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Comparative Repellency of 38 Essential Oils against Mosquito Bites

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The mosquito repellent activity of 38 essential oils from plants at three concentrations was screened against the mosquito *Aedes aegypti* under laboratory conditions using human subjects. On a volunteer's forearm, 0.1 mL of oil was applied per 30 cm^2 of exposed skin. When the tested oils were applied at a 10% or 50% concentration, none of them prevented mosquito bites for as long as 2 h, but the undiluted oils of *Cymbopogon nardus* (citronella), *Pogostemon cablin* (patchuli), *Syzygium aromaticum* (clove) and *Zanthoxylum limonella* (Thai name: makaen) were the most effective and provided 2 h of complete repellency.

From these initial results, three concentrations (10%, 50% and undiluted) of citronella, patchouli, clove and makaen were selected for repellency tests against *Culex quinquefasciatus* and *Anopheles dirus*. As expected, the undiluted oil showed the highest protection in each case. Clove oil gave the longest duration of 100% repellency (2–4 h) against all three species of mosquito. Copyright © 2005 John Wiley & Sons, Ltd.

Keywords: Mosquito repellent; *Cymbopogon nardus*; *Pogostemon cablin*; *Syzygium aromaticum*; *Zanthoxylum limonella*; *Aedes aegypti*; *Culex quinquefasciatus*; *Anopheles dirus.*

INTRODUCTION

Plants and plant-derived substances have been used to try to repel or kill mosquitoes and other domestic pest insects for a long time before the advent of synthetic chemicals (Curtis *et al.*, 1989). A review on the uses of botanical derivatives against mosquitoes has been presented by Sukumar *et al.* (1991). Essential oils of a large number of plants have been found to have repellent properties against various haematophagous arthropods; some have formed the basis of commercial repellent formulations (Curtis *et al.*, 1989). The repellency of these oils appears to be generally associated with the presence of one or more volatile mono-terpenoid constituents. Although they are effective when freshly applied, their protective effects dissipated relatively rapidly (Buescher *et al.*, 1982b; Rutledge *et al.*, 1983; Curtis *et al.*, 1989). The oils which have been reported as potential sources of insect repellents include citronella, cedar, verbena, pennyroyal, geranium, lavender, pine, cajeput, cinnamon, rosemary, basil, thyme, allspice, garlic and peppermint. Sharma *et al.* (1993) have reported the effectiveness of neem oil as a method of protection from mosquitoes which is safe and does not use synthetic chemicals. In laboratory tests in the USA (Barnard, 1999), thyme and clove oils provided 1.5–3.5 h of protection against *Aedes aegypti*. Citronella oil, in concentrations ranging from 0.05% to 15%, is used alone or in combination with cedarwood, lavender, peppermint, clove, eucalyptus and garlic in a number of commercial insect repellent products (Fradin, 1998). Currently, a lemon eucalyptus extract which comes from the plant *Eucalyptus maculata citriodon* with the principal active ingredient *p*-menthane-3,8-diol (PMD) has shown particularly good results in its mosquito repellent properties when tests were carried out under laboratory and field conditions. This repellent has been found to be effective against mosquitoes, midges, ticks and the stable fly (Curtis *et al.*, 1989; Trigg, 1996; Trigg and Hill, 1996; Govere *et al.*, 2000). Oils extracted from plants are widely used as fragrances in cosmetics, food additives, household products and medicines. The US Food and Drug Administration (FDA) generally recognize these as safe.

Recently, there have been many reports concerning the repellent properties of many kinds of essential oils; however, most of the results came from artificial (*in vitro*) testing methods using cloth, filter paper, animal membrane or olfactometry but some came from more realistic (*in vivo*) methods utilizing animals or human subjects (Rutledge *et al.*, 1964; Barnard, 2000). Results from different methods cannot be compared directly because these methods yield results strongly related to the laboratory conditions used. The evaluation of repellency should preferably be carried out using human subjects, because laboratory animals may simulate inadequately the condition of human skin to which repellents will eventually be applied (WHO, 1996; Barnard, 2000; Moore, 2003).

In the present study, attempts have been made to characterize the relationship between different concentrations of 38 selected essential oils against *Aedes aegypti* mosquitoes using human subjects with caged mosquitoes. The more promising of the oils were also studied for their repellent activities against *Culex quinquefasciatus* and *Anopheles dirus*.

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MATERIAL AND METHODS

Essential oils. The names and sources of 38 essential oils are presented in Table 1. *Ageratum conyzoides* (leaves and flowers), *Spilanthes acmella* (flowers), *Vitex negundo* (leaves) and *Zanthoxylum limonella* (seed and fruit) were obtained from northern Thailand. Herbarium specimens were identified by a botanist and deposited at the Forest Herbarium National Park, Wildlife and Plant Conservation Department, Thailand. They were subsequently extracted for essential oils by steam distillation. About 1 kg at a time of fresh plant material was cut into a small pieces and placed in a distillation flask with approximately three times as much water and 8–10 glass beads. The distillation chamber was heated to about 120 °C and allowed to boil until the distillation was completed. The distillate was collected in a separating funnel with which the aqueous portion could be separated from the oil. The yield of each essential oil is shown in Table 2. These oils were kept at 4 °C until they were tested for mosquito repellency.

Apart from the oils extracted from plant material as specified above, another 32 essential oils were purchased from the TCF Co. (Bangkok, Thailand).

Mosquitoes. The mosquito species tested were *Ae. aegypti*, *An. dirus* and *Cx quinquefasciatus*. These mosquitoes were uninfected laboratory strains and were reared in the insectary of the Insecticide Research Unit at the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University. The methods

Table 1. Name and source of essential oils

Scientific name	Family	English name	Thai name	Source
Ageratum conyzoides –	Asteraceae	–	Sap rang sap ka	Lab extract
Allium sativum – L.	Alliaceae	Garlic	Kra tearm	Commercial
A. tuberosum – Roxb.	Alliaceae	Oriental garlic	Kui chay	Commercial
Apium graveolens Linne	Umbelliferae	–	Khoun chay	Commercial
Boesenbergia pandurata Roxb Schltr	Zingiberaceae	–	Ka chai	Commercial
Canagium odoratum – Baill. Ex King	Annonaceae	Ylang Ylang	Kar dung nga	Commercial
Cedrus deodara	Pinaceae	Cedar	–	Commercial
Citrus hystrix – Dc.	Rutaceae	Leech lime	Ma krood	Commercial
C. reticulata – Blanco	Rutaceae	Tangerine orange	Soam keuw kwan	Commercial
Cupressus funebris	Cupressaceae	–	Opp chey cheen	Commercial
Curcuma longa – L.	Zingiberaceae	Turmeric	Kha min chan	Commercial
Cymbopogon citratus – (Dc.) Stapf	Poaceae	Lemon grass	Ta kai	Commercial
C. nardus – (L.) Rendle	Poaceae	Citronella grass	Ta kai hom	Commercial
Eucalyptus globulus – Labill.	Myrtaceae	Eucalyptus	Eucalyptus	Commercial
Lavandula angustifolia	Lamiaceae	Lavender	–	Commercial
Litsea cubeba	Lauraceeae	Cubeba	–	Commercial
Mentha arvensis – L.	Apiaceae	Japanese mint	–	Commercial
Mentha piperita – L.	Apiaceae	Pepper mint	–	Commercial
M. spicata – L.	Apiaceae	Spear mint	–	Commercial
Myristica fragrans – Houtt.	Myristicaceae	Nutmeg	Chan tade	Commercial
Ocimum basilicum – L.	Apiaceae	Sweet basil	Ho la pa	Commercial
O. sanctum – L.	Apiaceae	Holy basil	Ka prow	Commercial
Pelargonium graveolens	*Geraniaceae*	Geranium	–	Commercial
Pimpinella anisum	Umbelliferae	–	–	Commercial
Pinus sylvestris – L.	Pinaceae	–	Soan	Commercial
Piper betle – L.	Piperaceae	Betel pepper	Ploo	Commercial
P. nigrum – L.	Piperaceae	Black pepper	Prik tai	Commercial
Pogostemon cablin – Blanco	Apiaceae	Patchouli	Pim sane bai	Commercial
Sesamum indicum – L.	Pedaliaceae	Sesame	Nga	Commercial
Spilanthes acmella – (L.) Murr.	Asteraceae	Para cress	Pak kard hou wan	Lab extract
Syzygium aromaticum – (L.) Merr.	Myrtaceae	Clove	Khan ploo	Commercial
Vetiveria zizanioides – Nash	Poaceae	Vetiver	Yar fak hom	Commercial
Vitex negundo – L.	Labiatae	Indian privet	Khon tee kha mow	Lab extract
Zanthoxylum limonella – Alston	Rutaceae	–	Makaen, kam jad torn	Lab extract
Zingiber officinale – Roscoe	Zingiberaceae	Ginger	Khing	Commercial
Z. purpureum – Roscoe	Zingiberaceae	–	Plai	Commercial

Table 2. Description of 5 essential oils that were obtained from steam distillation in the laboratory

Plant	Collection place and date	Part used	Fresh plant (g)	Essential oil (g)	% Yield (w/w)
A. conyzoides	Chaing Rai, 22 January 2002	Leaves	1000	1.25	0.13
A. conyzoides	Chaing Rai, 23 January 2002	Flowers	950	1.14	0.12
S. acmella	Chaing Rai, 26 January 2002	Flowers	1200	1.68	0.14
V. negundo	Bangkok, 10 March 2002	Leaves	1000	1.10	0.11
Z. limonella	Chaing Rai, 10 February 2002	Seed	1000	20.80	2.08
Z. limonella	Chaing Rai, 12 February 2002	Fruit	1000	125.00	12.50

for mass rearing were slight modifications of the procedure described by Limsuwan *et al.* (1987).

Subjects. This study used three human subjects who agreed to take part in testing the repellency of each kind of oil.

Repellent assay. The repellency of the essential oils was evaluated by using an arm-in-cage test (Schreck and McGovern, 1989; WHO, 1996). Each oil was tested undiluted and also was diluted with 70% alcohol to 10% and 50% concentration. An arm was covered with a rubber sleeve with a 3 × 10 cm window and 0.1 mL of a 10% or 50% concentration or undiluted oil was applied. The treated arm was exposed for 1 min to 250 hungry female mosquitoes, 4–5 days old. Every 30 min after treatment the treated arm was re-exposed to mosquitoes and the time was recorded at which the first bite occurred. Following the method of Schreck and McGovern (1989), the arm exposure at 30-min intervals continued until two bites occurred and one further exposure was made to check that complete repellency had indeed failed. The duration (min) of complete repellency after application of repellent was used as a measure of the repellency of the essential oils. The arm treated with the solvent used for the essential oil was used as the control. This control arm was exposed before the start of each assay. The essential oils that provided the longest complete protection time were tested against *An. dirus* and *Cx quinquefasciatus* by the same methods.

RESULTS

The results of the initial screening tests showing the repellent activity of 38 essential oils from plants are given in Table 3.

Table 3. Repellent activity of 38 essential oils (undiluted or as 10% or 50% dilutions) against *Ae. aegypti* mosquitoes

	Duration (min) of complete repellency			
Oil	10%	50%	Undiluted	Control[a]
A. conyzoides (leaf)	0,30,30; (20)	30,30,30; (30)	60,60,60; (60)	0
A. conyzoides (flower)	0,0,30; (10)	30,30,30; (30)	30,30,60; (40)	0
A. sativum	0,0,30; (10)	30,30,60; (40)	60,60,90; (70)	0
A. tuberosum	0	0	0	0
A. qraveolens	0	60,60,60; (60)	30,30,60; (40)	0
B. pandurata	0	0	30,30,30; (30)	0
C. ordoratum	0	0	30,30,30; (30)	0
C. deodara	0	0	0	0
C. hystrix	0,0,30; (10)	30,30,30; (30)	60,60,60; (60)	0
C. reticulata	0	0	0	0
C. funebris	0	0	0,0,30; (10)	0
C. longa	0	0,0,30; (10)	0,0,30; (10)	0
C. citratus	0	30,30,30; (30)	30,30,30; (30)	0
C. nardus	0	60,60,60; (60)	120,120,120; (120)	0
E. globulus	0	0	30,30,30; (30)	0
L. angustifolia	0	0	0,0,30; (10)	0
L. cubeba	0	0	0	0
M. arvensis	0	30,30,30; (30)	30,60,60; (50)	0
M. piperita	0	0	30,30,30; (50)	0
M. spicata	0,0,30; (10)	30,30,30; (30)	30,30,30; (30)	0
M. fragrans	0	0	30,30,30; (30)	0
O. basillicum	0	0	60,60,90; (70)	0
O. sanctum	0	0,0,30; (10)	60,60,60; (60)	0
P. graveolens	0,0,30; (10)	30,30,60; (40)	30,60,60; (50)	0
P. anisum	0	0	0	0
P. sylvestris	0	30,30,60; (40)	60,60,60; (60)	0
P. betle	0	60,60,90; (70)	60,90,90; (80)	0
P. nigrum	0	0	90,90,90; (90)	0
P. cablin	0	60,60,60; (60)	120,120,120; (120)	0
S. indicum	0	0	0	0
S. acmella	30,30,30; (30)	0	30,30,30; (30)	0
S. aromaticum	30,30,30; (30)	60,60,90; (70)	120,120,120; (120)	0
V. zizaniodes	0	0,0,30; (10)	60,60,60; (60)	0
V. negundo	0	0	0,0,30; (10)	0
Z. limonella (seed)	30,30,30; (30)	60,90,90; (80)	90,90,120; (100)	0
Z. limonella (fruit)	30,30,30; (30)	60,90,90; (80)	120,120,120; (120)	0
Z. purpureum	0	0	60,60,60; (60)	0
Z. officinale	0	0	30,30,60; (40)	0

[a] In all cases many more than two bites were obtained on the untreated control arm during the first exposure just before the time of application of the repellent.
Duration of complete repellency (as defined in the methods section) recorded by three volunteers; (with mean of the three).

Table 4. Repellent activity of the promising essential oils in three concentrations against *Ae. aegypti, Cx quinquefasciatus* and *An. dirus* mosquitoes

Mosquito spp. and Oil	Duration (min) of complete repellency			
	10%	50%	Undiluted	Control[a]
Ae. aegypti				
C. nardus	0	30,60,60; (50)	120,120,120; (120)	0
P. cablin	0	60,60,60; (60)	120,120,120; (120)	0
Z. limonella	30,30,30; (30)	60,90,90; (80)	120,120,120; (120)	0
S. aromaticum	30,30,30; (30)	60,60,90; (70)	120,120,120; (120)	0
Cx quinquefasciatus				
C. nardus	30,30,60; (40)	60,90,90; (80)	90,90,120; (100)	0
P. cablin	60,60,60; (60)	60,90,120; (90)	150,150,150; (150)	0
Z. limonella	30,30,90; (50)	90,90,120; (100)	120,180,210; (170)	0
S. aromaticum	30,90,120; (80)	120,120,120; (120)	240,240,240; (240)	0
An. dirus				
C. nardus	30,30,60; (40)	30,30,30; (30)	60,60,90; (70)	0
P. cablin	30,90,120; (80)	90,120,150; (120)	150,180,180; (170)	0
Z. limonella	60,60,60; (60)	60,150,180; (130)	180,180,210; (190)	0
S. aromaticum	60,90,90; (80)	150,150,180; (160)	210,210,210; (210)	0

[a] In all cases many more than two bites were obtained from each mosquito species on the untreated control arm just before the tested arm was treated.
Duration of complete repellency (as defined in the methods section) recorded by three volunteers; (with mean of the three).

Of the essential oils tested, high concentrations of *C. nardus*, *P. cablin*, *S. aromaticum* and *Z. limonella* (fruit) were the most effective and provided at least 2 h complete repellency against *Ae. aegypti*. The protection times of these oils were less when they were diluted. At 50% concentration, *C. nardus, P. cablin*, *S. aromaticum* and *Z. limonella* (fruit) showed 50, 60, 70 and 80 min protection, respectively and, the repellent activity decreased to 30 min or less when diluted to 10%. Based on these results, *C. nardus*, *P. cablin*, *S. aromaticum* and *Z. limonella* (fruit) were further studied for effectiveness against two other mosquito species, *Cx quinquefasciatus* and *An. dirus* in comparison with *Ae. aegypti*. The results are presented in Table 4.

The undiluted oil showed the highest protection time in each case. Among the four kinds of oil tested, *S. aromaticum* demonstrated the longest protection time against all three species of mosquito and the order of potency based on the protection time was *Cx quinquefasciatus* > *An. dirus* > *Ae. aegypti*. The mean durations of protection from bites for *S. aromaticum* were 240, 210 and 120 min against *Cx quinquefasciatus*, *An. dirus* and *Ae. aegypti*, respectively. At a 50% concentration *S. aromaticum* provided 120 min of complete protection against both *An. dirus* and *Cx quinquefasciatus*. *P. cablin* and *Z. limonella* protected for 120 and 130 min, respectively, against *An. dirus*. The protection times of all oils at 10% concentration were less than 120 min against all three species of mosquito.

DISCUSSION

Repellency evaluation is preferably carried out using human subjects, as testing repellents on animals or artificial membranes may not give representative data of how the repellent will perform when applied to a human skin (Nicolaides *et al.*, 1968; Cockcroft *et al.*, 1998). Our studies evaluated the repellent activities of 38 oils against *Ae. aegypti* mosquitoes which are anthropophilic, are easy to rear under laboratory conditions and are avid biters. The tests showed that of 38 undiluted essential oils, the most effective were extracted from *C. nardus*, *P. cablin*, *S. aromaticum* and *Z. limonella* which provided complete repellency for 120 min. The results in the reports of United States Department of Agriculture (1952–1964) also documented the complete repellency of *S. aromaticum* and *C. nardus* for 120 min against *Ae. aegypti*.

According to the recommendation of the US Environmental Protection Agency (2003), using *Ae. aegypti* along with a representative human biting species from both the *Anopheles* and *Culex* genera for the laboratory studies of repellent efficacy can provide information on the difference in response of the main vector genera of mosquitoes. Rutledge *et al.* (1983) showed that patterns of sensitivity to repellent compounds varied between mosquito genera. Their experiments showed, for 31 repellents, that *Ochlerotatus* (*Ochlerotatus*) *taeniorhynchus* and *Cx pipiens* were significantly more sensitive than were *Ae. aegypti* and *An. albimanus*. Furthermore *Ae. aegypti*, the traditional test species for repellent studies, was an exceptionally poor predictor for the responses of *An. stephensi* to repellents. Curtis *et al.* (1987) showed that *Anopheles* mosquitoes were less sensitive to DEET and other repellent chemicals than *Ae. aegypti*. The present results showed that of the 38 oils tested, the undiluted oil of *C. nardus*, *P. cablin* and *Z. limonella* provided better protection against *Ae. aegypti, Cx quinquefasciatus* and *An. dirus*. The mean duration of repellency of *S. aromaticum* oil was slightly greater than from the other three oils against *Cx quinquefasciatus* (240 min) or *An. dirus* (210 min).

For oils manifesting mosquito repellency, the protection time generally increased with increasing oil concentration. None of the oils prevented mosquito biting for as long as 120 min when used at 10% or 50% concentration. It was reported by Li *et al.* (1974) (and summarized in English by Curtis *et al.* (1989)) that, against *Ae. aegypti*, Lemon Eucalyptus oil has a protection time of only 1 h but Table 4 showed that *P. cablin*, *S. aromaticum* and *Z. limonella* oils gave 2 h repellency (Table 4). However, Li *et al.* (1974) showed that the waste distillate of Lemon Eucalyptus contained an active repellent *p*-menthane diol (PMD) and he found that a 15% concentration of PMD obtained from Lemon Eucalyptus oil distillation showed 4.4 h protection. This was better than 10% of *P. cablin*, *S. aromaticum* and *Z. limonella* oils which gave no more than half an hour protection against *Ae. aegypti* (Table 4). A 50% concentration of PMD gave 13 h protection against this species (Li *et al.*, 1974). At a range of concentrations applied to humans, the protection time of PMD against *Ae. aegypti* was proportional to the amounts applied and was definitely higher than the protection time of the oils of *P. cablin*, *S. aromaticum* and *Z. limonella*.

For possible use by low-income rural communities, where the highest incidence of mosquito-borne diseases are reported, our studies have added the cheaply available *P. cablin*, *S. aromaticum* and *Z. limonella* to the list of effective plant based repellents.

Citronella from *C. nardus* belongs to the genus *Cymbopogon* which yield the most popular repellents in the world. In South Africa, *C. excavatus* gave 100% repellency for 2 h, when it was evaluated in the laboratory against *An. arabiensis* and its repellency decreased to 59.3% after 4 h (Govere *et al.*, 2000). In Thailand, *C. winterianus* oil, mixed with 5% vanillin, gave 100% protection for 6 h against *Ae. aegypti*, *Cx quinquefasciatus* and *An. dirus* and compared favourably with 25% DEET (Tawatsin *et al.*, 2001). The pure oil of *C. martinii martinii* (palmarosa) provided 100% repellency for 12 h against *Anopheles* mosquitoes in a field trial which was carried out by using pairs of volunteers who sat together, one of whom was treated with the oil and other was not (Ansari and Razdan, 1994).

These plants contain varying amounts of several insect repellent chemicals although environmental conditions cause the content of volatile oils in plants to vary greatly. The repellent compounds contained in this group include alpha pinene, camphene, camphor, geraniol and terpenen-4-ol. The most abundant repellent molecules found in the group are citronellal, citronellol and geraniol (Duke, 2000). Buescher *et al.* (1982a) and Rutledge *et al.* (1983) found a synthetic derivative of citronella (a mono-terpene aldehyde), is the main constituent of citronella oil and has been used as the active ingredient of commercial repellents. In addition, their high citronellal content makes the plants of this genus potential candidates for PMD production since citronellal is a precursor of this molecule. The grasses grow readily and rapidly throughout much of the tropics and a simple steam distillation is sufficient to extract the repellent fractions. The plants in this genus are pleasant smelling and are widely used in traditional medicine.

S. aromaticum or 'clove oil' was reported the most effective mosquito repellent in the comparison made by Barnard (1999) and in the present study. Barnard showed that this oil gave 90 to 225 min of protection against *Ae. aegypti* and 75 to 213 min of protection against *An. albimanus*, depending on oil concentration. The major constituents of clove oil are eugenol, eugenol-acetate and beta-caryophyllene (Leung and Foster, 1996). Eugenol is repellent to *Ae. aegypti* (USDA, 1954) and *An. gambiae* (Chogo and Crank, 1981). Neither eugenol-acetate nor beta-caryophyllene are repellent to *Ae. aegypti* (USDA, 1954), but neither has been tested for repellency to *Anopheles* mosquitoes. Eugenol also acts as an antioxidant in oleogenous foods, as an anticarminative, antispasmodic and antiseptic in pharmacy, and as an antimicrobial agent (Farag *et al.*, 1989a, 1989b). Clove oil is used in oriental medicine as a vermifuge, and as an antibacterial and/or antifungal agent (Awuah and Ellis, 2002; Dorman and Deans, 2000). Miyazawa and Hisama (2001) reported that a methanol extract from clove showed an antimutagenic effect. Clove oil is very widely used in clinical dentistry in root canal therapy and temporary fillings, and exhibits an antimicrobial activity against oral bacteria that are commonly associated with dental caries and periodontal disease (Cai and Wu, 1996).

For *P. cablin*, there has been no previous report of mosquito repellent activity. This plant has been used against the common cold and as an antifungal agent in traditional medicine. It is cultivated extensively in Indonesia, Malaysia, China and Brazil for its essential oil (patchouli oil), which is important to the perfumery industry. This oil contains many mono- and sesqui-terpenoids, and several flavonoids and alkaloids (Tsubaki *et al.*, 1967; Hikino *et al.*, 1968; Terhune *et al.*, 1973; Itokawa *et al.*, 1981). *P. cablin* has as strong an antimutagenic effect as *S. aromaticum* (Miyazawa *et al.*, 2000) and has antibacterial activity (Osawa *et al.*, 1990) and is used for the prevention of emphysema in the convalescent stage (Fu, 1989).

For *Z. limonella*, there is only one current report from India that showed the oil gave a protection time of 4–5 h against *Ae. albopictus* mosquitoes (Das *et al.*, 2003). There are no previous publications showing its mosquito repellent activity against *Ae. aegypti*, *Cx quinquefasciatus*, *An. dirus* and *Ma. uniformis*. This plant is mentioned in the website www.indmedplants-kr.org/Zanthoxylum_limonella.htm as some members of this genus have an insecticidal effect. A yield of 12.5% (w/w) of essential oil was obtained with this species which is higher than that of the other plants studied (Table 2) and is likely to make *Z. limonella* more cost effective than the other three plants. Itthipanichpong *et al*. (2002) reported the chemical compositions of the essential oil distilled from the fruit of *Z. limonella* in Thailand and found the presence of 33 chemical components. Limonene (31.1%), terpin-4-ol (13.9%) and sabinene (9.1%) were found to be the major components. They also reported that the essential oil from the fruit of this plant possessed a stimulatory effect on smooth muscle preparations by non-specific mechanisms.

These initial results clearly demonstrated that the essential oils from *P. cablin*, *S. aromaticum* and *Z. limonella* plants performed as mosquito repellents about as equally well as citronella oil. As indicated above, these oils are used in medicine, perfumery and flavouring of food and are considered non-toxic to humans and are environmentally friendly.

Further studies are needed to develop appropriate formulations including a fixative, which would increase their efficacy and cost effectiveness. Field trials should be carried out, particularly to evaluate the operational feasibility and dermal toxicity over a long period, especially to infants and children. It is important to determine whether widespread use of one of these repellents would produce an overall reduction of vector biting in a community or would simply divert biting from repellent users to non-users.

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