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Repellent activity of selected plant essential oils against the malarial fever mosquito *Anopheles stephensi*

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Abstract. In recent years, use of environment friendly and biodegradable natural insecticides of plant origin have received renewed attention as agents for vector control. In this study, essential oils extracted by steam distillation from leaves of five plant species *Centella asiatica* L., *Ipomoea cairica* L., *Momordica charantia* L., *Psidium guajava* L. and *Tridax procumbens* L. were evaluated for their topical repellency effects against malarial vector *Anopheles stephensi* in mosquito cages. All essential oils were tested at three different concentrations (2, 4 and 6%). Of these, the essential oils of *I. cairica*, *M. charantia* and *T. procumbens* exhibited relatively high repellency effect (>300 minutes at 6% concentration), followed by *C. asiatica* and *P. guajava* which showed less effective (<150 minutes at 6 % concentration). However, the ethanol applied arm served as control provided maximum 8.0 minutes repellency in this study. In general, clear dose–response relationships were established in all essential oils, with the highest concentration of 6% provided high repellency effect. The results obtained from this study suggest that essential oils of *I. cairica*, *M. charantia* and *T. procumbens* are promising as repellents at 6% concentration against *An. stephensi* and could be useful in the search for new natural repellent compounds.

INTRODUCTION

Anopheles stephensi Liston (Diptera: Culicidae) is a major vector in India as well as in some of the West Asian countries and has been shown to be directly responsible for about 40-50% of the annual malarial incidence (Curtis, 1994; Collins & Paskewitz, 1995). Globally, malaria kills 3 million person each year, including 1 child every 30 seconds (Shell, 1997). The search for effective vaccines against malaria is still in progress. Personal protective measures, including repellents are widely used to prevent the transmission of arthropod – borne diseases by minimizing contact between humans and vectors. The most common mosquito repellent formulations available on the market contain DEET (N,N-diethyl-3-methylbenzamide), which has shown excellent repellency against mosquitoes and other biting insects (Coleman *et al.*, 1993; Walker *et al.*, 1996). However, human toxicity reactions after the applications of DEET vary from mild to severe (Qui *et al.*, 1998). To avoid these adverse effect, research on repellents that are derived from plant essential oil to replace DEET. Botanical repellents are promising in that they are effective, safe to users, and also inexpensive (Senthil Nathan *et al.*, 2004).

Essential oils are natural volatile substances found in a variety of plants. Commercially, essential oils are used in four primary ways: as pharmaceuticals, as flavor enhancers in many food products, as odorants in fragrances, and as insecticides. Particular emphasis has been placed on their antibacterial, antifungal and insecticidal activities (Chang *et al.*, 2001; Chang & Cheng, 2002). Recently, extracts of several plants including neem (*Azadirachta indica* A. Juss), Citronella grass (*Cymbopogan nardus* Rendle), basil (*Ocimum basilicum* L., *Ocimum gratissimum* L., *Ocimum americanum* L.), clove (*Syzygium aromaticum* L.), prickly straggler (*Solanum trilobatum* L.), musk basil (*Moschosma polystachyum* L.) and thyme (*Thymus vulgaris* L.) have been studied as possible mosquito repellents (Chokechaijaroenporn *et al.*, 1994; Suwonkerd & Tantrarongraj, 1994; Rajkumar & Jebanesan, 2004, 2005). Therefore, the purpose of this study is to determine the repellent activity of essential oils from five plants against the malarial fever mosquito *An. stephensi*.

MATERIAL AND METHODS

Plant procurement and essential oils

Fresh leaves of experimental plants (Table 1) were collected from medicinal plant garden at Sivapuri village, in the district of Cuddalore, Tamil Nadu, India, between January and March 2005. After botanical identification, voucher specimens were deposited in the Department of Botany, Annamalai University, India. At least 10 kg of leaves of each experimental plant was extracted for essential oils by steam distillation. One kg of fresh leaves of each plant at a time was cut into small pieces and placed in a distillation flask with approximately five times as much water. The distillation chamber was heated at about 120°C and allowed to boil until the distillation was completed. The distillate was collected in a separating funnel in which the aqueous portion was separated from the essential oil. This procedure was repeated until at least 20 ml of oil had been recovered. The essential oil was collected and kept in amber coloured vials at 4°C until it was tested for mosquito repellency.

Mosquito culture

Adult *An. stephensi* were obtained from a laboratory colony maintained at 27±2°C, 70-80% relative humidity under 14:10 light and dark photo period cycle. Larvae were fed with dog biscuits and yeast powder in the ratio of 3:1. Adults were provided with 10% sucrose solution and were periodically blood fed on restrained 5-7 week – old chicks. Repellency assays were performed with 6-8 days old female *An. stephensi* that had been starved for 18h, but previously fed on 10% sucrose solution.

Repellency tests

The essential oils were evaluated for their repellent activities against *An. stephensi* using the human – bait technique (Fradin & Day, 2002). First, each essential oil was diluted in ethanol and prepared 2,4 and 6% concentrations. For each test, 10 disease free, laboratory-reared female mosquitoes were placed into separate laboratory cages (45 x 38 x 38 cm). Before each test, the volunteer's skin was washed with unscented soap and the tested essential oil was applied from the elbow to the finger tips. In each cage one arm was inserted for one test concentration and the other arm applied with ethanol served as control. The treated and control arms were interchanged regularly to eliminate bias. Each test concentration was repeated five times and in each replicate subject different volunteers to nullify any effect of colour of the skin on repellent.

Volunteers were asked to follow the testing protocol. Volunteers conducted their test of each concentration by inserting the treated and control arms alternatively into a same cage for one full minute for every five minutes. If they were not bitten within 20 minutes, then the arms were reinserted for 1 full minute for every 15 minutes, until the first bite occurred.

RESULTS AND DISCUSSION

Essential oils obtained from plants have been studied for use as natural repellents instead of DEET containing synthetic repellents. Table 1 shows the major chemical constituents and the yields of five essential oils distilled from leaves of five plants. The results indicated that the yields of essential oils ranged from 1.7 to 3.8 ml/kg. The yields of *Centella asiatica*, *Ipomoea cairica*,

Table 1. Essential oils from leaves of five plants tested for mosquito repellent activity

Plant species	Common name(s)	Family	Oil yield (ml/kg)	Major chemical constituents	Source
Centella asiatica	Indian penny wort	Apiaceae	1.7	Farnesene, Caryophyllene, P-cymol	(Fatope, 1993)
Ipomoea cairica	Morning glory Railway Creeper	Convolvulaceae	1.8	P-cymene and Carvone	(Roth *et al.*, 1998)
Momordica charantia	Bitter melon Bitter gourd	Cucurbitaceae	2.8	Momordenal, Momordicins, Momordicilin and Erythrodiol	(Takemoto, 1982)
Psidium guajava	Guava	Myrtaceae	1.9	Caryophyllene, a-Pinene and Eucalyptol	(Santos *et al.*, 1998)
Tridax procumbens	Coat buttons Tridax daisy	Asteraceae	3.8	α-Terpinene, α-Terpineol and β-Pinene (19)	(Tyagi *et al.*, 1998)

Momardica charantia, *Psidium guajava* and *Tridax procumbens* were 1.7, 1.8, 2.8, 1.9 and 3.8 ml/kg, respectively. Many papers on the yield and chemical constituents of essential oils have been published. The yield and chemical constituents, however, show much discordance between the same essential oil. The reasons for this variability due to climatic, seasonal and geographic conditions, harvest period and distillation technique, among others (Panizzi *et al.*, 1993). In this study, the discordance in yield between the same essential oil was not shown because above factors were kept in mind during the collection and distillation of leaves.

The relative repellency of five essential oils against *An. stephensi* under laboratory condition is given in Table 2. Among the five essential oils, *I. cairica*, *M. charantia* and *T. procumbens* provided repellency of 332.0, 323.0 and 317.0 minutes at 6% concentration, respectively, while *C. asiatica* and *P. guajava* gave only repellency of 140.0 and 119.0 minutes, respectively. At lowest concentration of 2%, the essential oils of *C. asiatica*, *I. cairica*, *M. charantia*, *P. guajava* and *T. procumbens* showed repellency of 62.0, 158.0, 143.0, 56.0 and 140.0 minutes, respectively. In the repellency results, increase in the concentration of the essential oils from 2% to 6% were found to increase the repellency time. There were significant differences in repellency among the different concentrations of each essential oil against *An. stephensi*. On the other hand, the ethanol applied arm served as control provided maximum 8.0 minutes repellency in this study. Among the five essential oils, *I. cairica*, *M. charantia* and *T. procumbens* provided more than 5 h repellency at 6% concentration, while *C. asiatica* and *P. guajava* gave repellency of less than 2.30 h. The essential oils of five plants used in this study did not cause skin irritation, hot sensations or rashes on the arms of the test volunteers during the study period. The results obtained in this work indicate that three of five essential oils which were distilled from *I. cairica*, *M. charantia* and *T. procumbens* showed promising repellent activity against *An. stephensi*. Nowadays, the use of plant essential oils in mosquito repellent is an alternative personal protective measures against the noxious effects of synthetic repellents on the users and environment [Thorsell *et al.*, 1998]. Unlike synthetic repellents, the literature on mosquitocidal botanical agents does not contain any evidence to suggest that resistance to these substances has emerged. This is most likely not due to any reason of consequence but because botanical agents are not often used in vector control (Shaalan *et al.*, 2005) and also they are mixtures of various related compounds with different modes of action and hence the development of resistance to such products is somewhat difficult (Mulla & Su, 1999).

In conclusion, the identification of these potential repellent plants from the local flora will generate local employment and

Table 2. Repellent activity of selected plant essential oils against *An. stephensi*

Plant species	Concentration (%)	Complete – protection time (min)	
		Treated	Control
Centella asiatica	2	62.0 ± 2.2a	7.0 ± 0.5
	4	107.0 ± 2.2b	8.0 ± 0.5
	6	140.0 ± 2.4c	8.0 ± 0.5
Ipomoea cairica	2	158.0 ± 2.4a	8.0 ± 0.5
	4	248.0 ± 3.5b	7.0 ± 0.5
	6	332.0 ± 3.7c	8.0 ± 0.5
Momordica charantia	2	143.0 ± 2.4a	8.0 ± 0.5
	4	236.0 ± 3.5b	7.0 ± 0.5
	6	323.0 ± 3.5c	8.0 ± 0.5
Psidium guajava	2	56.0 ± 1.5a	7.0 ± 0.5
	4	89.0 ± 2.2b	8.0 ± 0.5
	6	119.0 ± 2.2c	8.0 ± 0.5
Tridax procumbens	2	140.0 ± 2.4a	7.0 ± 0.5
	4	227.0 ± 3.1b	8.0 ± 0.5
	6	317.0 ± 3.5c	8.0 ± 0.5

Each value (mean ± SE) represents mean of five values. Values with different letters in a column are significantly different at P<0.05 level (Tukey's test).

stimulate local efforts to enhance public health. Further investigations are needed to elucidate the five essential oils against a wide range of mosquito species and also to identify active compound(s) responsible for repellent activity and to be utilized if possible, in preparing a commercial product / formulation to be used as mosquitocidal.

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