Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading doi:

Mosquito larvicidal and ovicidal properties of *Pemphis acidula* Frost. (Lythraceae) against *Culex tritaeniorhynchus* Giles and *Anopheles subpictus* Grassi (Diptera:Culicidae)

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ARTICLE INFO

Article history:
Received 15 August 2012
Received in revised from 10 September 2012
Accepted 5 December 2012
Available online 28 December 2012

Keywords:
larvicidal
ovicidal
Culex tritaeniorhynchus
Anopheles subpictus
Pemphis acidula

ABSTRACT

Objective: This study was undertaken to assess the larvicidal and ovicidal potential of the crude methanol, benzene and acetone solvent extracts from the medicinal plant *Pemphis acidula* (*Pe. acidula*) against the medically important mosquito vectors, *Culex tritaeniorhynchus* (*Cx. tritaeniorhynchus*) and *Anopheles subpictus* (*An. subpictus*) were exposed to various concentrations and (Diptera: Culicidae). **Methods:** Twenty five late third instar of *Cx. tritaeniorhynchus* and *An. subpictus* were exposed to various concentrations and were assayed in the laboratory by using the protocol of WHO 2005. The larval mortality was observed 24 h of treatment. Hundred eggs of *Cx. tritaeniorhynchus* and *An. subpictus* were exposed to various concentrations and were assayed in the laboratory by using the protocol of Su and Mulla 1998. The ovicidal activity was observed 48 h of treatment. **Results:** The LC_{50} and LC_{90} values being 10.81and 20.64 and 22.10 and 43.71 ppm and hundred percent of egg mortality was observed at 350 and 400 ppm methanol extract of *Pe. acidula* against *Cx. tritaeniorhynchus* and *An. subpictus*, respectively. **Conclusion:** These results suggest that the leaf extracts have the potential to be used as an ideal ecofriendly approach for the control of mosquitoes.

1. Introduction

Mosquitoes being vector for many tropical and subtropical diseases are the most important single group of insect well known for their public health importance. Despite progress in vaccine development, no effective and acceptable multivalent vaccines are currently available against mosquito borne diseases [1]. The approach to combat these diseases largely relies on interruption of the disease transmission cycle by either destruction of the aquatic stages or by killing the adult mosquitoes using chemical insecticides. The drastic effects of chemical insecticide-based intervention measures for the control of disease vectors have received wide public apprehension and have caused many problems like insecticide resistance, resurgence of pest species, environmental pollution, toxic hazards to humans, and other nontarget organisms. To alleviate these problems, major emphasis has been on the use of natural plant-based products as larvicides which can provide an alternate to synthetic insecticides. Plants are rich sources of bioactive compounds that can be used to develop environmentally safe vector and pest-managing agents. A number of plants and microbes have been reported as selective with little or no harmful effect on non-target organisms and the environment[2]. Botanical phytochemicals with mosquitocidal potential are now recognized as potent alternative insecticides to replace synthetic insecticides in mosquito control programs due to their excellent larvicidal, pupicidal, and adulticidal properties. Many synthetic insecticides and naturally occurring chemical cues have been shown to influence mosquito oviposition[3]. A few insecticides in common use have also exhibited high-deterrent activity, causing negative ovipositional response[4]. The effects of fruit and senescent leaf extracts of Melia azedarach[5] and the piperitenone oxide isolated from essential oil of Mentha spicata[6] were investigated for their larvicidal, ovicidal, and oviposition deterrence effects against Anopheles stephensi and Aedes aegypti. The active components dymalol, nymania-3, and triterpenes isolated from the extract of Dysoxylum malabaricum act as an oviposition repellent and/or deterrent to An. stephensi[7]. The chemicals derived from plants have been projected as weapons in future mosquito control program as they are shown to function as general toxicant, growth, and reproductive inhibitors, repellents, and oviposition deterrent.

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Methanolic leaf extract of Cassia fistula was tested for larvicidal and ovicidal activity against *Cx. quinquefasciatus* and An. stephensi[8]. The leaf extract of Acalypha indica with different solvents viz., benzene, chloroform, ethyl acetate, and methanol was tested for larvicidal, ovicidal activity, and oviposition attractancy against An. stephensi[9]. The leaf extract of *C. fistula* with different solvents viz., methanol, benzene, and acetone was studied for the larvicidal, ovicidal, and repellent activity against An. aegypti[10]. The larvicidal, ovicidal and repellent activities of *Pemphis acidula* Forst. (Lythraceae) against filarial and dengue vector mosquitoes[11]. The acetone, chloroform, ethyl acetate, hexane, and methanol leaf extracts of *A. indica*, Achyranthes aspera, Leucas aspera, Morinda tinctoria, and Ocimum sanctum were studied against the early fourth-instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus*[12]. Larvicidal activity of crude hexane, ethyl acetate, petroleum ether, acetone, and methanol extracts of the leaf of five species of cucurbitaceous plants, Citrullus colocynthis, Coccinia indica, Cucumis sativus, Momordica charantia, and Trichosanthes anguina was tested against the early fourth-instar larvae of Ae. aegypti L. and *Cx. quinquefasciatus*[13]. Elango et al. [14] have reported that the leaf acetone, chloroform, ethyl acetate, hexane, and methanol extracts of Aegle marmelos, Andrographis lineata, *A. paniculata*, Cocculus hirsutus, Eclipta prostrate and Tagetes erecta were tested against fourth-instar larvae of *Anopheles subpictus* and *Cx. tritaeniorhynchus*. The ethanol extract of Curcuma aeruginosa, Curcuma aromatica, and Curcuma xanthorrhiza was tested for repellent activity against Ae. togoi, Armigeres subalbatus, *Cx. quinquefasciatus*, and *Cx. tritaeniorhynchus*[15]. Murugan et al. [16] studied the interactive effect of botanicals (neem, pongamia) and L. aspera and *B. sphaericus* against the larvae of *Cx. quinquefasciatus*. Vahitha et al[17] studied the larvicidal efficacy of *Pavonia zeylanica* L. and Acacia ferruginea against *Cx. quinquefasciatus* Say. Shigeo et al [18] reported the larvicidal effect of neem against *Ae. aegypti* and chironomid larvae. Mullai et al [19] have reported that the leaf extract of Citrullus vulgaris with different solvents, viz., benzene, petroleum ether, ethyl acetate, and methanol, was tested for larvicidal, ovicidal, repellent, and insect growth regulatory activities against An. stephensi. Ovicidal effects of the seed extract of Atriplex canescens were reported against *Cx. quinquefasciatus*[20] and the larvicidal and repellent properties of essential oils from various parts of four plant species Cymbopogon citratus, Cinnamomum zeylanicum, Rosmarinus officinalis, and Zingiber officinale against *Cx. tritaeniorhynchus* and *An. subpictus*[21]. Su and Mulla[22] reported the ovicidal activity of the neem product azadirachtin against the mosquitoes Cx. tarsalis and *Cx. quinquefasciatus*. Leaf extracts of *Pe. acidula* are reported for strong insecticidal activity[23]. As far as our literature survey could ascertain, no information was available on the ovicidal and larvicidal activities of the experimental plant species given here against *Cx. tritaeniorhynchus* and *An. subpictus*. Therefore, the aim of this study was to investigate the mosquito ovicidal and larvicidal activities of the different solvent extracts of *Pe. acidula* plant species from Tamil Nadu, India.

2. Materials and methods

2.1. Collection of plants

Fully developed leaves of the *Pe. acidula* Forst. (Lythraceae) was collected from Gulf of Mannar Biosphere Reserve, (9° 14′47.2N lat. and 79° 12′38.6E long.) Tamilnadu, India. The plant was taxonomically identified at the Department of Botany, Annamalai University and voucher specimen is deposited at the herbarium of the plant photochemistry division, Annamalai University.

2.2. Preparation of plant extract

The leaves of *Pe. acidula* were washed with tap water, shade dried at room temperature, and powdered by electrical blender. The dried leaves (1.0 kg) were powdered by electrical blender. Three litre methanol, acetone and benzene separately were used for the extraction of 1.0 kg in the Soxhlet apparatus followed by the standard procedure[24]. The plant material was loaded in the inner tube of the Soxhlet apparatus and then fitted into a round bottomed flask containing methanol. The solvent was boiled gently (40℃) over a heating mantle using the adjustable rheostat. The extraction was continued until complete extraction was effected (8 hrs.) and the solvent was removed at the reduced pressure with the help of rotary vaccum evaporator to yield a viscous dark green residue (12.5 g) of each solvent of methanol, acetone and benzene leaf extracts. Standard stock solutions were prepared at 1% by dissolving the residues in dimethyl sulphoxide (DMSO), which was used for the bioassays.

2.3. Test organisms

To satisfy the enormous number of mosquitoes need for the day to day bioassays, a colony was essential. *Cx. tritaeniorhynchus* and *An. subpictus* were procured from Vector Control Research Centre (VCRC) at Puducherry, India. The mosquito colony maintained at 70-85% RH, 28 ± 2 ℃ temperature and 14:10 light and dark photoperiod cycle. The larvae were fed on powdered mixture of dog biscuits and yeast tablets in 3:1 ratio. The blood meal was given to the female adult mosquitoes and 5.0% glucose solution and honey were given to the male adult mosquitoes.

2.4. Larvicidal activity

Larvicidal evaluation was carried out at different concentrations by preparing the required stock solutions by following the standard procedure[25]. Appropriate serial dilutions were made from the stock solution and the desired concentrations of the test solution were achieved by adding 1.0 ml of an appropriate stock solution to 99 ml of tap water. The mosquito larvae were exposed to different concentrations of the test solution ranging from 50 to 300 ppm. Four replicates for each concentration were maintained. Batches of twenty five late third instar larvae were transferred into each disposable cup containing 100ml of water. DMSO was used as control. The bioassays were performed at room temperature (27±1℃). The larval mortality in both treated and control was recorded after 24h exposure period.

2.5. Ovicidal activity

Evaluation of the puffer fish extracts for ovicidal activity was carried out by following the method of Su and Mulla[22]. Eggs were exposed to different concentrations ranging from 150 to 500 ppm. The desired concentrations of the test solutions were achieved by adding 1.0 ml of an appropriate stock solution to 99 ml of tap water. Each egg raft containing 100 eggs of *Cx. tritaeniorhynchus* and hundred eggs of *An. subpictus* (14 to 18 hr old) were exposed to each dose of extract for 48hr. Counting of eggs was done under a microscope. DMSO served as control. Four replicates for each concentration were maintained. After 48hr of incubation, the egg rafts or eggs exposed to each concentration were transferred to distilled water cups. The hatch rates were calculated by the following formula.

$$\% \text{ Mortality} = \frac{\text{Mortality at treatment} - \text{Mortality at control}}{100 - \text{Mortality at control}} \times 100$$

2.6. Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC50, LC90, and other statistics at 95% confidence limits of upper confidence limit (UCL) and lower confidence limit (LCL), and chi-square values were calculated using the SPSS12.0 (Statistical Package of Social Sciences) software. Results with $p<0.05$ were considered to be statistically significant.

3. Results

The results of the larvicidal activity of crude methanol, benzene and acetone solvent extracts of leaf of *Pe. acidula* against the larvae of two important vector mosquitoes viz., *Cx. tritaeniorhynchus* and *An. subpictus* are presented in Tables 1. The experiments conducted for evaluating larvicidal efficacy of leaf of *Pe. acidula* revealed that leaf extract exerted effective larvicidal activity. Among the extracts tested, the highest larvicidal activity was observed in methanol extract of *Pe. acidula* against *Cx. tritaeniorhynchus* and *An. subpictus* with the LC_{50} and LC_{90} values being 10.81and 20.64 and 22.10 and 43.71 ppm, respectively. The chi-square values are significant at $P<0.05$ level. The chi-square values in the bioassays indicated probably the heterogeneity of the test population. The 95% confidence limits (LC_{50} (LCL-UCL)) and (LC_{90} (LCL-UCL)) were also calculated. The mean percent of egg hatchability of *Cx. tritaeniorhynchus* and *An. subpictus* were tested with three different solvents at different concentrations of *Pe. acidula* leaves extracts, and the results are listed in Table 2. The percent hatchability was inversely proportional to the concentration of extract and directly proportional to the eggs. Among the extracts tested for ovicidal activity against *Cx. tritaeniorhynchus* and *An. subpictus*, the methanol extract of *Pe. acidula* exerted 100% mortality (zero hatchability) at 350 and 400, respectively. The methanol extract of *Pe. acidula* was found to be most effective than others against larvae and eggs of two vector mosquitoes. Control eggs showed the 100% hatchability.

4. Discussion

Botanicals have been used traditionally by human communities in many parts of the world against pest species of insects. Many plants produce secondary metabolites that inhibit the growth of insects. Though several plants from different families have been reported for mosquitocidal activity, only very few botanicals have moved from the laboratory to field use. Simple crude extracts from plants

Table 1

Larvicidal activity of different solvent leaf extracts *Pe. acidula* against *Cx. tritaeniorhynchus* and *An. subpictus*

Species	Solvents	LC_{50}	LC_{90}	Regression	95% confidence limit		Chi-square
					LCL	UCL	
Cx. tritaeniorhynchus	Methanol	10.81	20.64	Y=8.41+3.77x	8.10	13.33	9.21*
	Benzene	41.07	81.89	Y=11.14+0.91x	31.46	49.93	10.11*
	Acetone	53.22	104.55	Y=8.28+0.75x	41.17	64.6	8.42*
An. subpictus	Methanol	22.10	43.71	Y=9.61+1.80x	16.52	27.29	10.54*
	Benzene	43.99	84.89	Y=8.47.0.93x	33.47	53.8	10.17*
	Acetone	57.66	106.51	Y=5.48+0.76x	45.11	69.81	10.39*

*- Significant at $P<0.05$ level. LC50- Lethal concentration; LCL- Lower confidence limit; UCL- Upper confidence limit.

Table 2

Ovicidal activity of different solvent leaf extracts *Pe. acidula* against *Cx. tritaeniorhynchus* and *An. subpictus*

Species	Solvents	Percentage of egg hatchability								
		Concentration (ppm)								
		Control	150	200	250	300	350	400	450	500
Cx. tritaeniorhynchus	Methanol	98±1.2	72.3±1.3	55.8±2.1	36.6 ±1.3	18.8±2.2	NH	NH	NH	NH
	Benzene	99.9±0.1	92.1±2.1	70±1.2	48.1±1.2	32.4±2.2	8.2±1.8	NH	NH	NH
	Acetone	100	100	90.1±2.0	69.1±2.2	37.1±1.2	12.4±1.1	NH	NH	NH
An. subpictus	Methanol	99.0±1.2	99±1.2	92.1±2.2	68.2±1.2	49±1.1	30.1±1.2	8.1±2.3	NH	NH
	Benzene	100	100	88.1±2.2	65.3±3.1	42±2.8	20.1±2.3	3.3±1.1	NH	NH
	Acetone	100	100	100	88.1±	69.6±2.4	49.6±2.3	33.5±2.0	11.8±2.2	NH

NH- No hatchability; values are mean of four replicates ±SE

have been used as insecticides in many countries for centuries[26]. Crude plant extracts often consist of complex mixtures of active compounds. Advances of using complete mixture may act synergistically, they may show greater overall bioactivity compared to the individual constituents[29] and insect resistance is much less likely to develop with mixtures. These reasons support the use of crude chemically unrefined plant extracts, containing mixtures of bioactive plant compounds rather than the use of the pure individuals. *Pe. acidula* leaf extracted with three different solvents namely methanol, benzene and acetone. Stock solution was prepared using acetone, because basic toxicological investigation and screening for insecticidal activity, acetone is commonly used as a solvent. Earlier authors reported that the effect of water extract of citrus seed extract showed LC50 values of 135,319.40 and 127,411.88 ppm against the larvae of Ae. aegypti and *Cx. quinquefasciatus*[29]. Larvicidal activity of Saraca indica, Nyctanthes arbor-tristis, and Clitoria ternatea extracts against three mosquito vector species[30]. The aqueous extract of *R. nasutus* showed LC50 values of 5,124 and 9,681 mg/l against *Cx. quinquefasciatus* and *Ae. aegypti*, respectively[31]. A preliminary screening of crude acetone extract of Cuscuta hyalina was conducted against the laboratory-reared preadult stages of common house mosquito *Cx. quinquefasciatus* (Say) (Diptera: Culicidae). Twenty-four-hour LC_{50} of third and fourth instar larvae and pupae were 303, 306.44, and 97.66 ppm, respectively[32]. Sharma et al. [33] reported that the acetone extract of Nerium indicum and Thuja orientelis have been studied with LC_{50} values of 200.87, 127.53, 209.00, and 155.97 ppm against third-instar larvae of *An. stephensi* and *Cx. quinquefasciatus*, respectively. Mullai and Jebanesan[34] have reported that the methanol leaf extracts of *C. colocynthis* and Cucurbita maxima showed that the LC_{50} values were 117.73 and 171.64 ppm, respectively, against *Cx. quinquefasciatus* larvae. Larvicidal efficacies of methanol extracts of *M. charantia*, *T. anguina*, Luffa acutangula, Benincasa cerifera, and *C. vulgaris* tested against the late third larval age group of *Cx. quinquefasciatus*[35]. The methanol leaf extracts of *V. negundo*, *V. trifolia*, *V. peduncularis*, and *V. altissima* were used for larvicidal assay against the early fourth-instar larvae of *Cx. quinquefasciatus*[36]. The leaf extract and active compound cryptolepine showed negligible mortality against early third-instar larvae of *An. subpictus* and *Cx. tritaeniorhynchus*[37]. Compared with earlier reports, our results revealed that the experimental plant extracts were effective to control *Cx. tritaeniorhynchus* and *An. subpictus*. In the present study, extracts of *Pe. acidula* was found to possess ovicidal activity against two mosquito species of *Cx. tritaeniorhynchus* and *An. subpictus*. Even though the ovicidal activity was moderate, the larvae which hatched out of the treated eggs immediately scumbed to death. In the laboratory treatment of the eggs of various age groups of three species of mosquitoes with two synthetic pyretheroids, permethrin and deltamethrin, which caused only moderate ovicidal activity but inflicted delayed effects such as high larval and low pupal and adult mortality[38]. Su and Mulla[23] reported that the egg rafts aged for 0, 4, 8, 12 and 24 hr were exposed to 10 ppm neem suspensions for 36 hrs and the ovicidal activity was only attained in the egg rafts deposited directly (0 hr old) in neem suspensions, not in those with ages of 4-24 hr. In this study, the exposure period also played a crucial role in causing toxicity. Ouda *et al*.[39] reported that a 1000 ppm concentration of the seed extract of Atriplex canescens killed all the eggs of *Cx. quinquefasciatus*. Govindarajan et al.[40] stated that essential extracts from Delonix elata were evaluated for larvicidal and ovicidal activities against the filarial mosquito An. stephensi and Ae. aegypti. The efficacy to act on the embryo inside the egg shell depends on an efficient penetration of the insecticide, which in turn influenced by the exposure period. The use of locally available plants for mosquito control could generate employment, reduce dependency on expensive imported products and stimulate local efforts to enhance public health. As reported earlier, the insecticidal compounds solanidine and abrine isolated from Solanaceae are toxic to mosquitoes[41]. From these results, it was concluded that the plant *Pe. acidula* exhibits larvicidal and ovicidal activity against two important vector mosquitoes. The flora of India has rich aromatic plant diversity with potential for development of natural insecticides for control of mosquito and other pests. These results could encourage the search for new active natural compounds offering an alternative to synthetic insecticides from other medicinal plants. Further analysis to isolate the active compound for the mosquito control is under way our laboratory.

Acknowledgements

The author is grateful to Director, Centre of Advanced study in Marine Biology, Annamalai University for the laboratory facilities provided. We acknowledge the staff members of the VCRC (ICMR), Pondicherry for their cooperation.

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