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Susceptibility of *Culex quinquefasciatus* larvae against chloroform fraction of ethanol extract of *Pongamia pinnata* leaves as mosquito repellent and larvicidal agents

Anbu Jeba Sunilson J*[1,2], Anandarajagopal K[2], Anita Gnana Kumari AV[2], Abdullah Khan[2]

1.*Centre for Postgraduates Studies, KPJ Healthcare University College, Lot PT 17010, Persiaran Seriemas, Kota Seriemas, Nilai, Malaysia*

2.School of Pharmacy, KPJ Healthcare University College, Lot PT 17010, Persiaran Seriemas, Kota Seriemas, Nilai, Malaysia

ABSTRACT

Organic repellents and insecticides derived from plant products have been evaluated as alternatives to synthetic products used as repellents and chemical insecticides. Leaf extracts of *Pongamia pinnata* were evaluated for repellent and insecticide activity against *Culex quinquefaciatus*. 100% mortality and no survival of *C. quinquefaciatus* was observed in the ethanol extract (1000mg/l) treated group. Also the ethanol extract exhibited significant ($P<0.05$) larvicidal activity at the concentration of 250 mg/l. The chloroform soluble fraction of ethanol extract (25mg/l) exhibited significant ($P<0.05$) larvicidal activity against *C. quinquefaciatus*, and also showed significant ($P<0.05$) repellent against adult *C. quinquefasciatus* mosquitoes. The larvicidal activity of the leaf extracts was similar to the synthetic insecticide, malathion at different concentrations. Results demonstrate a potential alternative application for leaves of *P. pinnata* for the control of *C. quinquefaciatus*.

Keywords: *Pongamia pinnata, Culex quinquefaciatus,* Ethanol extract, Chloroform fraction, Larvicidal, Repellent

*Corresponding Author Email: anbujsunil@gmail.com
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INTRODUCTION

Plants are rich source of bioactive chemicals that are considered as an alternative to synthetic insecticides to control anthrapod vectors and pests. Malaria, lymphatic filariasis, schistosomiasis, dengue, trypanosomiasis and leishmaniasis affect more than 500 million people every year[1]. These diseases, besides causing high levels of morbidity and mortality, produce great economic loses and social disturbances for countries with high endemic profile. Lymphatic filariasis is the second leading cause of disability in the world ranked by World Health Organization[2].

Malaria and lymphatic filariasis are transmitted by *Anopheles* and *Culex* spp, respectively. These mosquitoes are found in many under developed tropical countries that have ecosystems favorable for high populations of mosquitoes. Resistance at the currently used synthetic and botanical insecticides and the emergence of multidrug resistant strains of parasites enhances the malaria problem in the affected countries. On the other hand, management of lymphatic filariasis through chemotherapy is usually effective when it is applied early after infection, but toxic side effects are often experienced during such treatment. Moreover, difficulties in early detection and unavailability of vaccines further contribute to the difficulties in the treatment of these conditions. Consequently, the most reliable approach to eventually eradicate both malaria and lymphatic filariasis would require interruption of the disease transmission cycle, by either targeting mosquito larvae through application of insecticides to water breeding sites or by application of adulticides for reducing mosquito population[3]. The most successful way of reducing mosquito densities to a level where dengue or yellow fever epidemics do not occur is by attacking the larval breeding places[4, 5]. Numerous botanical extracts have been traditionally used as insecticides and can be considered as potential sources of natural novel mosquito control agents.

The mangrove plant, *Pongamia pinnata* (Leguminosae) is a fast growing glabrous, deciduous tree that is widely distributed along Southeast Asia to the sandy coast of the Pacific Ocean; it is also found in limestone shrub forests[6]. Leaf extracts are traditionally used symptoms of cold, cough, diarrhea, dyspepsia, flatulence, gonorrhea and leprosy, diabetes, cleaning gums, teeth and ulcers[7] and digestive disorder, laxative, anthelmintic and cure piles, wounds and inflammation[8]. Twigs are used as a chew stick for cleaning the teeth[7]. The present study was aimed to evaluate the mosquito repellent and larvicidal activity of *Pongamia pinnata* leaf extracts against *C. quinquefasciatus*, a vector of lymphatic filariasis.

MATERIALS AND METHOD

Collection of plant material

Leaves of the plant *Pongamia pinnata* were collected from Alam Jaya, Cheras, Selangor, Malaysia during early August 2014 and authenticated by Dr. J Anbu Jeba Sunilson, Pharmacognosist, KPJ Healthcare University College, Nilai, Malaysia. Voucher specimen was deposited in the KPJ Healthcare University College herbarium.

Preparation of extracts

P. pinnata leaves were washed, dried in the shade and ground to coarse powder. The leaf powder (500 g) was extracted successively with petroleum ether, ethanol and water using soxhlet apparatus. The extract was filtered and concentrated using Rotary vacuum evaporator under reduced pressure[9]. The colour, consistency of and percentage yield were recorded in Table 1. Except water extract all other extracts were suspended in DMSO.

Table 1: Colour, consistency and percentage yield of various extracts of *Pongamia pinnata* leaves

Extract	Colour	Consistency	Yield (%)
Petroleum ether	Greenish	Sticky	6.5
Ethanol	Brownish green	Powder	12.3
Chloroform	Brownish green	Sticky	5.6
Water	Brownish	Mucilaginous	26.4

Mosquito larvae

Larvae of *C. quinquefasciatus* were obtained from the Forest Research Institute of Malaysia, Selangor, Malaysia and reared in plastic trays (36 cm x 24 cm x 7 cm) containing tap water at KPJUC Vivarium, KPJ Healthcare University College, Nilai, Malaysia. Larvae were fed a diet of yeast and powdered dog biscuits in the ratio of 2:1, kept at 26 ± 2º C and 75% - 80% relative humidity, with a photoperiod of 12:12 Light and Dark h for the larval growth.

Mosquito larvicidal bioassay

Early fourth instar larvae were used for bioassay. Screening of the extracts was performed by placing 20 fourth instars in cups containing a series of three dilutions of 250, 500 or 1000mg/L in distilled water and conducted in triplicates. Mortality was observed after 24 hr, 48 hr and 72 hr (Table 2). Dead larvae were acknowledged when they failed to move after probing with a needle. The percent mortality for each concentration of active extract was plot for LC_{50} determinations (Table 3). Fourth instars exposed similarly to a mixture of 1ml of DMSO diluted to 1L with

distilled water were used as control[10]. Malathion purchased from Behn Meyer & Co (M) Sdn Bhd, Kuala Lumpur, Malaysia served as standard.

Table 2: Efficacy of different concentrations of extracts of leaves of *P. pinnata* on fourth instar larvae of *Culex quinquefasciatus*. M, mortality (%); S, survival (%)

Treatment	Concentration (mg/l)	Larval Mortality and Survival (%)					
		24hr		48hr		72hr	
		M	S	M	S	M	S
Petroleum ether extract	250	0	100	0	100	3.3	96.7
	500	0	100	11.7	88.3	21.7	78.3
	1000	5	95	18.3	81.7	25	75
Ethanol extract	250	18.3	81.7	28.3	71.7	33.3	66.7
	500	23.3	76.7	38.8	61.7	60	40
	1000	46.6	53.4	100	0	100	0
Water extract	250	13.3	86.7	23.3	76.7	33.3	66.7
	500	16.6	83.4	31.6	68.4	40	60
	1000	25	75	58.3	41.7	100	0
Malathion (ml/L)	250	100	0	100	0	100	0
	500	100	0	100	0	100	0
	1000	100	0	100	0	100	0
Water + DMSO	250	0	100	0	100	0	100
	500	0	100	0	100	0	100
	1000	0	100	0	100	0	100

Table 3: Larvicidal activity of ethanol extract of *P. pinnata* against *C. quinquefasciatus*

Concentration of ethanol extract (mg/L)	24 hr mortality (%)	LC_{50} (mg/l)
62.5	30.0 ± 1.00^{a}	
125	45 ± 1.00^{b}	
250	63.3 ± 0.57^{c}	170
500	76.7 ± 1.15^{d}	
Malathion (62.5ml/L)	100 ± 0.00^{e}	
Water + DMSO	1.6 ± 0.57^{f}	

Values in a column with a different superscript are significantly different at $P < 0.05$ level (DMRT test). Each value (Mean ± S.D.) represents mean of five values

Repellent activity

The repellent activity was assessed using the method described by Samuel *et al*., [10]. Protection in minutes for two different concentration of active fraction was used. A total of 100 3-day-old female *C. quinquefasciatus* mosquitoes starved for 24 hr were maintained in two separate cages (45cm × 30cm × 45cm). The arms of the test person were washed with distilled water. After air drying, 25 cm^2 of the dorsal side of the arm was exposed the remaining was being covered by rubber gloves. Chloroform fraction at concentration of 5.0 and 2.5 mg/cm^2 in coconut oil was

applied. The control and treated arms were introduced simultaneously into the same cage at 29 ± 2 ◦C and relative humidity of 80 ± 2% and repeated five times for each dilution. The test was repeated at every 30 min interval. The average time before the first occurrence of three bites was taken as protection time against the bites afforded by each of the concentrations of the test repellents. There was no skin irritation indicated for each of the leaf extract dilutions.

The percentage of protection was calculated using the following formula.

% Protection = (Minutes of protection time for treated arm - Minutes of protection time for control arm / Minutes of protection time for treated arm × 100

Statistical analysis

Data were expressed as Mean ± S.E.M. and subjected to ANOVA followed by Duncan mortality range test (DMRT) performed. Values of $P<0.05$ was considered statistically significant[10].

RESULTS AND DISCUSSION

The ethanol extract of P. pinnata leaf showed maximum efficacy against *C. quinquefaciatus* larvae at all concentrations and exhibited significant activity (LC_{50} = 170) at 250mg/L (Tables 2 and 3). Among the n-hexane, ethyl acetate and chloroform fractions of ethanol extract tested larvicidal activity, the chloroform fraction exhibited 100% mortality (Table 4). The chloroform fraction showed maximum mortality (LC_{50} = 47) (Table 5). Malathion at all concentrations had 100 % mortality. Repellent activity for the 5% chloroform fraction of ethanol extract provided 96.45% protection for up to 83 minutes (Table 6).

Table 4: Larvicidal activity of different concentrations of ethanol fractions of the leaves of *P. pinnata* on fourth instar larvae of *C. quinquefasciatus*

Types of fraction	Concentration of extract (mg/L)	Larval Mortality and Survival (%)					
		24hr		48hr		72hr	
		M	S	M	S	M	S
n-hexane	25	0	100	0	100	0	100
	50	0	100	0	100	0	100
	100	0	100	1.6	98.4	1.6	100
Ethyl acetate	25	0	100	0	100	0	100
	50	0	100	8.3	91.7	25	75
	100	15	85	41.6	58.4	53.3	46.7
Chloroform	25	43.3	56.7	71.6	28.4	80	20
	50	61.7	38.3	93.3	6.7	100	0
	100	90	10	100	0	100	0
Remaining fraction	25	0	100	13.3	86.7	23.3	76.7
	50	21.6	78.4	38.3	61.7	46.6	53.4
	100	33.3	66.7	40	60	58.3	41.7
Malathion(ml/L)	25	100	0	100	0	100	0
	50	100	0	100	0	100	0

100	100	0	100	0	100	0

Table 5: Larvicidal activity of Chloroform fraction of ethanol extract of the leaves of *P. pinnata* against *C. quinquefasciatus*

Concentration of Chloroform fraction (mg/L)	24 hr mortality (%)	LC_{50} (mg/l)
6.25	21.6 ± 1.52^{a}	
12.5	30.0 ± 1.00^{b}	
25	43.3 ± 0.57^{c}	47
50	63.3 ± 0.57^{d}	
Malathion (6.25 ml/L)	100 ± 0.00^{e}	

Values in a column with a different superscript are significantly different at $P < 0.05$ level (DMRT test). Each value (Mean ± S.D.) represents mean of five values.

Table 6: Repellent activity of Chloroform fraction of ethanol extract of the leaves of *P. pinnata* against *C. quinquefasciatus*

Concentration of Chloroform fraction (mg/cm^2)	Protection time (min)		% Protection
	Treated	Control	
2.5	52.6 ± 4.4^{b}	6.9 ± 0.7^{b}	86.8 ± 2.0^{b}
5	83.5 ± 4.8^{a}	3.0 ± 0.6^{a}	96.4 ± 0.8^{a}

Values in a column with a different superscript are significantly different at $P < 0.05$ level (DMRT test). Each value (Mean ± S.D.) represents mean of five values.

Mosquito control is directed against larval and adult population to reduce pest activity and transmission of pathogens of medical and veterinary importance. Larval control is an effective method for the control of larval populations since breeding sites can often be easily identified[11]. An earlier study on methanol extract of *P. pinnata* bark showed the highest larval mortality against *C. quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi*[12]. Another study on hexane extract of *P. pinnata* seeds demonstrated the maximum insecticidal activity against *Earias vitella*[13] while methanol extract was effective against the American bollworm (*Helicoverpa armigera*)[14]. A review on *P. pinnata* reported that the leaves were used to repel insects in stored grains and seeds extracts were used as insecticides[15]. Romen reported that pongam oil exhibited the highest insecticidal efficiency against *Spodoptera littoralis*, *Myzus persicae* and *Tetranychus urticae*[16] and *Plutella xylostella*[17]. Pratibhav *et al*.,[18] reported that *P. pinnata* leaves extracts demonstrated the insecticidal properties against *Spodoptera litura*. Elena *et al*.,[19] reported *P. pinnata* seed oil formulations were effective against *Myzuz persicae*, the aphid adults.

Plant extracts make up a vast repository of phytoconstituents that have a wide range of biological activities. Mosquito repellents and larvicidal activities of *Pongamia pinnata* leaves may be due

to the presence of various phytoconstituents. Chopade *et al*., revealed the presence of sterols and fatty acids in *P. pinnata* seed extracts and flavonoids in the root bark[8]. The presence of alkaloids, flavonoids, saponins, steroids and tannins of *P. pinnata* were reported by Behera *et al*., [20].

CONCLUSION

The results conclude that the ethanol extract and its chloroform fraction are active against *C. quinquefasciatus* larvae and exhibited significant repellent activity against adults. These results indicate that *P. pinnata* leaf extracts are potentially useful as botanical larvicides for the control of *Culex* mosquitoes. Leaf extracts of P. pinnata are an attractive products to be further investigated of their larvicidal and repellent activity.

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