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ADULTICIDAL ACTIVITY AGAINST *Stegomyia aegypti* (DIPTERA: CULICIDAE) OF THREE *Piper* spp.

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SUMMARY

Three *Piper* species, *Piper longum*, *P. ribesoides* and *P. sarmentosum*, were selected for investigation of adulticidal potential against *Stegomyia aegypti*, a main vector of dengue and dengue haemorrhagic fever. Successive extraction by maceration with 95% ethanol showed percentage yields of ethanolic extracts, which derived from *P. longum*, *P. ribesoides* and *P. sarmentosum*, of 8.89, 3.21 and 5.30% (w/w), respectively. All *Piper* extracts illustrated an impressive adulticidal activity when tested against female mosquitoes by topical application. The susceptibility of *St. aegypti* females to ethanol-extracted *Piper* was dose dependent and varied among the plant species. The highest adulticidal effect was established from *P. sarmentosum*, followed by *P. ribesoides* and *P. longum*, with LD_{50} values of 0.14, 0.15 and 0.26 µg/female, respectively. The potential of these *Piper* species, as possible mosquitocides, established convincing activity for further researches to develop natural substances for combat against adult mosquitoes.

KEYWORDS: *Piper*; *Piper longum*; *Piper ribesoides*; *Piper sarmentosum*; *Stegomyia aegypti*; Adulticidal activity; Topical application.

INTRODUCTION

Stegomyia aegypti (redirected from *Aedes aegypti*)[18] mosquitoes are the primary carrier of dengue and dengue haemorrhagic fever in many developing countries in the tropical world[33]. In Thailand, annual dengue incidences are quite high and have been increasing and spreading recently to newer areas[10]. Like the case of malaria, a vaccine for dengue and dengue haemorrhagic fever is not yet available. Consequently, a reliable way to diminish the incidence of these diseases is to eradicate the mosquito vectors through continued applications of synthetic insecticides. However, the problems of high cost, toxic hazards to humans and other non-target creatures, and development of resistance to conventional insecticides in many mosquito species have revived great interest in exploiting the vector control potential of natural origins. Research and the development of mosquito control alternatives have focused on plant-derived constituents or phytochemicals[6,27,30,34].

Botanical insecticides may serve as suitable alternatives to synthetics in future, as they are relatively safe, degradable and readily available in many parts of the world[24]. Recently, plants in the family, Piperaceae, have been of interest because they contain insecticidal principles[2,9]. Little work has been carried out on mosquito adulticidal activity, although some *Piper* spp. and their bioactive constituents are reported to have noteworthy larvicidal potential against various species of mosquitoes such as *Culex pipiens pallens*, *Aedes aegypti*, *Ae. togoi* and *Ae. atropalpus*[8,13,14]. In a search for effective and affordable natural substances for use in the control of mosquito-borne diseases, three *Piper* spp. available in Thailand including *Piper longum*, *P. ribesoides* and *P. sarmentosum* were chosen to evaluate their adulticidal potential against the *St. aegypti* mosquito.

The Piperaceae family contains approximately 2,000 species, which are widely distributed and commonly used in tropical and subtropical regions as food, spice, traditional medicines and pest control agents[11,12,21]. *P. longum*, *P. ribesoides* and *P. sarmentosum*, widely grown throughout Thailand, have been locally known as De-plee, Ta-khaan and Cha-plu, respectively. These *Piper* species have been traditionally used in Thailand for food and medicinal purposes[16,19]. *P. longum* is reported as good remedy used for treatment of gonorrhea, menstrual pain, tuberculosis, sleeping problems, respiratory tract infections, chronic gut related pain and arthritic conditions[22]. Additionally, analgesic and diuretic effects, relaxation of muscle tension and alleviation of anxiety have also been reported[23]. *P. ribesoides* and *P. sarmentosum* are commonly used in folk medicine as a carminative. *P. sarmentosum* has also been used as an expectorant, and to relieve muscle pain[16,19]. Methanol extracts of *P. longum* fruit and *P. sarmentosum* root have been proved to possess the anti-amoebic effects against *Entamoeba histolytica* infecting the caecum of mice[20]. *P. longum* and its component piperine have accounted as a non-toxic immunomodulator which possess antitumor property also[28]. The water extract of whole plant of

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P. sarmentosum has been noted for a hypoglycemic effect in rats[15]. Brachyamide B and 3',4',5'-trimethoxycinnamoyl pyrrolidine, bioactive compounds derived from *P. sarmentosum* fruits, have been reported to exert antituberculosis activity and antiplasmodial potential against *Plasmodium falciparum* parasite[17].

MATERIALS AND METHODS

Plant preparation and extraction: Three *Piper* species of the Piperaceae family, *Piper longum* Linn., *Piper ribesoides* Wall. and *Piper sarmentosum* Roxb. were obtained from E.A.R. Samunpri, a traditional herb supplier in Chiang Mai province, Thailand. Taxonomic identification of plants was performed by J.F. Maxwell, botanists at the CMU Herbarium, Department of Biology, Faculty of Science, Chiang Mai University, Thailand. The voucher specimens (PARA-PI-001/1, PARA-PI-002/1, and PARA-PI-003/1, respectively) were preserved at the Department of Parasitology, Faculty of Medicine, Chiang Mai University. Dried material of each plant (1 kg) was crushed and successively extracted three times by maceration, with 3 L of 95% ethanol at room temperature for seven days. The ethanolic extracts were separated by suction filtered through a Büchner funnel. Solvent in combined filtrates was removed by rotary evaporator at temperatures below 60 °C until the extract was rather concentrated. The resulting crude extracts were transferred to an open container set on a hot plate and the residual solvent was removed for at least 24 hours. The ethanolic extract of each plant was thus obtained, lyophilized and then refrigerated at -20 °C until testing for adulticidal activity.

Test mosquitoes: Laboratory colonies of *Stegomyia aegypti*, which originated from larvae collected at various places in Chiang Mai province, northern Thailand, had been reared continuously for several generations in a laboratory free of exposure to pathogens and insecticides. They were maintained at 25-30 °C and 80-90% relative humidity under a photoperiod of 14:10 h (light/dark) in the insectarium of the Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai province. Larvae were fed on a ground commercial dog biscuit. The adults were reared in humidified cages and supplied with 10% sucrose and 10% multivitamin syrup. Female mosquitoes were periodically blood-fed on restrained rats to obtain protein used principally for egg production. Under these conditions, the full development from egg to adult lasted about 3-4 weeks. Batches of 2-5 day-old healthy female mosquitoes were used in the adulticidal bioassay.

Adulticidal bioassay: The adulticidal activity of the *Piper* extracts was determined by topical application of the insecticide to the adult female mosquitoes (Fig. 1), following slightly modified versions of the WHO standard protocols[32]. Each plant extract was dissolved in acetone yielding a graded series of concentrations. Non-blood fed females were briefly anaesthetized with carbon dioxide (CO_2), weighed and placed on a cold plate. Treatment was performed with the aid of a dissecting microscope. A 0.1 µL droplet of plant solution in acetone was applied onto the upper part of the immobilized mosquito's pronotum using a Hamilton's digital syringe (700 series MICROLITER™, Hamilton Company, USA). Dosages were expressed in the µg of plant material per mg of mosquito body weight. A total of 25 individuals was used at each concentration, with 4-6 concentrations providing a range of 0-100% mortality. Controls were divided into two groups including acetone treated and untreated groups. Both groups were treated in a similar manner to that of *Piper* treated groups. After application, the females in all groups were maintained at 27 ± 3 °C and 80% ± 10% RH in plastic cups, with 10% sucrose and 10% multivitamin syrup provided. At the end of a 24-hour recovery period, the mosquitoes were considered dead if they showed no sign of movement such as lying on the bottom of the plastic cup and not responding to mechanical stimulation. Eight replicates were carried out for each plant extract with mosquitoes from different rearing batches, and the results were pooled.

Fig. 1 - Topical application of the *Piper* extracts to *St. aegypti* female mosquitoes with the aid of a dissecting microscope.

Data management and statistical analysis: It was essential to obtain not less than three mortality counts of between 10% and 90%. Experimental tests that demonstrated more than 20% control mortality were discarded and repeated. However when the control mortality ranged from 5-20%, the observed percentage mortality (%M) was corrected by Abbott's formula[1]:

$$\%M = \frac{\%\text{ test mortality - }\%\text{ control mortality}}{100\text{- }\%\text{ control mortality}} \times 100$$

The 95% confidence intervals (CI) of the lethal dosage of 50% and 95% (LD_{50} and LD_{95}, respectively) calculated by a computerized

log-probit analysis (Harvard Programming; Hg1, 2) were used to measure differences between test samples.

RESULTS

Successive extraction by maceration with 95% ethanol showed the percentage yield of ethanolic extracts, which derived from *P. longum*, *P. ribesoides* and *P. sarmentosum* in relation to the starting dry material that averaged 8.89, 3.21 and 5.30% (w/w), respectively. These alcoholic extracts showed various strengths of odor and a high solubility in absolute ethanol, DMSO or acetone (Table 1). Investigating against female *St. aegypti* by topical application in this study revealed a pronounced toxic effect in all *Piper* extracts (Table 2). The susceptibility of *St. aegypti* females to a graded series of concentrations of each ethanol-extracted *Piper* was dose dependent. Following treatment with increasing concentrations of *P. longum*, *P. ribesoides* and *P. sarmentosum* from 0.10 - 0.50, 0.025-0.40 and 0.05-0.30 µg/mg female, respectively, the mortality values increased from 6.5-84.5, 6.0-97.5 and 3.0-89.5%, respectively. Comparison of adulticidal activity of these ethanolic-extracted peppers demonstrated that the highest potential was established from *P. sarmentosum*, followed by *P. ribesoides* and *P. longum* with LD_{50} values of 0.14, 0.15 and 0.26 µg/mg female, respectively. Although *P. sarmentosum* provided slightly better activity than *P. ribesoides*, no statistically significant difference was found, based on the overlapping of 95% confidence interval at LD_{50} values. No mortality was detected in any acetone treated or untreated groups.

Table 1
Botanical data, physical characters, solubility and percentage yields of ethanolic extracts of *Piper* spp.

Botanical name	Common name	Part used	Physical character			Solubility	Yield (%)
			Appearance	Color	Odor		
P. longum	Javanese long pepper	Fruit	Semi-solid	Dark brown	Sweet-spicy	Soluble in ethanol & acetone	8.89
P. ribesoides	Jakhang pepper	Wood	Semi-solid	Dark brown	Raw smell	Soluble in DMSO & acetone	3.21
P. sarmentosum	Yariegatum	Whole plant	Semi-solid	Dark green	Sweet-herbal	Soluble in DMSO & acetone	5.30

Table 2
Adulticidal activity of the ethanolic extracts derived from three *Piper* species against adult female *St. aegypti*

Piper species extract (µg/mg female)	% Mortality (Mean ± SE)	Adulticidal activity (95% C.I., µg/mg female)		
		LD_{50}	LD_{95}	LD_{99}
P. longum		0.26	0.71	1.27
0.10	6.5±1.41	(0.23-0.28)	(0.60-0.95)	(0.95-2.02)
0.20	30.5±3.15			
0.30	65.5±3.25			
0.40	79.5±2.03			
0.50	84.5±2.42			
Control	0			
Untreated	0			
P. ribesoides		0.15	0.83	2.17
0.025	6±1.77	(0.13-0.17)	(0.60-1.38)	(1.32-4.65)
0.05	15.5±2.42			
0.10	29.5±2.77			
0.20	62.5±4.40			
0.30	78±2.67			
0.40	97.5±1.06			
Control	0			
Untreated	0			
P. sarmentosum		0.14	0.42	0.79
0.05	3±0.89	(0.12-0.15)	(0.34-0.58)	(0.57-1.32)
0.10	30.5±1.77			
0.20	71±3.57			
0.30	89.5±0.74			
Acetone	0			
Untreated	0			

DISCUSSION

Piperaceae plants are widely used in tropical and subtropical regions throughout the world as medicines, spice, food flavoring and pest control substances[21,25]. Recently, there has been a growing interest in plants belonging to the family, Piperaceae, as potential sources of bioactive chemical compounds against insect pests. While excellent effects on mosquito vectors from larvicidal constituents that derived from some *Piper* species have been reported[8,13,14,35], little work has been carried out on *Piper* toxicity against adult mosquitoes. The potential of three *Piper* species, *P. longum*, *P. ribesoides* and *P. sarmentosum* against *St. aegypti*, as observed at 24 hours following treatment in this investigation, was strong and found to have various degrees of adulticides. *St. aegypti* adults were most susceptible to *P. sarmentosum* followed by *P. ribesoides* and *P. longum* (LD_{50}: 0.14, 0.15 and 0.26 µg/mg female, respectively). However, the adulticidal activity of the two former species, *P. sarmentosum* and *P. ribesoides*, showed no statistically significant difference, and was considered to be approximately equal, and higher than that of *P. longum*. The variety in adulticidal activity of these extracts is probably due to variation in the types and levels of active ingredients that depend on not only the genetic characteristics of the plant species, but also the conditions under which they were grown and harvested[29,31].

The literature offers no publication on the toxicity of natural plant products against adult *St. aegypti* or other mosquito species, measured by topical application. KHAN & AHMED[7] revealed the toxicity of crude neem leaf extract against the adult of housefly *Musca domestica*, investigated by topical application, with LD_{50} and LD_{95} values of 8.4 and 169.8 µg/fly, respectively. The LD_{50} values of male and female *M. domestica* treated with commercial eucalyptol were 118 and 177 µg/fly, respectively, whereas those of male and female *Chrysomya megacephala* were 197 and 221 µg/fly, respectively[26]. The *St. aegypti* mosquito is actually smaller in size and lower in weight (≈ 10-fold) than *M. domestica* and *C. megacephala*. With regard to the size and weight of insect and lethal dosage parameter, although insect susceptibility to each plant product generally demonstrates an interspecies variability, it could be expected that the toxic effect of *Piper* products was greater than that of eucalyptol and neem, the popular plant-based insecticide. The adulticidal potential of *P. ribesoides*, *P. sarmentosum* and *P. longum* investigated in this study is encouraging, but not as much as that of conventional synthetic insecticides. CORBEL *et al.*[5] reported the excellent intrinsic toxicity of some chemical insecticides measured by topical application against many mosquito species. The LD_{50} of bifenthrin, permethrin and temephos were 0.077, 0.24 and 195 ng/mg female, respectively, when tested against *Ae. aegypti* adults. The adulticidal activity of the *Piper* spp. seemed much less toxic against female *St. aegypti* (*Ae. aegypti*) than these commonly used insecticides by more than 10-fold at the LD_{50} level. Pyrethroids and organophosphates resistance, however, is now widely developed in mosquitoes of medical importance[5].

Aerial toxicants are partially effective in eradicating *Ae. aegypti*, which are highly domesticated, and many adults hide indoors[4]. Insecticide space-spraying against adult mosquitoes is still necessary during epidemics of dengue. However, the use of aerosolized pyrethroid insecticides in public health programs can cause allergies in the human population[3]. There have also been questions raised regarding the long-term persistence and other possible hazards to human health from conventional applications. The results from this research are encouraging and clearly demonstrate the potential of these *Piper* species as possible mosquitocides against *St. aegypti*. The pepper plants, *P. longum*, *P. ribesoides* and *P. sarmentosum* may be used as alternatives for managing adult flying mosquitoes. Further work on *Piper*-derived constituents is needed to develop effective formulations for controlling adult mosquitoes. Moreover, further research to identify the biologically active compounds in *Piper* extracts, which showed potent adulticidal activity, is already in progress.

RESUMO

Atividade de três *Piper* spp. contra adultos de *Stegomyia aegypti* (Diptera: Culicidae)

Três espécies de *Piper*, *Piper longum*, *P. ribesoides* e *P. sarmentosum*, foram selecionadas para investigação da potencialidade contra *Stegomyia aegypti* adultos, principal vetor de dengue e febre do dengue hemorrágico. Sucessivas extrações por maceração com etanol a 95% mostraram uma porcentagem de extratos etanólicos, derivados de *P. longum*, *P. ribesoides* e *P. sarmentosum*, de 8,89, 3,21 e 5,30% (w/w), respectivamente. Todos os extratos de *Piper* mostraram atividade adulticida expressiva quando testados contra fêmeas de mosquitos através de aplicação tópica. A suscetibilidade das fêmeas do *St. aegypt* ao extrato de *Piper* etanólico foi dose dependente e variou entre as espécies de plantas. O mais elevado efeito adulticida foi demonstrado a partir do *P. sarmentosum*, seguido pelo *P. ribesoides* e *P. longum*, valores LD_{50} de 0,14, 0,15 e 0,26 µg/fêmea, respectivamente. O potencial destas espécies de *Piper*, como possíveis mosquiticidas, estabeleceu atividade convincente para futuras pesquisas a fim de desenvolver substâncias naturais para o combate a mosquitos adultos.

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