HDMs control should be done by multiple ways which including: physical method, by heat, washing, cold condition, or using mite cover such as tightly woven fabric places over mattresses. In addition, regarding the usage of the chemical methods in the chemical acaricides category such as benzyl benzoate, pyrethroids and natural products acaricides were also alternative[2-5].

Recently, the effects of herb essential oils on HDMs have received much more attention with a view of producing natural mite-killing agents because they are not harmful to human and animal, such as clove bud oil (*Syzygium aromaticum* L.)[6], *Chamaecyparis obtusa* (Siebold & Zucc.) Endl.[7], *Pinus densiflora* Siebold & Zucc. and *Cryptomeria japonica* (L.f.) D.Don[8], pennyroyal oil (*Mentha pulegium* L.)[9], *Taiwania cryptomerioides* Hayata[10], citronella oil (*Cymbopogon nardus* Rendle)[11], tea tree oil [*Melaleuca alternifolia* (Maiden & Betche) Cheel][12], eucalyptus oil (*Eucalyptus globulus* Labill.)[13], and cinnamon oil (*Cinnamomum zeylanicum* L.)[14]. Thai herbal essential oils have numerous varieties which make them very substantial for anti HDMs studies.

In this study, we investigate the acaricidal activity of twenty four Thai herbal essential oils against *Dermatphagoides pteronyssinus* (*Dp*) house dust mite, toxicity to brine shrimp, chemical compositions and physicochemical properties of active essential oils.

MATERIALS AND METHODS

Sample of Thai herbal essential oils

Twenty four Thai herbal essential oils (Table 1) and twelve standards were purchased from Thai-China Flavors and Fragrances Industry Co., Ltd. (TCFF), except the ma-khwann oil obtained from water distillation from *Zanthoxylum myriacanthum* Wallich ex Hook.f., collected from Chiangmai province by Assoc. Prof. Noppamas Soonthornchareonnon and identified by Assist. Prof. Paritat Trisonthi (BKF Number 121179). The sample was deposited at herbarium of Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University.

The physicochemical properties of essential oils

The active essential oils were further examined for physicochemical properties which were relative density (ρ_{20}), optical rotation [α], and refractive index. The methods were modified from British Pharmacopoeia (BP).

Chemical qualitative analysis of essential oils

Fifteen active essential oils were analyzed for their chemical compounds. The instrument was gas column chromatography (GC) combined with mass spectrometry (MS) (Shimadzu), capillary column DB5, 30 m. x 0.25 mm. id., with a film thickness of 0.25 μm (J&W Scientific, USA). All oven temperature programs were used depended on each essential oil as follows:

Holy basil oil : initial temperature 50°C which was held for 5 min, ramped at 4°C/min to 110°C which was held for 1 min, ramped at 2°C/min to 170°C which was held for 2 min and ramped at 40°C/min to 250°C which was held for 5 min.

Betel vine oil : see Holy basil oil

Ma-khwann oil : initial temperature 50°C which was held for 2 min, ramped at

1°C/min to 100°C and ramped at 3°C/min to 250°C which was held for 5 min.

Hairy basil oil : initial temperature 50°C which was held for 5 min, ramped at 2°C/min to 120°C, ramped at 4°C/min to 160°C which was held for 1 min and ramped at 45°C/min to 250°C which was held for 5 min

Clove bud oil : initial temperature 50°C which was held for 5 min, ramped at 3°C/min to 170°C which was held for 2 min and ramped at 40°C/min to 250°C which was held for 5 min.

Citronella oil : see Clove bud oil

Kaffir lime leaf oil : see Clove bud oil

Ylang Ylang oil : initial temperature 65°C which was held for 5 min, ramped at 3°C/min to 200°C which was held for 2 min and ramped at 25°C/min to 250°C which was held for 5 min.

Kaffir lime oil : initial temperature 50°C which was held for 2 min, ramped at 2°C/min to 110°C, ramped at 3°C/min to 170°C which was held for 1 min and ramped at 40°C/min to 250°C which was held for 5 min.

Jasmine ABS : initial temperature 65°C which was held for 5 min and ramped at 3°C/min to 250°C which was held for 5 min.

Sweet basil oil : see Clove bud oil

Cinnamon leaf oil : initial temperature 50°C which was held for 2 min, ramped at 3°C/min to 120°C, ramped at 2°C/min to 170°C which was held for 1 min and ramped at 40°C/min to 250°C which was held for 5 min.

Clove leaf oil : see Clove bud oil

The type of injection was split in volume 1 µl, and helium was used as the carrier gas at a linear velocity flow of 3 ml/min. The GC-MS interface was held at 250°C, injection port at 200 °C, ion source at 200°C and mass spectra were obtained at 70 eV. The effluent of the capillary column was introduced directly into the MS ion source. The mass analyzer was set to SCAN from 40 to 440 amu every 0.5 sec. The chemical components were identified by comparing of mass spectral fragmentation patterns with WILEY7 and NIST147 library database.

***Anti house dust mite activities test*[15,16]**

House Dust Mites (HDMs)

The mites, *Dermatophargoides pteronyssinus* (*Dp*) species were used in this study. The mites have been cultured in the Siriraj Dust Mite Center for Service and Research (SDMC), Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University Thailand. The HDMs were cultured in rat chow mixed with liver extract media in cultured bottle where the air can be penetrated, stored at 25 °C and 75% relative humidity (by using saturated sodium chloride solution). After being cultured for 2 months, adult mites of either gender were isolated from the culture medium by mite isolator.

Preparation of test solution

Fifteen suitable essential oils, and reference compounds were prepared in serial dilutions using 95% ethanol or acetone as solvent according to the range cover LC_{50} values as the concentration of 0.1, 0.05, 0.025, 0.0125, 0.01, 0.005, 0.0025, 0.00125, 0.000625 and 0.0003125 ml/ml, except *trans*-sabinene hydrate was diluted to the concentration of 12,500, 25,000, 50,000 and 100,000 ppm. The positive control, benzyl benzoate, was diluted similar to other samples and 95% ethanol was used as negative control.

Bioassay

The undiluted oils were preliminary screened for anti-HDM activity. The oils which were active, and easily found in local were continued to be tested for LC_{50} by preparing as serial dilutions and tested by detection at 24 and 48 hours. Anti HDMs activities were performed in the well of "Siriraj chamber" (capacity 0.79 cm^2/well). Ten mites were placed in each well, then applied 10 µl of the undiluted essential oils (for preliminary screening) and 45 ml of the serial dilution samples (for testing of LC_{50} value) on the mites. Covered the well with glass plate of Siriraj Chamber and motality was observed using stereobionocular microscope after 24 and

48 hrs incubation time. Mites that did not move when prodded with a fine brush were considered as dead, and percent deaths at each concentration were determined. Benzyl benzoate and 20% acetone in water were used as positive and negative control, respectively. All experiments were replicated five times. The LC_{50} values and 95% confident intervals were determined using Probit analysis method and calculated by Finney program.

Preliminary toxicity test using brine shrimp lethality test[17,18]

Preliminary toxicity was studied using brine shrimp lethality test (BST) against brine shrimp (*Artemia salina* Leach). It is a simple, comfortable, non-aseptic technique and low cost bioassay. BST has been previously utilized in various bioassay systems for analysis of pesticide residues, mycotoxins, water pollutants, dinoflagellate toxins, and preliminary test for toxicity, antitumor, insecticide, etc.

Preparation of test solution

The active fifteen essential oils were prepared by dissolving 160 µl in 100 µl dimethylsulfoxide (DMSO). The essential oils were prepared in dilutions according to the range cover LD_{50} values as 6.2196×10^{-3} to 79345.45 ppm. Positive control was DMSO 100 µl mix ed with seawater 1,500 µl and negative control as 0.1-100 ppm of berberine solution.

Table 1. The list of twenty four Thai essential oils

No.	Scientific name of plants	Family	Part uses	Name of oils
1	*Alpinia officinarum* Hance	Zingiberaceae	rhizome	galangal oil
2	*Boesenbergia pandurata*	Zingiberaceae	rhizome	lesser galangal oil
3	*Cananga odorata* (Lam.) Hook.f.&Thomson var. *odorata*	Annonaceae	flower	ylang ylang oil
4	*Cinnamomum zeylanicum Blume*	Lauraceae	leaf	cinnamon leaf oil
5	*Citrus hystrix* DC.	Rutaceae	peel	kaffir lime oil
6	*Citrus hystrix* DC.	Rutaceae	leaf	kaffir lime leaf oil
7	*Citrus aurantifolia* (Christm.) Swingle	Rutaceae	peel	lime oil
8	*Citrus reticulata* Blanco	Rutaceae	peel	tangerine oil
9	*Citrus sinensis* (L.) Osbeck	Rutaceae	peel	orange oil
10	*Curcuma longa* L.	Zingiberaceae	rhizome	turmeric oil
11	*Cymbopogon citratus* Stapf	Lamiaceae	aerial part	lemongrass oil
12	*Cymbopogon nardus* Rendle	Lamiaceae	aerial part	citronella oil
13	*Eucalyptus globulus* Labill.	Myrtaceae	leaf	eucalyptus oil
14	*Jasminum sambac* (L.) Aiton	Oleaceae	flower	jasmine ABS
15	*Ocimum americanum* L.	Lamiaceae	aerial part	hairy basil oil
16	*Ocimum basilicum* L.	Lamiaceae	aerial part	sweet basil oil
17	*Ocimum sanctum* L.	Lamiaceae	aerial part	holy basil oil
18	*Piper betle* L.	Piperaceae	leaf	betel vine oil
19	*Psidium guajava* L.	Myrtaceae	leaf	guava leaf oil
20	*Rosa damascena* Mill	Rosaceae	flower	rose oil
21	*Syzygium aromaticum* (L.) Merr.&L.M.Perry	Myrtaceae	flower bud	clove bud oil
22	*Syzygium aromaticum* (L.) Merr.&L.M.Perry	Myrtaceae	leaf	clove leaf oil
23	*Zanthoxylum myriacanthum* Wallich ex Hook.f.	Rutaceae	fruit	ma-khwann oil
24	*Zingiber montanum* (Koenig) Link ex Dietr.	Zingiberaceae	rhizome	phlai oil

Bioassay

The procedure for BST was modified from the assay described by Solis et al.[13] and berberine sulfate was used as positive control. The 200 µl of each sample dilution was added to each well of 96-wells microplate, repeatedly 6 wells, and 4-8 shrimps in 50 µl artificial seawater solutions were added to each well, and the 96-well was incubated at room temperature under illumination. After 24 hours the dead and live larvae were counted under stereomicroscope, and the LD_{50} values and 95% confident intervals of each dose and control were determined by using Finney-probit analysis program[19].

RESULTS

The preliminary screening for acaricidal activity against Dp of the undiluted essential oils

The preliminary screening for acaricidal activity against *Dp* of the undiluted essential oils was found that among twenty four herbal essential oils, fifteen oils were shown the activities against *Dp* which were essential oils number 1-15. They caused 100% mortality after 10 minutes. The most active oil was jasmine ABS that could kill all *Dp* within 30 seconds followed by clove bud oil within 1 minute. The others were shown as following: ylang ylang oil, citronella oil and clove leaf oil can kill all mites within 3 minutes; hairy basil oil, holy basil oil, lemongrass oil, kaffir lime leaf oil, betel vine oil, mae-khwann oil and cinnamon leaf oil can kill all mites within 5 minutes; phlai oil, eucalyptus oil and kaffir lime oil can kill all mites within 10 minutes (Table 2).

The LC_{50} of active essential oils

The results of the acaricidal activity of fifteen oils at 24/48 h on *Dp* were shown that at the concentration 0.1% v/v, all essential oils can kill 100% *Dp* in 24/48 h, while at this concentration, clove bud oil, cinnamon leaf oil, jasmine ABS and hairy basil oil killed all mites within 10 min, lemongrass oil within 15 min and holy basil oil within 20 min (Table 3). In addition, at the concentration of 0.025% v/v, jasmine ABS, betel vine oil, lemongrass oil, holy basil oil and hairy basil oil killed all mites at the same period of time. On the basis of LC_{50} values, clove bud oil was the most active oil followed by cinnamon leaf oil, turmeric oil, jasmine ABS, betel vine oil, lemongrass oil, holy basil oil and hairy basil oil with LC_{50} values in 24 h at 0.0026, 0.0041, 0.0069, 0.0079, 0.0091, 0.0091, 0.0101 and 0.0113 ml/ml, respectively.

The physicochemical properties of active essential oils

The active essential oils were further examined for physicochemical properties. This study was consisted of relative density (ρ_{20}), optical rotation [α], refractive index. The experiment data were shown in Table 4.

Chemical qualitative analysis of active essential oils using GC-MS

The major compounds and % area from GC-MS data of chemical components of each essential oil were shown in Table 5.

Anti house dust mite activity of major components

The major components of active essential oils were tested for anti house dust mite activity against *Dp*. It was found that among the LC_{50} values in 24 h, cinnamaldehyde was the most active, followed by eugenol, *ar*-turmerone, methyl eugenol and citral with LC_{50} values at 0.0003, 0.0018, 0.0056, 0.0119 and 0.0127 ml/ml, respectively (Table 6).

Preliminary toxicity test using Brine shrimp lethality test

The preliminary toxicity test showed that turmeric oil was the most active against *Artemia salina*. In addition, jasmine ABS was less active and eucalyptus oil was inactive. This data shown that the LD_{50} values of these oils did not relative to acaricidal activities (Table 7).

Table 2. The acaricidal activity against *Dermatophagoides pteronyssinus* (*Dp*) of undiluted oils

No.	Name of oil	Mite death time (min)
1	Jasmine ABS	0.5
2	Clove bud oil	1
3	Ylang Ylang oil	3
4	Citronella oil	3
5	Clove leaf oil	3
6	Hairy basil oil	5
7	Holy basil oil	5
8	Lemongrass oil	5
9	Kaffir lime leaf oil	5
10	Betel vine oil	5
11	Ma-khwann oil	5
12	Cinnamon leaf oil	5
13	Phlai oil	10
14	Eucalyptus oil	10
15	Kaffir lime oil	10
16	Lime oil	15
17	Galangal oil	25
18	Sweet basil oil	30
19	Lesser galangal oil	60
20	Orange oil A	60
21	Tangerine oil	60
22	Rose oil	60
23	Guava leaf oil	60
24	Turmeric oil	1,440

Table 3. The LC_{50} values (ml/ml) in 24/48 hours of active essential oils against *Dermatophagoides pteronyssinus* (*Dp*)

No.	Name of oil	LC_{50} value (ml/ml)	
		24 h	**48 h**
1	Clove bud oil	0.0026	0.0023
2	Cinnamon leaf oil	0.0041	0.0037
3	Turmeric oil	0.0069	0.0045
4	Jasmine ABS	0.0079	0.0072
5	Betel vine oil	0.0091	0.0086
6	Lemongrass oil	0.0091	0.0088
7	Holy basil oil	0.0101	0.0113
8	Hairy basil oil	0.0113	0.0091
9	Ylang Ylang oil	0.0128	0.0133
10	Kaffir lime leaf oil	0.0214	0.0218
11	Citronella oil	0.0271	0.0227
12	Ma-khwann oil	0.0264	0.0160
13	Phlai oil	0.0490	0.0461
14	Kaffir lime oil	0.0675	0.0691
15	Eucalyptus oil	0.0798	0.0767
16	95% EtOH (negative control)	inactive	inactive
17	Acetone (negative control)	inactive	inactive
18	Benzyl benzoate (positive control)	0.0003	<0.0001

DISCUSSIONS

The anti house dust mite activity of each essential oil mostly depends on their chemical compounds, especially major compounds. The most active essential oils based on LC_{50} values such as clove bud oil, cinnamon leaf oil, turmeric oil, holy basil oil, lemongrass oil, hairy basil oil, betel vine oil, jasmine ABS and ylang ylang oil are composed of these active compounds. Some of them have large amount of active compounds such as the content of eugenol, which are found in cinnamon leaf oil and clove bud oil more than 82.83 and 95.52%, respectively. While cinnamon leaf oil also has potential anti HDMs compounds, benzyl benzoate (2.64%) and cinnamaldehyde (0.76%), which were shown lower LC_{50} values than eugenol. Lemongrass oil and hairy basil oil also contain large amount of 84.55 and 67.20% citral, respectively. Moreover, some essential oils consist of fewer proportion of compounds which are active against *Dp* such as *ar*-turmerone in turmeric oil, eugenol in holy basil oil and betel vine oil and benzyl benzoate in jasmine ABS and ylang ylang oil. Jasmine ABS contains only 2.27% benzyl benzoate but it is very active against *Dp*, it may be due to the effect of the other minor compounds which has strong activity against *Dp*.

Table 4. Physicochemical properties of the active essential oils

Name of oil	Relative density (20°C)	Optical rotation (20°C)	Refractive index (20°C)
Clove bud oil	1.0910	-1.0999	1.5374
Cinnamon leaf oil	0.9560	-1.8029	1.5320
Turmeric oil	0.9326	-61.5055	1.5030
Jasmine ABS	1.0050	-1.2438	1.5146
Betel vine oil	1.0580	-2.0416	1.5148
Lemongrass oil	0.9096	-2.1807	1.4871
Holy basil oil	0.9866	-12.5279	1.5149
Hairy basil oil	0.9036	-9.9159	1.4836
Ylang Ylang oil	0.9434	-73.0973	1.4981
Kaffir lime leaf oil	0.8620	-13.0394	1.4509
Citronella oil	0.8780	-3.9801	1.4660
Ma-khwann oil	0.8552	35.3134	1.4710
Phlai oil	0.8760	-52.7397	1.4986
Kaffir lime oil	0.8734	17.1743	1.4722
Eucalyptus oil	0.9070	3.5913	0.9030

In addition, the activity of essential oils that have effect to *Dp* could be considered in many ways for example, they were either stimulated by compounds in essential oils or the species of plant of herbal essential oils. The different species could affect causing changes to the physicochemical properties of essential oils and also changing their potency.

In a normal circumstance, the death of HDMs *Dp* would show foreleg was extended forward together but the usage of some essential oil such as eucalyptus oil also involves the mite shriveling before dead. HDMs survive using the exchange of gas between their cell and atmosphere, it was possible that the essential oils disrupt the passage of gas to mite cell.

The preliminary toxicity test of essential oils to brine shrimp could be useful to consider the safety of active oils for further study and product development of those essential oils as a acaricidal product in the future.

CONCLUSION

The twenty four Thai essential oils were preliminary tested for acaricidal activity against *Dp* which is the major cause of allergy in Thaialnd. The fifteen active essential oils were selected for further studies for LC_{50} in 24/48 h, preliminary toxicity test using BST (LD_{50}), chemical analysis of major compositions using GC-MS and their acaricidal activity. The result showed that the potential oils for product development were clove bud oil, cinnamon leaf oil, and turmeric oil.

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Table 5. The major components of the active essential oils analysed by GC-MS

Name of oil	**Major compound**	**%Area**
Clove bud oil	eugenol	95.52
Cinnamon leaf oil	eugenol	82.83
	benzyl benzoate	2.64
	cinnamaldehyde	0.76
Turmeric oil	turmerone	39.05
	ar-turmerone	21.49
	curlone	15.80
Jasmine ABS	3-hexenyl benzoate	23.79
	benzyl acetate	12.12
	methyl anthranilate	11.14
Betel vine oil	acetyl eugenol	28.81
	eugenol	28.44
	ethanone	23.58
Lemongrass oil	citral a, citral b	49.05, 35.50
Holy basil oil	methyl eugenol	44.18
	caryophyllene	27.57
	eugenol	11.88
Hairy basil oil	citral a, citral b	36.49, 30.71
Ylang Ylang oil	gurjunene	40.54
	benzyl benzoate	11.86
Kaffir lime leaf oil	citronellal	75.31
Citronella oil	citronella	35.87
	geraniol	12.98
Ma-khwann oil	sabinene	21.57
	terpinen-4-ol	20.62
	γ-terpinene	11.86
Phlai oil	sabinene	54.59
	terpinen-4-ol	12.48
Kaffir lime oil	limonene	26.16
	β-pinene	21.43
Eucalyptus oil	eucalyptol	72.28

Table 6. The LC_{50} values (ml/ml) in 24/48 hours of compounds from active essential oils against *Dermatophagoides pteronyssinus* (*Dp*)

No.	Compound	LC_{50} value (ml/ml)	
		24 h	48 h
1	Cinnamaldehyde	0.0003	0.0008
2	Benzyl benzoate	0.0003	<0.0001
3	Eugenol	0.0018	0.0018
4	*ar*-Turmerone	0.0056	0.0040
5	Methyl eugenol	0.0119	0.0111
6	Citral	0.0127	0.0127
7	α-Terpineol	0.0227	0.0206
8	Citronellal	0.0474	0.0367
9	Eucalyptol	0.0650	0.2486
10	Terpinene-4-ol	0.0679	0.0612
11	Limonene	0.0804	0.0642
12	β-Pinene	0.1222	0.1390
13	*trans*-Sabinene hydrate (μg/ml)	0.1622	0.2471
14	Linalool	0.2942	0.1095

Table 7. The LD_{50} values in 24 hours of active essential oils from brine shrimp lethality test (BST) against *Artemia salina* Leach

No.	Name of oil	LD_{50} value (µg/ml)
1	Turmeric oil	<0.0001
2	Citronella oil	0.03
3	Phlai oil	0.08
4	Ma-khwann oil	0.31
5	Lemongrass oil	0.37
6	Cinnamon leaf oil	0.50
7	Betel vine oil	0.80
8	Holy basil oil	0.89
9	Ylang Ylang oil	1.65
10	Hairy basil oil	4.68
11	Kaffir lime oil	7.21
12	Clove bud oil	7.55
13	Kaffir lime leaf oil	8.95
14	Jasmine ABS	13.72
15	Eucalyptus oil	>1,000
16	Berberine (positive control)	13.27
17	diluents (negative control)	inactive

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