

Research Article

LARVICIDAL POTENTIAL OF *HYDNOCARPUS PENTANDRA* (BUCH.-HAM.) OKEN SEED EXTRACTS AGAINST *Aedes Aegypti* LINN. AND *Culex quinquefasciatus* SAY (DIPTERA: CULICIDAE)

**G. Sivaraman, M. Gabriel Paulraj, M. Rajiv Gandhi,
A. Daniel Reegan and S. Ignacimuthu***

Entomology Research Institute, Loyola College, Chennai-600 034, Tamil Nadu, India

Article History: Received 2nd January 2014; Accepted 19th May 2014; Published on 21st May 2014

ABSTRACT

A study was conducted to evaluate the larvicidal activity of solvent extracts of *Hydnocarpus pentandra* (Family: Flacourtiaceae) seed against two mosquito species, *Aedes aegypti* L. and *Culex quinquefasciatus* Say. Crude seed extracts were prepared by hexane, chloroform, ethyl acetate and methanol solvents and larvicidal activity was studied at 62.5ppm, 125ppm, 250ppm and 500ppm concentrations against the late third instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus*. Results clearly showed that the chloroform extract was the most effective treatment against both species. The median lethal concentration (LC₅₀) values of chloroform extract were calculated as 248.28ppm and 89.52ppm for *Ae. aegypti* and *Cx. quinquefasciatus*, respectively. The LC₉₀ values were 731.62ppm and 678.48ppm for *Ae. aegypti* and *C. quinquefasciatus*, respectively.

Keywords: *Hydnocarpus pentandra*, filarial vector, dengue vector, lethal concentrations.

INTRODUCTION

Unrestrained application of synthetic insecticides in vector mosquito control has resulted in many environmental problems, pesticide resistance, destruction of non-target organisms and human health problems (Rawani *et al.*, 2009; Mathew *et al.*, 2009). Botanicals offer an eco-friendly way of mosquito management. Mosquitoes can be effectively controlled at their immature stages. Destruction of mosquitoes at larval stages is an easy and effective way. So far many botanicals have been screened for their mosquito larvicidal activities (Das *et al.*, 2003; Choochote *et al.*, 2004; Chowdhury *et al.*, 2008). Ample literature on larvicidal activity of seed extracts against different mosquito species is available (Zebitz Claus, 1984; Choochote *et al.*, 2004; Akram *et al.*, 2010; Kundu *et al.*, 2013).

Botanical insecticides are generally pest specific and are harmless to non-target organisms including humans (Khater, 2012). *H. pentandra* is a common tree found in wet evergreen forests and along streams. It is endemic to the Western Ghats of India. The seed oil of *H. pentandra* is used to treat leprosy (Jain and Srivastava, 2005).

With this pharmacological background, the present study was conducted to evaluate the mosquito larvicidal activity of *H. pentandra* seed extracts against *Ae. aegypti* and *Cx. quinquefasciatus* larvae.

MATERIALS AND METHODS

Plant seed collection

H. pentandra dried seed were obtained from market in Parrys at Chennai, Tamil Nadu. The plant seed was identified by taxonomist at Entomology Research Institute, Loyola College, Chennai. The voucher specimen (ERI-BP-GS-001) was deposited at the Entomology Research Institute.

Extraction

The seed shells were removed and the kernels were powdered coarsely. The powder was soaked in hexane (1:3 ratio of power: solvent, respectively) for a period of 48 h with intermittent shaking and filtered. The junk was air dried and sequentially extracted in chloroform, ethyl acetate and methanol solvents. The extracts were concentrated under reduced pressure using rotary evaporator and stored at 4°C.

*Corresponding author e-mail: entolc@hotmail.com, Phone: +9144-28178348, Fax: +9144-28175566

Mosquitoes

Laboratory reared *Ae. aegypti* and *Cx. quinquefasciatus* mosquito larvae were used in the bioassay experiments. The rearing conditions were: 27±1°C, 75-85% RH and 14:10 h photoperiod.

Mosquito larvicidal bioassay

Larvicidal activity of the extracts was evaluated using the method recommended by the World Health Organization (WHO, 2005). A range of concentrations viz., 62.5, 125, 250 and 500 ppm of each solvent extract were prepared with emulsifier and taken in 100 ml plastic cups separately. Third instar larvae (20 larvae) of each *Ae. aegypti* and *Cx. quinquefasciatus* were introduced into each cup that contained test solutions. Emulsifier control and water control were also maintained separately. Five replicates were maintained for each treatment and control. Larval mortality was recorded after 24h of treatment. Larvae were considered dead when they did not respond to stimulus or did not rise to the surface of the solution.

5. Statistical analysis

The lethal concentration values (LC₅₀ and LC₉₀) were calculated by probit analysis software (SPSS 11.5 version) (Finney, 1971).

RESULTS

Larvicidal effect of *H. pentandra* seed extracts on the third instar larvae was recorded and the results are presented in Table 1. The median lethal concentration (LC₅₀ and LC₉₀) values were least in chloroform extract that means it was the most effective treatment. The LC₅₀ and LC₉₀ values of chloroform extract were 248.28 and 731.62 ppm, respectively for *Ae. aegypti* and 89.52 and 678.48 ppm respectively for *Cx. quinquefasciatus*. LC₅₀ and LC₉₀ values of hexane extract were 853.45 and 1538.83 ppm, respectively for *Ae. aegypti* and 130.74 and 1062.34 ppm respectively for *Cx. quinquefasciatus*. Ethyl acetate extract recorded LC₅₀ and LC₉₀ values of 543.72 and 1035.46 ppm respectively for *Cx. quinquefasciatus*. Methanol extract showed LC₅₀ and LC₉₀ values of 936.29 and 1776.32 ppm respectively for *Ae. aegypti* and 1144.76 and 2202.70 ppm respectively for *Cx. quinquefasciatus*. The larvicidal activity was found to be concentration dependent. From the results it is clear that the chloroform extract was the most potent treatment against *Ae. aegypti* and *Cx. quinquefasciatus* larvae.

Table 1. Larvicidal activity of *H. pentandra* seed extracts against *Ae. aegypti* and *Cx. quinquefasciatus*.

Mosquito species	Solvent extracts	LC ₅₀ (ppm)	95% confidence limit		LC ₉₀ (ppm)	95% confidence limit		Intercept ± SE	x ²
			Lower (ppm)	Upper (ppm)		Lower (ppm)	Upper (ppm)		
<i>Ae. aegypti</i>	Hexane	853.44611	602.68	2093.58	1538.83	1020.40	4226.85	-0.5±0.2	12.4*
	Chloroform	248.28	176.55	326.61	731.62	574.38	1096.26	-0.6±0.1	16.4*
	Ethyl acetate	-	-	-	-	-	-	-	-
	Methanol	936.29	616.77	4076.01	1776.32	1087.40	8836.05	-1.4±0.2	16.9*
	Hexane	130.74	-294.70	260.99	1062.34	686.24	3930.61	-0.1±0.1	9.5*
<i>Cx. quinquefasciatus</i>	Chloroform	89.52	-263.24	201.50	678.48	470.07	1756.49	0.2±0.2	8.2*
	Ethyl acetate	543.72	437.34	784.11	1035.46	791.82	1656.06	-1.4±0.1	10.4*
	Methanol	1144.763	-	-	2202.70	-	-	-1.4±0.2	11.9*

LC₅₀-lethal concentration that kills 50% of the exposed larvae; LC₉₀-lethal concentration that kills 90% of the exposed larvae. *p 0.5, level of significance of chi-square values.

DISCUSSION

Plants are excellent sources of natural pesticides. Different types of phytochemicals are present in plants and these phytochemicals are the main toxicants to many insect pests and vector mosquitoes. In the present study the chloroform extract showed very high larvicidal activity against the two test mosquito species. Jang *et al.* (2002) and Cavalcanti *et al.* (2004) reported that pure plant extracts are less expensive and highly efficacious for the control of mosquitoes. Seed extracts of many plants

possess mosquito larvicidal activity as evidenced from the studies of Zebitz Claus (1984), Choochote *et al.* (2004), Akram *et al.* (2010), Kundu *et al.* (2013) and Musman *et al.* (2013). In some plants the seed extracts did not present significant larvicidal activity. For example, Musman *et al.* (2013) have studied the larvicidal activity of roots, stem, leaves, flowers and seeds of *Ipomoea pes-caprae* against *Ae. aegypti*. They found that the leaf extract only showed significant larvicidal activity at lower concentrations.

H. pentandra is traditionally used in the treatment of leprosy and skin diseases (Lima *et al.*, 2005). This plant is also reported for its anticancer activity, hypolipidemic activity, *in vivo* anti-inflammatory and anti-neoplastic activities (ICMR, 2008). In the present study, the most effective treatment was identified as chloroform extract, which was found to be better than previous reports. The treatments were found to be species specific. Chloroform extract was more toxic to *Cx. quinquefasciatus* than *Ae. aegypti*. In the present study the chloroform extract presented LC₅₀ and LC₉₀ values of 89.52 and 678.48 ppm respectively against *Cx. quinquefasciatus*, which was comparatively less than the values reported by Mathew *et al.* (2009). They reported that the *Clitoria ternatea* seed methanol extract presented LC₅₀ and LC₉₀ values of 148.2 and 681.4 ppm, respectively at 24 h against *Cx. quinquefasciatus*.

Similarly *H. pentandra* seed chloroform extract recorded higher toxicity in *Ae. aegypti* larvae compared to previous studies. In the present study the LC₅₀ and LC₉₀ values of *H. pentandra* seed chloroform extract against *Ae. aegypti* were 248.28 and 731.62 ppm, respectively and these values were very less than the LC₅₀ (195.0 ppm) and LC₉₀ (599.2 ppm) values of *Clitoria ternatea* seed methanol extract against *Ae. aegypti* (Mathew *et al.* 2009). Similarly the effect of *H. pentandra* seed chloroform extract in the present study was significantly higher than the activity of the acetone extract of *Ageratina adenophora* leaves as reported by Mohan *et al.* (2007). They obtained a LC₅₀ value of 227.20 ppm against *Cx. quinquefasciatus* and 356.70 ppm against *A. aegypti* at 24 h when the acetone extract of *A. adenophora* leaves was screened. Akram *et al.* (2010) have studied the larvicidal effect of seed extracts of 10 varieties of citrus against *Aedes albopictus*. They observed that *Citrus jambhiri* was the most effective seed extract with the lowest LC₅₀ of 119.993 and 108.85 ppm after 24 and 48 h respectively. They found that larvicidal activity increased when the exposure time was extended. In the present study the larvicidal activity was recorded after 24 h only.

CONCLUSION

H. pentandra seed chloroform extract produced high larval mortality against third instar larvae of

Ae. aegypti and *Cx. quinquefasciatus* at 500 ppm concentration. So *H. pentandra* seed extract can be successfully used as effective larvicide in mosquito control programmes.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this article.

ACKNOWLEDGEMENT

The authors are thankful to Entomology Research Institute, Loyola College, Chennai, for financial support to carry out this work.

REFERENCES

- Akram, W., Ali Khan, H.A., Hafeez, F., Bilal, H., Kim, Y.K. and Lee, J.J., 2010. Potential of Citrus seed extracts against dengue fever mosquito, *Aedes albopictus* (Skuse) (Culicidae: Diptera). *Pak. J. Bot.*, 42(4): 3343-3348
- Cavalcanti, E.S.B., Morais, S.M., Ashley, A.L.M and William, P.S.E., 2004. Larvicidal activity of essential oils from Brazilian plants against *Aedes aegypti* L. *Mem. Inst. Oswaldo Cruz*, 99: 541-544.
- Choochote, W., Tuetun, B., Kanjanapothi, D., Rattanachanpichai, E., Chaithong, U., Chaiwong, P., Jitpakdi, A., Tippawangkosol, P., Riyong, D. and Pitasawat, B., 2004. Potential of crude seed extract of celery, *Apium graveolens*, against the mosquito *Aedes aegypti* L. (Diptera: culicidae). *J. Vec. Ecol.*, 29: 340-346.
- Chowdhury, N., Ghosh, A. and Chandra, G. 2008. Mosquito larvicidal activities of *Solanum villosum* berry extract against the dengue vector *Stegomyia aegypti*. *BMC Comp. Alt. Med.*, 8:10 doi:10.1186/1472-6882-8-10.
- Das, N.G., Baruah, I., Talukdar, P.K., and Das S.C., 2003. Evaluation of botanicals as repellents against mosquitoes. *J. Vector Borne Dis.*, 40: 49-53.
- Finney, D.J., 1971. Probit analysis, Cambridge University Press, London, p. 256.
- Indian Council of Medical Research (ICMR), 2008. Medicinal Plant Unit New Delhi. *Quality Standards of Indian Medicinal Plants*, 5: 256–264.

- Jain, S.K. and Srivastava, S., 2005. Traditional uses of some Indian plants among islanders of the Indian Ocean. *Ind. J. Trad. Knowledge*, 4(4): 345-357.
- Jang, Y.S., Kim, M.K., Ahn, Y.J. and Lee, H.S., 2002. Larvicidal activity of Brazilian plants against *Aedes aegypti* and *Culex pipiens* Pallens (Diptera: Culicidae). *Agric. Chem. Biotechnol.*, 44: 23-26.
- Kundu, M., Rawani, A. and Chandra, G. 2013. Evaluation of Mosquito Larvicidal Activities of Seed Coat Extract of *Cassia sophera* L. *J. Mosq. Res.*, 3(11). doi: 10.5376/jmr.2013.03.0011
- Kundu, M., Rawani, A. and Chandra, G. 2013. Evaluation of Mosquito Larvicidal Activities of Seed Coat Extract of *Cassia sophera* L. *J. Mosq. Res.*, 3(11). doi: 10.5376/jmr.2013.03.0011
- Lima, J.A., Oliveira, A.S., Miranda, A.L.P., Rezende, C.M. and Pinto, A.C., 2005. Anti-inflammatory and antinociceptive activities of an acid fraction of the seeds of *Carpotroche brasiliensis* (Raddi) (Flacourtiaceae). *Braz. J. Med. Biol. Res.* 38: 1095–1103.
- Mathew, N., Anitha, M.G., Bala, T.S.L., Sivakumar, S.M., Narmadha, R. and Kalyanasundaram, M., 2009. Larvicidal activity of *Saraca indica*, *Nyctanthes arbor-tristis* and *Clitoria ternatea* extracts against three mosquito vector species. *Parasitol. Res.*, 104:1017–1025.
- Mohan, D.R. and Ramaswamy, M., 2007. Evaluation of larvicidal activity of the leaf extract of a weed plant, *Ageratina adenophora*, against two important species of mosquitoes, *Aedes aegypti* and *Culex quinquefasciatus*. *Afric. J. Biotech.*, 6 (5): pp. 631-638.
- Musman, M., Karina, S. and Almuksin, S., 2013. Larvicide of *Aedes aegypti* (Diptera: Culicidae) from *Ipomoea pes-caprae* (Solanales: Convolvulaceae), *AAFL Bioflux*, 6(5): 446-452.
- Rawani, A., Haldar, K.M., Ghosh, A. and Chandra, G. 2009. Larvicidal activities of three plants against filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae). *Parasitol. Res.* 105:1411-1417.
- WHO, 2005. Guidelines for laboratory and field testing of mosquito larvicides. World Health Organization, Geneva WHO/CDS/WHOPES/GCDPP/13.
- Zebitz Claus, P.W. 1984. Effect of some crude and azadirachtin-enriched neem (*Azadirachta indica*) seed kernel extracts on larvae of *Aedes aegypti*. *Entomol. Exp. Appl.*, 35:11-16.