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Adulticide efficacy of essential oil from *Piper retrofractum* Vahl against *Aedes aegypti* and *Culex quinquefasciatus*

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Abstract. Essential oil was extracted from the fresh fruit of *Piper retrofractum Vahl* by water distillation method. The yield of extraction was estimated at 0.125%. Adulticidal activity was tested against 2-5 days-old non-blood-fed female *Aedes aegypti* and *Culex quinquefasciatus* by topical application. Six concentrations of essential oil dissolved in acetone were prepared in 5%, 7%, 9%, 11%, 13% and 15%. The solution (0.5 µL) was on the pronotum of mosquito, and mortality was observed after 24 hours of exposure. The LD_{50} and LD_{99} of essential oil in acetone against *Ae. aegypti* (8.86%, 23.21%) and *Cx. quinquefasciatus* (6.95% and 17.35%,) was measured. This is the first report of adulticidal activities of essential oil from *P. retrofractum* against mosquito vectors.

INTRODUCTION

Mosquito vectors cause health problems in almost all regions of the world, and global warming has contributed to their growing numbers by increasing their habitable environments. The use of chemical insecticides is an effective and popular method to control their numbers. However, particularly in urban areas, these can result in an increase of insecticide resistance development in insect vectors (Gakhar *et al.*, 2013). Insecticides from plants are an alternative way to control insect vectors, and the high biodiversity of plants in the tropics means that there is a good possibility that some plants can be developed into a new generation of insecticide.

The Piperaceae family, plants with a spicy flavor, contains roughly 3,920 species in 13 genera. There are two main genera, genus Piper which contains 2,000 species, and genus Peperomia which contains 1,600 species (Stevens, 2001). The plant is commonly found in South East Asia and has been used in cooking and medicine for a long time (Khaewphrom, 2008). Many plants in the Piperaceae family have been studied for their adulticidal activity, such as *Piper nigera* (Newaz *et al.*, 2011), *Piper longum* (Chaiyasit *et al.*, 2006; Choochote *et al.*, 2006) and *Piper aduncum* (Hidayatulfathi *et al.*, 2004). The major chemical constituents in the plants' essential oils are Beta-sitosterol, Cineol terpinan-4,1-betacaryophylene, Piperodione, Sitisterol and Venerol (Banerji, 2002; Kubo, 2013). During the past decade, many studies have been conducted about the adulticidal activities of medicinal plants against mosquito vectors. Essential oils were extracted from several plants that have adulticidal activity against mosquito vectors, such as *Polygonum hydropiper* (Maheswaran & Ignacimuthus, 2013) and *Lantana camara* (Dua *et al.*, 2010). The plants in Piperaceae family such as *Piper nigera* (Newaz *et al.*, 2011) and *Piper longum* (Chaiyasit *et al.*, 2006; Choochote *et al.*, 2006) were also studied for adulticidal effect on other insects. However, only the essential oil from *P. longum* has been studied for adulticidal activity against mosquito

vectors (Chaiyasit *et al.*, 2006). The essential oil extracted from *Piper retrofractum Vahl* is spicy and has a pungent odor (Choochote *et al.*, 2006), and the fruit of this plant is cheap and available in local markets across Thailand. Therefore, this study investigated the adulticide activity of the essential oil from *P. retrofractum Vahl* against mosquito vectors.

MATERIAL AND METHODS

Plant Collection and Preparation

The fruits of *P. retrofractum* were collected from the Thami district, Chanthaburi province, Thailand during April – May 2014. The plant specimens were identified by Dr. Chalermphol Suwanphakdee from Kasetsart University, Thailand and the plants specimens were kept at Queen Sirikit Botanical Garden Mae-rim District, Chiangmai Province, Thailand as voucher specimen QBG No. 79271.

Essential Oil Preparation

One kilogram of the fresh fruits, size approximately 5 x 1 cm, was washed with tap water and air dried. They were ground in a blender machine and then 2 liters of distilled water was added to distillation apparatus. The essential oil was extracted for 3 hours by water distillation method and kept at 4ºC until used.

Mosquitoes

Female *Ae. aegypti* and *Cx quinquefasciatus* susceptable strains were used in this experiment. They were maintained at 28±2ºC at the Insecticide Research Unit, Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University.

Adult Bioassay

The adulticidal activity of essential oil extracted from *P. retrofractum* was determined by topical application against *Ae. aegypti* and *Cx. Quinquefasciatus*, following a slightly modified WHO protocol (WHO, 2006). The dosage is expressed in percent of essential oil in acetone. Six concentrations were prepared in 5%, 7%, 9%, 11%, 13% and 15%. The non-blood fed female mosquitoes, aged 2-5 days, were anesthetized with ether and placed on a cold plate. A 0.5µl droplet of essential oil dissolved in acetone was applied on the pronotum of the mosquitoes by Berkard hand Microapplicator®. Mortality was observed after 24 hours of exposure. Fifty female mosquitoes were used for each concentration. There were two control groups, one acetone-treated group and one untreated group. The experiment was replicated three times.

Data Analysis

The Median Lethal Dose (LD_{50}) values of an essential oil against female *Ae. aegypti* and *Cx. quinquefasciatus* were analyzed by a probit analysis program (Finney, 1971).

RESULTS AND DISCUSSION

The essential oil from the fresh fruit of *P. retrofractum* is clear in color, has a sweet-spicy odor and is soluble in acetone. The yield of extraction was estimated at 0.125%, five times lower than that extracted by the same method from its closely related species *P. longum* (Choochote, *et al.*, 2006). There are several plants in the Piperaceae family containing essential oils with insecticidal activity against mosquito vectors, such as *Piper klotzschianum* (do Nascimrnto *et al.*, 2013) and *Piper marginatum Jacq* (Autranes *et al.*, 2009). This study investigated the efficacy of essential oil from *P. retrofractum* against adult mosquitoes. The mortality rate of *Ae. aegypti* and *Cx. quinquefasciatus* after 24 hour exposure with different percentage of essential oil showed in Table 1. The mortality of *Ae. aegypti* and *Cx. quinquefasciatus* at 15% was 92.7% and 98.7%, respectively. There was no mortality in the acetone control group and untreated groups in both mosquito species.

Table 2 shows that less than 20% of essential oil in acetone can kill over 90% of both *Ae. aegypti* and *Cx. quinquefasciatus. Cx. quinquefasciatus* was found to be more sensitive to the essential oil than *Ae. aegypti*

Table 1. The mortality rate of female *Aedes aegypti* and *Culex quinquefasciatus* after 24 hours exposure by topical application

Percent of essential oil in acetone	Number of tests	Mortality rate of female mosquito	
		Aedes aegypti	*Culex quinquefasciatus*
5	150	17.3	24.7
7	150	26.7	50.0
9	150	44.0	70.7
11	150	65.3	84.0
13	150	80.0	90.7
15	150	92.7	98.7
Acetone treated	150	0	0
Untreated	150	0	0

Table 2. The LD_{50} LD_{90} LD_{99} of *Piper retrofractum Vahl* essential oil against female *Aedes aegypti* and *Culex quinquefasciatus* after 24 hour exposure by topical application

Species	Lethal Dose (%) (95% C.I.)		
	LD_{50}	LD_{90}	LD_{99}
Aedes aegypti	8.9 (8.1- 9.5)	16.2 (14.4-18.0)	23.2 (20.4-24.5)
Culex quinquefasciatus	7.0 (6.6-7.5)	12.3 (10.2-13.4)	17.4 (13.3-19.8)

with LD_{50} values (Table 2). A previous study by Choochote *et al*. (2006) showed the adulticidal effects of essential oil extract from *P. longum* against *Ae. aegypti* females in laboratory and field strains being LD_{50} of 6.21 and 6.35 µg/mg. This closely related species appears to give a higher adulticidal activity than our study of *P. retorfractum* (LD_{50} = 8.9% is equivalent to 16.44 µg/mg). The essential oil extracted from *P. retrofractum* is suitable to control the polluted breeder species, *Cx quinquefasciatus.* The application in the form of spraying formulation can be used as household insecticide. It requires further study against other mosquito species such as *Ae. albopictus*, *Cx. tritaeniorhynchus*, *Anopheles* spp. and agricultural insects to develop a new commercial insecticide in the future. The development and synthesis of these compounds can be one candidate for a new generation of insecticides.

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