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Egg hatchability and larvicidal activity of *Swertia chirata* Buch. - Hams. ex Wall. against *Aedes aegypti* L. and *Culex quinquefasciatus* Say.

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Abstract: Crude *Swertia chirata* extracts (hexane, ethyl acetate & methanol) were tested for egg hatchability and larvicidal activity against two vector mosquitoes, *Aedes aegypti* and *Culex quinquefasciatus*. The lowest egg hatchability was recorded at 1000 ppm of ethyl acetate extract which showed 12.6, 20.4 and 25.4 % at 0-6, 6-12 and 12-18 h respectively against *Cx. quinquefasciatus*; it was 11.4, 17.8 and 23.4 % at 0-6, 6-12 and 12-18 h respectively against *Ae. aegypti*. The LC_{50} values for the larvicidal activity of ethyl acetate extract of *S. chirata* against first, second, third and fourth instar larvae of *Cx. quinquefasciatus* were 164.91, 220.10, 284.05 and 326.46 ppm and against *Ae. aegypti* 192.67, 237.30, 339.06 and 329.29 ppm respectively.

Keywords: *Swertia chirata*, *Culex quinquefasciatus*, *Aedes aegypti*, egg hatchability, larvicidal activity

Introduction

Mosquito is one of the blood sucking insects in the group of arthropods and it transmits parasites and pathogens which cause devastating impact on human beings. It spreads many dreadful diseases such as filariasis, malaria, dengue, yellow fever and japanese encephalitis, which contribute significantly to disease burden, death, poverty, and social debility in tropical countries (Jang *et al*., 2002). *Cx. quinquefasciatus* is an important vector of Bancraftian filariasis. There are 45 million cases of lymphatic filariasis in India alone (Bowers *et al*., 1995). Estimates suggest that about 120 million people over 73 countries are infected with human lymphatic filariasis (Southgate, 1984; WHO, 1997).

Aedes aegypti is a vector of arboviruses responsible for major diseases like dengue, dengue haemorrhagic fever and chikungunya. It infects more than 100 million people every year. Dengue fever has become an important public health problem as the number of reported cases continue to increase, especially with more severe forms of the disease, dengue haemorrhagic fever and dengue shock syndrome, or with unusual manifestations (Hendarto & Hadinegoro, 1992; Pancharoen *et al*., 2002). About two-fifth of the world's population are at risk of catching dengue according to the World Health Organization (WHO, 2003).

Global use of chemical insecticides viz., organophosphates such as Temephos and Fenthion and insect growth regulators such as Diflubenzuron and Methoprene are generally used for the control of mosquito larvae (Yang *et al*., 2002). Although effective, their repeated use has disrupted natural biological control systems led to outbreaks of insect species, widespread development of resistance, and undesirable effects on nontarget organisms, and fostered environmental and human health concerns (Yang *et al*., 2002; Pitasawat *et al*., 2007). Natural products are preferred because of their innate biodegradability and harmlessness (Ananthakrishnan, 1988). A large number of plant essential oils may be potential sources of mosquito larvicides, because they constitute a rich source of bioactive components (Lahlou *et al*., 2001; Cetin *et al*., 2004). Over the last 50 years, thousands of plants have been screened as potential sources of larvicidal, repellents and insecticidal activities (Sukumar *et al*., 1991).

Swertia chirata belongs to the family Gentianaceae, native of temperate Himalayas, found at an altitude of 1200-3000 m, from Kashmir to Bhutan. The entire plant is used in traditional medicine; however root is documented as the most powerful part for the pharmacological effect (Kirtikar & Basu, 1984). Phytochemical studies have revealed the presence of secondary metabolites such as xanthones, triterpenoids, glycosides, triterpenoid and alkaloid (Jensen & Schripsema, 2002; Joshi & Dhawan, 2005). The present study deals with the egg hatchability and larvicidal activity of crude solvent extracts of *S. chirata* against the larvae of *Ae. aegypti* and *Cx. quinquefasciatus*.

Materials and methods

Plant collection and extraction

Healthy, disease free plants of *S. chirata* (whole plant) were collected from Darjeeling (West Bengal, India). The species was identified and authenticated by Dr. Jeya Jyothi, Taxonomist, Department of Plant Biology and Biotechnology, Loyola College, Chennai and the voucher specimen (LCH 1002) was deposited at the departmental herbarium, Loyola College, Chennai. Freshly collected plant was washed thoroughly, shade dried in open air and grounded into powder. The *S. chirata* plant powder (3 kg) was sequentially soaked in hexane, ethyl acetate and methanol for 72 h respectively with intermittent shaking. After 72 h the solution was filtered and the filtrate was concentrated under reduced pressure using rotary vacuum evaporator. The filtrate was air dried to yield 38 g of hexane extract, 68 g of ethyl acetate extract and 55 g of methanol extract. All the crude extracts above obtained are stored at 4^0C in air tight containers until assay.

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Table 1. Larvicidal activity of Swertia chirata against different larval stages of Aedes aegypti

Instars	Solvents	LC_{50}	95% Confidence limit		LC_{90}	Chi-square
			LFL	UFL		
4th instar	Hexane	1076.94	903.40	1374.21	2181.42	26.50
	Ethyl acetate	329.29	247.28	414.98	816.83	58.38
	Methanol	1489.90	1194.34	2101.58	2728.44	14.31
3rd instar	Hexane	955.74	808.35	1195.14	2081.92	28.60
	Ethyl acetate	339.06	302.19	490.64	766.77	67.28
	Methanol	1307.01	1068.12	1764.58	2504.69	18.07
2nd instar	Hexane	866.19	699.38	1173.78	1923.52	37.26
	Ethyl acetate	237.30	125.09	337.30	733.96	83.54
	Methanol	1279.15	1023.31	1807.38	2653.54	28.23
1st instar	Hexane	875.89	707.09	1189.42	1784.37	41.13
	Ethyl acetate	192.67	127.46	253.68	494.91	69.41
	Methanol	996.74	775.08	1498.81	2242.25	38.35

Values were based on four concentrations and four replications with 25 larvae in each. Significant at p<0.05 level.

Mosquito culture

Ae. aegypti and *Cx. quinquefasciatus* larvae, were derived from various places with stagnant water bodies within Chennai, India, and were colonized and maintained continuously for generations in a laboratory free of exposure to pathogens, insecticides or repellents. They were maintained at 27±2^{0}C, 75-85% Relative humidity under a photoperiod of 14:10 h (light/dark) in the insectary. Larvae were fed on finely ground dog biscuit and yeast extract in the ratio of 3:1. Water was changed everyday to avoid scum formation, which might create toxicity. Pupae were transferred from the trays to a cup containing tap water and placed in screened cages (30x30x30 cm dimension) where the adults emerged. The adults of Ae. aegypti and Cx. quinquefasciatus were reared in the glass cages of 30x30x30 cm dimension. The adult colony was provided with 10% sucrose solution and was periodically blood-fed on restrained rats. After three days, ovitrap was kept in the cages and the eggs were collected and transferred to the enamel trays. Two developmental stages, larvae and adult females, were continuously available for the experiments and were maintained at the same condition.

Egg hatchability test

Egg hatchability was studied following the method of Su and Mulla (1998). Hundred freshly layed (0-6, 6-12 and 12-18 h old) eggs of *Ae. aegypti* and *Cx. quinquefasciatus* were exposed to four (1000, 500, 250 and 125 ppm) concentrations. *Cx. quinquefasciatus* eggs were separated with the help of fine brush. Each concentration was replicated five times. Each crude extract was dissolved in water with an emulsifier (Tween 80) to get the experimental concentrations. Tween 80 was used as negative control and egg hatchability was observed under the microscope. The hatch rate was assessed in percentage at 120 h post treatment using the following formula.

Number of hatched larva / Total number of eggs in treated water × 100

Larvicidal bioassay

Larvicidal activity was evaluated using WHO method (1975) with slight modifications. Twenty larvae of first, second, third and fourth instar were released separately in a 500 ml glass beaker containing 249 ml of dechlorinated water and 1.0 ml of the desired plant extract concentration. Five replicates for each concentration was run at a time. Tween 80 was used as negative control. Mortality and survival were recorded after 24 hours of the exposure period. The moribund and dead larvae in four replicates were combined and expressed as a percentage of larval mortality for each concentration. Dead larvae were identified when they failed to move after probing with a needle in the siphon or cervical region. Moribund larvae were those incapable of rising to the surface (within reasonable period of time) or showing the characteristic diving reaction when the water was disturbed. The mortality was calculated by EPA Probit analysis programme version 1.5.

Table 2. Larvicidal activity of Swertia chirata against different larval stages of Culex quinquefasciatus

Instars	Solvents	LC_{50}	95% Confidence limit		LC_{90}	Chi-square
			LFL	UFL		
4th instar	Hexane	962.89	829.57	1167.74	1905.51	24.51
	Ethyl acetat	326.46	224.59	429.15	887.45	67.85
	Methanol	1588.13	1244.69	2362.12	2937.77	15.89
3rd instar	Hexane	930.70	772.88	1202.66	1928.60	30.38
	Ethyl acetat	284.05	187.98	379.08	765.55	75.40
	Methanol	1297.58	1064.64	1737.66	2469.81	20.65
2nd instar	Hexane	896.75	715.00	1253.61	2003.06	38.78
	Ethyl acetat	220.10	120.78	311.50	620.70	94.72
	Methanol	1240.01	964.37	1888.76	2548.19	30.85
1st instar	Hexane	789.31	633.67	1065.92	1790.04	42.84
	Ethyl acetat	164.91	86.34	231.31	464.21	85.50
	Methanol	1030.91	789.85	1622.23	2295.62	42.11

Values were based on four concentrations and four replications with 25 larvae in each. Significant at p<0.05 level.

Results and discussion

In the present investigation the toxicity of *S. chirata* was tested at four different concentrations against the first, second, third and fourth instar larvae of *Cx. quinquefasciatus* and *Ae. aegypti*. The mortality rate was recorded and statistical data regarding LC_{50}, LC_{90}, 95% confidence limit and chi-square were calculated. The LC_{50} values of S. chirata against first, second, third and fourth instar larvae of *Cx. quinquefasciatus* were 164.91, 220.10, 284.05 and 326.46 ppm and against *Ae. aegypti*

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Table 3. Egg hatchability of Swertia chirata against Culex quinquefasciatus

Age of eggs	Solvent	Concentration			
		1000 ppm	500 ppm	250 ppm	125 ppm
00-06 h	Hexane	36.8±2.38	46.0±3.60	56.6±2.30	64.4±2.40
06-12 h		46.6±3.20	56.4±3.57	64.4±3.20	73.8±4.20
12-18 h		55.6±3.84	66.6±2.88	79.2±4.54	92.4±4.39
00-06 h	Ethyl acetate	12.6±2.40	18.6±3.04	26.4±3.78	33.2±3.83
06-12 h		20.4±2.70	26.8±2.58	33.0±3.80	36.2±2.58
12-18 h		25.4±3.84	36.6±2.70	39.6±2.07	56.2±3.70
00-06 h	Methanol	61.2±3.34	66.2±3.70	71.4±4.92	74.4±4.03
06-12 h		71.4±3.36	75.4±3.36	82.6±3.57	86.8±3.56
12-18 h		84.4±2.96	86.4±3.36	90.0±4.63	93.6±2.96
Control		98.0±2.12			

Values are mean of five replications of four concentrations. (Mean ±S.D.)

192.67, 237.30, 339.06 and 329.29 ppm respectively (Table 1 & 2). No mortality was observed in negative control. Maximum mortality was observed in ethyl acetate extract followed by hexane and methanol extract. In the present study first and second instar larvae were highly susceptible than third and fourth instar larvae of Cx. quinquefasciatus and *Ae. aegypti*. Maheswaran *et al*. (2008) also have reported similar findings as mentioned above in hexane extract of *Leucas aspera*. Redwane *et al*. (2002) reported that the ethyl acetate extract of *Quercus lusitania* showed 497 and 1531ppm against second and fourth instar larvae of *Cx. pipiens*.

Table 4. Egg hatchability of Swertia chirata against Aedes aegypti

Age of eggs	Solvent	Concentration			
		1000 ppm	500 ppm	250 ppm	125 ppm
00-06 h	Hexane	35.4±3.64	43.6±4.61	54.2±4.08	62.2±4.02
06-12 h		46.6±3.64	53.0±3.16	63.2±3.56	70.4±5.94
12-18 h		53.6±2.96	63.2±3.70	78.2±4.54	91.4±2.50
00-06 h	Ethyl acetate	11.4±1.81	16.2±2.77	24.6±2.07	31.2±3.03
06-12 h		17.8±1.48	23.6±2.70	28.8±2.16	32.8±4.96
12-18 h		23.4±4.39	34.6±3.04	37.6±2.96	54.0±4.47
00-06 h	Methanol	62.6±3.84	64.2±3.83	70.2±2.28	74.2±3.96
06-12 h		76.8±2.58	80.2±1.92	83.8±2.48	89.2±1.92
12-18 h		84.0±4.35	87.2±3.11	90.6±3.71	95.8±1.92
Control		99.0±1.34			

Values are mean of five replications of four concentrations. (Mean ±S.D.)

In the laboratory test three different age groups of the eggs of *Cx. quinquefasciatus* and *Ae. aegypti* were tested at four different concentrations of *S. chirata* leaf extract. At 1000 ppm concentration, ethyl acetate extract showed 12.6, 20.4 and 25.4 % egg hatchability at 0-6, 6-12 and 12-18 h against *Cx. quinquefasciatus* and 11.4, 17.8 and 23.4 % egg hatchability at 0-6, 6-12 and 12-18 h against *Ae. aegypti* respectively. At 125 ppm concentration the hatchability was 33.2, 36.2 and 56.2 % at 0-6, 6-12, 12-18 h in *Cx. quinquefasciatus* and 31.2, 32.8 and 54.0 % at 0-6, 6-12, 12-18 h in *Ae. aegypti* followed by hexane and methanol extracts (Table 3 & 4). In the present study the ethyl acetate extract of *S. chirata* retarded embryonic growth and delayed hatch. The treated eggs contained developed embryos the eclosion of the egg was incomplete (Miura *et al*., 1976). The ovicidal efficacy compared well with an earlier report; the bioactive compound Azadirachtin (*Azadirachta indica*) showed complete ovicidal activity in eggs of *Cx. tarsalis* and Cx. quinquefasciatus exposed to 10 ppm concentration (Su & Mulla 1998). Ouda *et al*. (1998) reported that the seed extract of *Atriplex canescens* showed complete ovicidal at 1,000 ppm concentration in eggs of *Cx. quinquefasciatus*. The present findings corroborated with earlier studies of ethyl acetate extracts of *Glycosmis pentaphylla* leaves which reduced egg hatchability of *Cx. quinquefasciatus* and prolonged developmental duration of mosquito larvae (Muthukrishnan *et al*., 1999). The botanical insecticides are generally pest-specific and are relatively harmless to non-target organisms including man, easily biodegradable and also harmless to the environment (Rembold, 1994). To the best of our knowledge this is the first report of effective ovicidal and larvicidal activity of different solvent extracts of *S. chirata* against *Cx. quinquefasciatus* and *Ae. aegypti*. Ethyl acetate extract of *S. chirata* showed potential larvicidal activity. In short, our findings suggested that leaves of *S. chirata* and its effective constituents may be explored as a potential natural larvicide. Further investigations for the mode of the constituents' actions, effects on non-target organisms and field evaluation are necessary.

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References

1. Ananthakrishnan TN (1988) Insect plant interactions problems and perspectives, In: Dynamics of insect plant interaction (Eds. Ananthakrishnan TN & Raman A). New Delhi. Oxford & IBH Publishing Co. pp: 1-11.
2. Bowers WS, Sener B, Evans PH, Bingol F and Erdogan I (1995) Activity of Turkish medicinal plants against mosquitoes *Aedes aegypti aind Anopheles gambiae*. *Insect Sci. & Appl*. 16 (3&4): 330-342.
3. Cetin H, Erler F and Yanikoglu A (2004) Larvicidal activity of a botanical natural product, AkseBio2, against *Culex pipiens*. *Fitoterapia*. 75, 724-728.
4. Hendarto SK and Hadinegoro SR (1992) Dengue encephalopathy. *Acta. Paediatr. Jpn*. 34, 350-357.
5. Jang YS, Kim MK, Ahn YJ and Lee HS (2002) Larvicidal activity of Brazilian plants against *Aedes aegypti and Culex pipiens pallens* (Diptera:Culicidae). *Agric. Chem. Biotechnol*. 45(3), 131-134.

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6. Jensen SR and Schripsema J (2002) Gentainaceae systematics and natural History (Eds. Struwe L & Albert V), Cambridge University Press. London. pp: 573-632.
7. Joshi P and Dhawan V (2005) *Swertia chirayita* – an overview. *Cur. Sci*. 89, 635-640.
8. Kirtikar KR, and Basu BD (1984) Indian medicinal plants. (Lalit Mohan Basu, 49, Leader Road, Alahabad 3). pp: 1664-1666.
9. Lahlou MR, Berrada M, Hmamouchi and Lyagoubi M (2001) Effect of some Moroccan medicinal plants on mosquito larvae. *Thérapie*. 56, 193-196.
10. Maheswaran R, Sathish S and Ignacimuthu S (2008) Larvicidal activity of *Leucas aspera* (Willd.) against the larvae of *Culex quinquefasciatus* Say. and *Aedes aegypti* L. *Int. J. Integrative Biol*. 2 (3), 214 – 217.
11. Miura T, Schafer CH, Takahashi RM and Mulligan FS (1976) Effects of insect growth inhibitor, Dimilin, on hatching of mosquito eggs. *J. Econ. Ent*. 69, 655 – 658.
12. Muthukrishnan J, Karlheinz S, Klaus HH and Matthias WL (1999) Inhibition of juvenile biosynthesis in *Gryllus bimaculatus* by *Glycosmis pentaphylla* leaf compounds. *Phytochem*. 50, 249-254.
13. Ouda NAA, A1-chalabi BBM, A1- charchafchi FFMR, Mohsen ZZH (1998) Extract of *Atriplex canescens* against *Culex quinquefasciatus*. *Pharm. Biol*. 36(1), 69-71.
14. Pancharoen C, Kulwichit W, Tantawichien T, Thisyakorn U and Thisyakorn C (2002) Dengue infection: a global concern. *J. Med. Assoc. Thai*. 85, S25-33.
15. Pitasawat B, Champakaew D, Choochote W, Jitpakdi A, Chaithong U, Kanjanapothi D, Rattanachanpichai E, Tippawangkosol P, Riyong D, Tuetun B and Chaiyasit D (2007) Aromatic plant-derived essential oil: An alternative larvicide for mosquito control. *Fitoterapia*. 78, 205-210.
16. Redwane A, Lazrek HB, Bouallam S, Markouk M, Amarouch H and Jana M (2002) Larvicidal activity extracts from *Quercus lusitania* var. infectoria galls (Oliv.). *J. Ethnopharmacol*. 79, 261-263.
17. Rembold H (1994) Advances in invertebrate reproduction. Elsevier Sci. Publ. 3, 481-491.
18. Southgate BA (1984) Recent advances in the epidemiology and control of filarial infections including entomological aspects of transmission. *Trans. R. Soc. Trop. Med. Hyg*., 78, 19-28.
19. Su and Mulla (1998) Ovicidal activity of neem products (Azadirachtin) against *Culex tarsalis* and *Culex quinquefasciatus* (Diptera: Culicidae). *J. Am. Mosq. Control. Assoc*., 14(2), 204-209.
20. Sukumar K, Perich MJ and Booba LR (1991) Botanical derivatives in mosquito control: A Review. *J. Am. Mosq. Control Assoc*., 7, 210-237.
21. World Health Organization (1975) Instruction for determining the susceptibility or resistance of mosquito larvae to insecticides. Mimeographed document. WHO/VBC/1975, 583.
22. World Health Organization (1997) UNDP/World Bank/ WHO Special Programme for Research and Training in Tropical Diseases (TDR), Tropical Disease Research: Progress 1995-96. Thirteenth Programme Report of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, Geneva, Switzerland. pp: 141.
23. World Health Organization (2003) Dengue (Online, access in 03/06/2003), Available at http://w.w.w.who.int/inf-fs/en/fact117.html.
24. Yang YC, Lee SG, Lee HK, Kim MK, Lee SH and Lee HS (2002) A Piperidine amide extracted from *Piper longum* L. fruit shows activity against *Aedes aegypti* mosquito larvae. *J. Agric. Food Chem*. 50, 3765 – 3767.

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