Available online at www.elixirpublishers.com (Elixir International Journal)

Applied Zoology

Elixir Appl. Zoology 64 (2013) 19394-19397

ISSN: 2229-712X

Larvicidal, Ovicidal and Repellent activities of *Opuntia dillenii* Haw extracts against dengue vector *Aedes aegypti* L. (Diptera: Culicidae)

K.T. Kamakshi[1], R. Raveen[1] and A. Daniel Reegan[2*]

[1]Department of Zoology, Madras Christian College, Chennai – 600 059, Tamil Nadu, India.

[2]Entomology Research Institute, Loyola College, Chennai – 600 034, Tamil Nadu, India.

ARTICLE INFO

Article history:

Received: 16 September 2013;

Received in revised form:

5 November 2013;

Accepted: 14 November 2013;

Keywords

Opuntia dillenii,

Solvent extracts,

Biological activities,

Aedes aegypti.

ABSTRACT

Solvent extracts of xerophytic plant, *Opuntia dillenii* Haw were screened for larvicidal, ovicidal and repellent properties against the dengue vector *Ae. aegypti* L. Five different extracts viz., hexane, petroleum ether, ethyl acetate, carbon tetrachloride and distilled water were tested for larvicidal, ovicidal activity in five different concentrations ranging from 62.5, 125, 250, 500 and 1000 ppm. Among the five solvent extracts of *O. dillenii*, petroleum ether extract showed the highest larvicidal activity at 1000 ppm against the fourth instar larvae of *Ae. aegypti*. The LC_{50} and LC_{90} values of *O. dillenii* petroleum ether extract were recorded as 323.76 and 829.76 ppm against *Ae. aegypti* larvae respectively. High ovicidal activity of 64% was recorded at 1000ppm concentration of petroleum ether extract. The petroleum ether extract was also found to be the most effective protectant against the adult female mosquitoes of *Ae. aegypti*. The mean protection time recorded in petroleum ether extract was up to 107 min at 5 mg/cm^2 dosage against *Ae. aegypti* adults. The potential of petroleum ether extract of *O. dillenii* could be used in dengue vector control.

Introduction

Mosquitoes are insects with public health importance. Several mosquito species belonging to the genera *Anopheles*, *Culex* and *Aedes* are vectors for the pathogens of various diseases like malaria, filariasis, Japanese encephalitis, dengue fever, dengue hemorrhagic fever and yellow fever causing millions of deaths every year (Becker *et al*, 2003; Chaithong *et al*, 2006; Das *et al*, 2007). Mainly, tropical countries are at risk of mosquito borne diseases due to climate change and it was estimated that over two billion people at risk (Muthu et al 2012). The yellow fever mosquito, *Aedes aegypti* is the primary vector responsible for dengue fever, dengue hemorrhagic fever and chikungunya in India, where the number of dengue fever cases has increased significantly in recent years (Pruthvi et al 2012). It is reported to infect more than a hundred million people every year in more than 110 countries in the tropics (Halstead 2000; Malavige et al., 2004). Control of the mosquito vectors is frequently dependent on continued applications of synthetic insecticides (mostly organophosphates). Repeated use of these synthetic insecticides has disrupted natural biological system and of human health concerns (Yang et al., 2002). Plant extracts are good alternative source for mosquito control. Many researchers have reported the insecticidal activity of plant extracts against various stages of vector mosquito and recently many plant extracts had been screened against *Ae. aegypti* (de Mendonça et al 2005; de Omena et al 2007; Garcez et al 2009).

O. dillenii is a widely distributed perennial plant. *O. dillenii* is well-known in India as its fruits are edible (Singh et al 2001). The pharmacological importance of this plant has been investigated by many authors (Feugang et al 2006; Rodriguez-Fragoso et al 2007) and the cladodes are used in the treatment of gastric ulcer and anti-infective agents (Park et al 2001; Galati et al 2001). However, the mosquitocidal activity of cladodes of this plant not yet been investigated. Therefore, in this study we examine the larvicidal, ovicidal and repellent activities of crude extracts taken from the cladodes of *O. dillenii* against dengue vector *Ae. aegypti*.

Materials and methods

Plant collection and extraction

Matured fresh cladodes of *O. dillenii* were collected in Kelambakam, Tamil Nadu, India and shade dried at room temperature for one week and then powdered. 50 g of powder were mixed sequentially with 1 litre of hexane, petroleum ether, ethyl acetate, carbon tetra chloride and distilled water for a period of 96 hours each and filtered using soxhlet extractor. The extract was concentrated at reduced temperature on a rotary evaporator and the yield were 5g hexane extract, 4g petroleum ether extract, 2g ethyl acetate extract, 2g carbon tetra chloride extract and 6g distilled water extract. All the extracts were stored in 4^{o}C until use.

Test insects

The fourth instar larvae and eggs of dengue vector *Ae. aegypti* were obtained from Entomology Research Institute, Loyola College, Chennai, India. The repellent experiments were carried out at Entomology Research Institute laboratory for all the five extracts against the adults of *Ae. aegypti*.

Preparation of test solution

Stock solutions were prepared in acetone (10%) for all extracts. Test solutions were prepared from the stock solution for each extract separately at different concentrations ranging from 62.5 ppm to 1000 ppm and then subjected to bioassay screening.

Larvicidal activity

Larvicidal bioassay was carried out as per the guidelines of WHO (2005) with slight modifications. Twenty five numbers of early fourth instar larvae of *Ae. aegypti* were introduced into each test containers. The extracts taken in five different concentrations were 62.5ppm, 125ppm, 250ppm, 500ppm,

Tele:

E-mail addresses: danielreegan85@gmail.com

1000ppm. Control and solvent control (Acetone in water) were maintained separately. Mortality rate were registered after 24 h exposure period and calculated for each concentration. The bioassays were performed at room temperature of 27 ± 2°C with five replicates for each concentration. Mortality was converted into percent mortality and corrected mortality was calculated using Abbott's formula (1925).

Ovicidal activity

Ovicidal bioassay was carried out as per the guidelines of Elango at al. (2009) with slight modifications. Twenty five numbers of eggs of *Ae. aegypti* were introduced into each test containers. The extracts taken in five concentrations were 62.5ppm, 125ppm, 250ppm, 500ppm, 1000ppm. Acetone in water was used as solvent control. Non-hatched eggs were registered after 96 h exposure period and mortality rate was calculated for each concentration. The bioassays were performed at room temperature of 27 ± 2°C with five replicates for each concentration. Mortality was converted into mean percent mortality and tabulated.

Repellent activity

Repellent activity was conducted as per the guidelines of WHO (2009) with slight modifications. 3 to 6 day old hundred blood-starved adult mosquitoes introduced into separate laboratory cages (45×45×50 cm). Before each test, the forearms of human volunteers were washed with unscented neutral soap, thoroughly rinsed, and allowed to dry before the application of the extract. Three concentration were taken (1, 2.5, 5 mg/cm^2) for each extract and five replication (5 volunteers) were maintained for each concentration. The extracts being tested applied on the right upper forearm and remaining regions covered with gloves. The left arm served as control. *N,N*-Diethyl-*meta*-toluamide (DEET 12%, w/w) was used as standard reference control (on the 6th volunteer forearm). The mosquito bites observed for three full minute of every fifteen minutes by inserting hand inside the cage. Protection duration recorded as the time elapsed between the extract application and the first confirmed bite obtained. The protection time of each concentration of each extract was calculated.

Statistical analysis

Statistical analysis of all the data obtained in larvicidal activity were corrected in Abbott's formula (Abbott's 1925) and evaluated using Probit analysis (SPSS Probit analysis Program; Version15.0).The differences were considered as significant at $P \leq 0.05$. The mean percent ovicidal activity, mean repellent duration and Standard deviation (SD) were calculated in excel spreadsheet.

Results

Larvicidal activity

The larvicidal activity varied between the solvent extracts and the activity was moderate. Table 1 shows the results on effective lethal concentration (LC_{50} and LC_{90}) values of hexane, petroleum ether, ethyl acetate, carbon tetrachloride and distilled water extracts of *O. dillenii* after 24 h treatment period. The results showed that the petroleum ether extract recorded the maximum larvicidal activity against *Ae. aegypti* larvae. The LC_{50} and LC_{90} values of petroleum ether extract were 323.76 and 829.76 ppm respectively. Significant chi-square values were recorded in all the extracts (Table 1). In control and solvent control all the larvae were active. But in the treated, restless movement was observed as reported earlier (Senthil Nathan 2007; Maheswaran and Ignacimuthu 2012).

Ovicidal activity

The ovicidal activities of *O. dillenii* extracts on *Ae. aegypti* eggs are given in Table 2. Petroleum ether extract was found to be highly lethal to the eggs of *Ae. aegypti* than other extracts. Petroleum ether extract showed 64% ovicidal activity at 1000 ppm concentration in 96 h post treatment period. The lowest concentration (62.5 ppm) of petroleum ether extract caused 8.8% egg mortality against the eggs of *Ae. aegypti*. The ovicidal effects of all the five extracts were directly proportional to the concentration.

Repellent activity

The complete protection times for all the five extracts of *O. dillenii* against *Ae. aegypti* mosquitoes were recorded and the results are given in Table 3. The repellence was directly proportional to the dose and protection time (min) for each extract and showed variations against *Ae. aegypti* mosquitoes. In general petroleum ether extract gave maximum protection time against *Ae. aegypti* compared to other extracts. Petroleum ether extract gave 100% protection up to 107 min against *Ae. aegypti* at a dose of 5 mg/cm^2 followed by carbon tetrachloride provided 41 min protection at 5 mg/cm^2 dosage (Table 3). These results were compared with negative control (*N,N*-Diethyl-*meta*-toluamide 12%, w/w), which showed maximum of 224 min protection at 5 mg/cm^2 dosage against *Ae. aegypti* mosquitoes.

Discussion

Results on the larval mortality of the petroleum ether extract reported in the present study, confirm the larvicidal potential against *Ae. aegypti* larvae. After petroleum ether extract treatment at a higher dose (1000ppm), the larvae showed abnormal and irregular in movement and died immediately before the pupal stage. Our LC_{50} result (323.76ppm) of petroleum ether extract against *Ae. aegypti* is comparable with the earlier report of Bilal et al (2012), who reported the LC_{50} value of 363.7 ppm, 377.5 ppm and 403.4 ppm for *Coriandrum sativum, Nigella sativa* and *Syzygium aromaticum* respectively, against *Ae. albopictus* after 24 h exposure period. In another study, Rahuman et al (2008) have reported that the LC_{50} value of petroleum ether extracts of *Euphorbia hirta* was 272.36 against *Ae. aegypti*.

Results of ovicidal activity showed that the petroleum ether extract was potent and recorded 64% ovicidal at 1000ppm concentration. All the other four extracts of *O. dillenii* did not show significant ovicidal activity (Table 2). Further, all the five extracts of *O. dillenii* showed moderate repellent activity and among them petroleum ether extract recorded maximum repellent duration of 107min (Table 1) at 5mg/cm^2 concentration. This result is comparable with the earlier report of Govindarajan (2010) who have reported that the repellent activity of methanol extract of *Ferronia elephantum* leaves up to 150min against *Ae. aegypti* at 5mg/cm^2 concentration.

Plant extracts are being widely used for biological activity against various stages of vector mosquitoes. The use of plant extracts may be considered as an important alternative insecticide for the control of dengue vector *Ae. aegypti*, since they constitute a rich source of bioactive compounds that are biodegradable, nontoxic, and cost effective. In conclusion, *O. dillenii* petroleum ether extract may be used as an ecologically safe alternative larvicide and repellent against *A. aegypti*.

Acknowledgements

Authors are thankful to Entomology Research Institute, Loyola College, Chennai, India for providing eggs and larvae of *A. aegypti* and providing facilities to carryout repellent experiments.

References

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. *J Econ Entomol.* 18: 265–267.

Table 1: Lethal concentration (in ppm) of opuntia extracts against *Aedes aegypti* larvae

Mosquito species	Treatment	LC_{50} (ppm)	95% confidence limit		LC_{90} (ppm)	95% confidence limit		Intercept ± SE	df	χ^2
			LL	UL		LL	UL			
Aedes aegypti	Hexane	1313.53	1054.47	1841.01	2805.20	2163.12	4174.33	-1.1 ± 0.09	23	8.7*
	Ethyl acetate	1349.31	1049.65	2040.47	3094.86	2298.08	5024.03	0.9 ± 0.08	23	6.2*
	Carbon tetrachloride	1047.28	857.59	1399.50	2487.63	1954.37	3558.55	-0.9 ± 0.08	23	4.2*
	Petroleum ether	323.76	280.19	368.45	829.76	745.11	943.71	-0.8 ± 0.08	23	15.7*
	Dist water	1326.18	1053.69	1904.14	2898.73	2207.40	4435.35	-1.1 ± 0.08	23	12.1*

LC_{50} lethal concentration that kills 50 % of the exposed larvae, LC_{90} lethal concentration that kills 90 % of the exposed larvae, LL lower limit (95 % confidence limit), UL upper limit (95 % confidence limit)

*$p \leq 0.05$, level of significance of chi-square values

Table 2: Percent ovicidal activity of opuntia extracts against *Aedes aegypti* eggs

Solvent	Ovicidal (in %)					
	Concentration (ppm)					
	Solvent Control	62.5 ppm	125ppm	250ppm	500 ppm	1000 ppm
Hexane	2.6 ± 0.54	2.6 ± 0.54	3.2 ± 0.44	3.8 ± 0.83	5.0 ± 0.70	6.6 ± 0.89
Ethyl acetate	0.8 ± 1.09	2.6 ± 0.54	2.8 ± 0.83	4.2 ± 0.83	5.4 ± 1.14	6.6 ± 1.14
Carbon tetrachloride	0.0 ± 0.0	2 ± 0.70	3.4 ± 0.54	4.2 ± 0.83	4.8 ± 0.83	7 ± 1.22
Petroleum ether	0.0 ± 0.0	8.8 ± 0.54	13.6 ± 0.83	20.8 ± 1.30	34.4 ± 1.14	64 ± 1.87
Dist water	0.2 ± 0.44	2.4 ± 0.89	2 ± 0.70	2.6 ± 0.54	3 ± 0.70	5.6 ± 1.14

Each value represents mean percentage of five replicates ± SD.

Table 3: Complete protection time of different solvent extracts of opuntia against *Aedes aegypti* female adults

Extract	Concentration mg/cm^2	Complete protection time (min)	
		Control	Treated
Hexane	1.0	2.0 ± 0.70	00 ± 00
	2.5	2.2 ± 0.30	16 ± 0.89
	5.0	1.2 ± 0.44	32 ± 1.41
Petroleum ether	1.0	2.0 ± 1.41	31 ± 1.09
	2.5	1.5 ± 0.44	64 ± 1.41
	5.0	2.0 ± 0.70	107 ± 0.89
Carbon tetrachloride	1.0	1.6 ± 0.54	16 ± 0.89
	2.5	1.5 ± 0.44	33 ± 1.67
	5.0	1.6 ± 0.54	41 ± 1.09
Ethyl acetate	1.0	1.8 ± 0.44	00 ± 00
	2.5	1.5 ± 0.44	16 ± 1.09
	5.0	2.0 ± 0.70	33 ± 1.41
Distilled water	1.0	1.6 ± 0.89	00 ± 00
	2.5	1.6 ± 0.54	00 ± 00
	5.0	2.2 ± 0.44	16 ± 0.89
N,*N*-Diethyl-*meta*-toluamide 12%	1.0	2.2 ± 1.09	42 ± 1.58
	2.5	2.0 ± 0.70	100 ± 2.91
	5.0	2.4 ± 0.89	224 ± 2.54

Each value represents mean of five replicates ± SD.

Becker, N., Petriae, D., Zgomba, M., Boase, C., Dahe, C., Lane, J. and Kaiser, A. Mosquitoes and their control. New York; Kluwer Academic / Plenum publisher. 2003.

Bilal, H.W., Akram, S., Din, I.A., Khan, S.A., Hassan, and Arshad, M. Larvicidal Activity of Selected plant extracts against *Aedes albopictus* Skuse (Diptera: Culicidae). *African Entomology*. 2012; 20(1): 8–12.

Chaithong, U., Choochote, W., Kamsuk, K., Jitpakdi, A., Tippawangkosol, P., Chaiyasit, D., Champakaew, D., Tuetun, B. and Pitasawat, B. Larvacidal effect of pepper plants on *Aedes aegypti* (L.) (Diptera: Culicidae). *J. of Vector Ecology*. 2006; 31(1): 138–144.

Das, N.G., Goswami, D and Rabha, B. Preliminary evaluation of mosquito larvicidal efficacy of plant extract. *J. Vect Borne Dis.* 2007; 44: 145–148.

De Mendonça F.A.C., da Silva, K.F.S., dos Santos, K.K., Ribeiro Júnior, K.A.L. and Sant'Ana, A.E.G. Activities of some Brazilian plants against larvae of the mosquito *Aedes aegypti. Fitoterapia*. 2005; 76(7): 629–636.

De Omena M.C., Navarro, D.M.A.F., de Paula, J.E., Luna, J.S., Ferreira de Lima, M.R. and Sant'Ana A.E.G. Larvicidal activities against *Aedes aegypti* of some Brazilian medicinal plants. *Bioresource Technology*. 2007; 98: 2549–2556.

Elango, G., Bagavan, A., Kamaraj, C., Abduz Zahir, A. and Rahuman, A.A., Oviposition deterrent, ovicidal, and repellent activities of indigenous plant extracts against *Anopheles subpictus* Grassi (Diptera: Culicidae). *Parasitol. Res*. 2009; 105: 1567–1576.

Feugang, J.M., Konarski, P., Zou, D., Stintzing, F.C. and Zou, C. Nutritional and medicinal use of Cactus pear (*Opuntia* spp.)

cladodes and fruits. *Frontiers in Bioscience*. 2006; 11: 2574–2589.

Galati, E.M., Monforte, M.T., Tripodo, M.M., d'Aquino, A. and Mondello, M.R. Antiulcer activity of *Opuntia ficus indica* (L.) Mill. (Cactaceae): ultrastructural study. *Journal of Ethnopharmacology.* 2001; 76: 1–9.

Garcez, W.S., Fernanda, R., Garcez, Lilliam M.G.E., da Silva, and Hamerski, L. Larvicidal activity against *Aedes aegypti* of some plants native to the West-Central region of Brazil. *Bioresource Technology*. 2009; 100(24): 6647–6650.

Govindarajan, M. Larvicidal and repellent activities of *Sida acuta* Burm. F. (Family: Malvaceae) against three important vector mosquitoes. *Asian Pacific Journal of Tropical Medicine*. 2010; 691–695.

Halstead, S.B. Global perspective on Dengue Research. *Dengue Bulletin*. 2000; 24: 77–82.

Maheswaran, R. and Ignacimuthu, S. A novel herbal formulation against dengue vector mosquitoes *Aedes aegypti* and *Aedes albopictus. Parasitol. Res*. 2012; 110: 1801–1813.

Malavige, Lapcharoen, P., Apiwathnasorn, C., Komalamisra, N., Dekumyoy, P. and Palakul. Three indigenous Thai medicinal plants for control of *Aedes aegypti*. *J. Southeast Asian Trop. Med. Public Health*, 2004; 36(4): 167–17

Muthu, C., Daniel Reegan, A., Kingsley, S. and Ignacimuthu, S. Larvicidal activity of pectolinaringenin from *Clerodendrum phlomidis* L. against *Culex quinquefasciatus* Say and *Aedes aegypti* L. (Diptera: Culicidae). *Parasitol. Res*. 2012; 111: 1059–1065.

Park, E.H., Kahng, J.H., Lee, S.H. and Shin, K.H. An anti-inflammatory principle from cactus. *Fitoterapia.* 2001; 72: 288–290.

Pruthvi, D., Shashikala, P. and Shenoy, V. Evaluation of Platelet count in Dengue fever along with Seasonal Variation of Dengue Infection. *J. Blood Disorders Transf.* 2012; 34: 1–4.

Rahuman, A.A., Gopalakrishnan, G., Venkatesan, P. and Geetha, K. Larvicidal activity of some Euphorbiaceae plant extracts against *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitol. Res.* 2008; 102: 867–873.

Rodriguez-Fragoso, L., Reyes-Esparza, J., Burchiel, S., Herrera-Ruiz, D. and Torres, E. Risks and benefits of commonly used herbal medicines in Mexico. *Toxicology and Applied Pharmacology*. 2008; 227(1): 125–135.

Senthil Nathan, S. The use of Eucalyptus tereticornis Sm. (Myrtaceae) oil (leaf extract) as a natural larvicidal agent against the malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae). *Bioresource Technology*. 2007; 98: 1856–1860.

Singh, Gurbachan, Tiwari, R.K., Bajpai, C.K. and Solanki, K.R. Studies on exploitation of edible cactus in red soils of Bundelkhand. *Indian J. Soil Cons*. 2001; 29(2):184–186.

WHO, 2005. Guidelines for laboratory and field testing of mosquito larvicides. Geneva, WHO/CDS/WHOPES/GCDPP/ 2005; 13.

WHO, 2009. Guidelines for efficacy testing of household insecticide products.

Yang, Y.C., Lee, S.G., Lee, H.K., Kim, M.K., Lee, S.H. and Lee, H.S. A piperidine amide extracted from *Piper longum* L. fruit shows activity against *Aedes aegypti* mosquito larvae. *J. Agric. Food Chem*. 2002; 50: 3765–3767.