Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Asian Journal of Plant Science and Research, 2012, 2 (5):633-637

ISSN : 2249-7412
CODEN (USA): AJPSKY

Mosquito larvicidal activity of *Cadaba indica lam* leaf extracts against the dengue vector, *Aedes aegypti*

Kandasamy Kalimuthu[1], Kadarkarai Murugan[1], Chellasamy Panneerselvam[1] and Jiang- Shiou Hwang[2]

[1]*Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore - 641 046, India*
[2]*Institute of Marine Biology, National Taiwan Ocean University, 202 Keelung, Taiwan*

ABSTRACT

Mosquitoes are associated with the transmission of malaria, dengue, chikungunya, Japanese encephalitis, filariasis and other viral diseases throughout the globe. The present study was carried out to establish the properties of leaf extract of Cadaba indica lam with different solvents Ethanolic, Hexane, Chloroform and Petroleum ether - were tested for larvicidal activity against mosquitoe such as dengue vector, Aedes aegypti. The Ethanolic, Hexane, Chloroform and Petroleum ether was moderate considerable mortality; however, the highest larval mortality was ethanolic extract observed in mosquito vector. The ethanolic extract had higher mortality with the values of LC50= 115.70, 96.09, 144.50, and 143.75 ppm and LC90= 215.46, 204.98, 233.82 and 260.86 ppm was observed. The results suggest ethanolic extract of C.indica lam have potential to be used as an good larvicidal activity an ideal eco-friendly approach for the control of dengue vector, A. aegypti as target species of vector control programs.

Keywords: *Aedes aegypti*, *Cadaba indica* lam, larvicidal, different solvent,

---

INTRODUCTION

Mosquito-borne diseases, such as malaria, filariasis, dengue, yellow fever, and Japanese encephalitis, contribute significantly to disease burden, death, poverty, and social debility in tropical countries [11], [10]. Mosquito-borne diseases have an economic impact, including loss in commercial and labor outputs, particularly in countries with tropical and subtropical climates; however, no part of the world is free from vector-borne diseases [8]. An obvious method for the control of mosquito-borne diseases is the use of insecticides, and many synthetic agents have been developed and employed in the field with considerable success. However, one major drawback with the use of chemical insecticides is that they are non-selective and could be harmful to other organisms in the environment. It has also provoked undesirable effects, including toxicity to nontarget organisms, and fostered environmental and human health concerns [14]. The toxicity problem, together with the growing incidence of insect resistance, has called attention to the need for novel insecticides [16] and for more detailed studies of naturally occurring insecticides [5]. Natural products of plant origin with insecticidal properties have been tried in the recent past for control of variety of insect pests and vectors. Plants are considered as a rich source of bioactive chemicals [29] and they may be an alternative source of mosquito control agents. Ehanobotnical searchreveals use of many traditional herbs in treatment of various diseases, which are usually free fromside effects, are economical and also accessible to humans and provide significant potential for the development of novel biomolecules [19]. Herbal medicine for curing number of disease, *Cassia tora* [25] *Cassia obtusifolia* [26]. However 80% of the world's population use

plant as their primary source of medication [2]. Natural products are generally preferred because of their less harmful nature to non-target organisms and due to their innate biodegradability.

Many studies on plant extracts against mosquito larvae have been conducted around the world. Extracts or essential oils from plants may be alternative sources of mosquito larval control agents, as they constitute a rich source of bioactive compounds that are biodegradable into nontoxic products and potentially suitable for use in control of mosquito larvae. In fact, many researchers have reported on the effectiveness of plant extracts or essential oils against mosquito larvae [27], [3], [4]. [18] studied the effect of some indigenous plants on the larvicide and ovipositional properties on *A. stephensi*. [6] investigated that the larvicidal effect of resinous exudate from the tender leaves of *Azadirachta indica*. *Citrullus colocynthis* is an annual herb found in wild as well as cultivated throughout India in the warm areas. The leaf and fruit extracts of bel (*Aegle marmelos*) and leaf extracts of *Opuntia*, *Nochi*, and *Jatropha* are potential against various pests [28]. The ethyl acetate extracts of the stem bark exhibited moderate insecticidal activity against *Phaedon cochleariae* and *Musca domestica* [24]. A 23% mortality was noted at I instar larvae by the treatment of *A. ilicifolius* at 20 ppm, whereas it was increased to 89% at 100 ppm of *A. ilicifolius* leaf extract treatment [13].

Hence, the present study has been attempted to make a survey of widely distributed plant, whose extracts may be of great biocontrol value in the context of integrated vector control management. The results of the present study would be useful in promoting research aimed at the development of new agents for mosquito control based on bioactive from indigenous plant source. In view of the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide, this study was undertaken to assess the larvicidal potential of the extracts from the medicinal plant *Cadaba indica* lam against medically important species of mosquito vector, *A. aegypti*.

MATERIALS AND METHODS

Collection of eggs and maintenance of larvae

The eggs of *A. aegypti* were collected from National Centre for Disease Control (NCDC) field station of Mettupalayam, Tamil Nadu, India, using an ‘O’ type brush. These eggs were brought to the laboratory and transferred to 18×13×4 cm enamel trays containing 500 ml of water for hatching. The mosquito larvae were pedigree dog biscuits and yeast at 3:1 ratio. The feeding was continued until the larvae transformed into the pupal stage.

Maintenance of pupae and adults

The pupae were collected from the culture trays and transferred to plastic containers (12×12 cm) containing 500 ml of water with the help of a dipper. The plastic jars were kept in a 90×90×90 cm mosquito cage for adult emergence. Mosquito larvae were maintained at 27+2°C, 75–85% RH, under a photoperiod of 14 L:10D. A 10% sugar solution was provided for a period of 3 days before blood feeding.

Blood feeding of adult mosquito vectors

The adult female mosquitoes were allowed to feed on the blood of a rabbit (a rabbit per day, exposed on the dorsal side) for 2 days, to ensure adequate blood feeding for 5 days. After blood feeding, enamel trays with water from the culture trays were placed in the cage as oviposition substrates.

Plant bioassay

Cadaba indica lam was collected from the Kaveri river bank, Manappalli, Namakkal, India. The plants were authenticated at BSI (Botanical Survey of India), and the voucher specimens were deposited at Zoology Department, Bharathiar University, Coimbatore, Tamil Nadu, India. *C. indica* lam plant was washed with tap water and shade-dried at room temperature. The dried leaves (800 g) were powdered mechanically using commercial electrical stainless steel blender and extracted with ethanolic, hexane, chloroform and petroleum ether in a Soxhlet apparatus (boiling point range 60–80°C for 8 h. The extract was concentrated under reduced pressure 22–26 mmHg at 45°C and the residue obtained was stored at 4°C. The extracts filtered through a Buchner funnel with Whatman number 1 filter paper. The yield of extracts was ethanolic (12.22 g), hexane (9.34 g), chloroform (9.64 g) and petroleum ether (7.64 g). One gram of the plant residue was dissolved in 100 ml of acetone (stock solution) considered as 1% stock solution. From the stock solution, 50 to 250 ppm various concentration was prepared with decholorinated tap water, respectively.

Larval toxicity test

A laboratory colony of *A. aegypti* larvae was used for the larvicidal activity. Twenty-five individuals of early-fourth instar larvae were kept in 500-ml glass beaker containing 249 ml of dechlorinated water and 1 ml of desired concentration of plant extracts were added. Larval food was given for the test larvae. At each tested concentration, two to five trials were made and each trial consists of five replicates. The control was setup by mixing 1 ml of acetone with 249 ml of dechlorinated water. The larvae exposed to dechlorinated water without acetone served as control. The control mortalities were corrected by using Abbott's formula [1]. LC50 and LC90 were calculated from toxicity data by using probit analysis [7].

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \times 100$$

$$\text{Percentage mortality} = \frac{\text{ObserNumber of dead larvaeved mortality in treatment} - \text{Observed mortality in control}}{\text{Number of larvae introduced}} \times 100$$

Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC50 and LC90, and other statistics at 95% fiducidal limits of upper confidence limit and lower confidence limit, and $\chi 2$ values were calculated using Finney's method. SPSS software package 16.0 Version was used.

Table 1. Larvicidal activity of Ethanolic solvent extracts of *C. indica* lam against the dengue vector, *A. aegypti*

Larval stage	Slope	LC50(LC90) (ppm)	95%Confidence Limit				X^2 (df=3)
			LC50		LC90		
			LCL	UCL	LCL	UCL	
I	0.3904	115.70(215.46)	104.76	125.75	200.42	235.12	3.420
II	0.3276	96.09(204.98)	61.15	120.15	174.31	264.80	5.432
III	0.3364	144.50(233.82)	101.40	126.15	215.58	258.52	1.241
IV	0.3296	143.75(260.86)	104.97	180.83	213.65	386.55	9.829

LCL - lower confidence limit, UCL - upper confidence limit, χ2 - chi square value, df - degrees of freedom

Table 2. Larvicidal activity of Hexane solvent extracts of *C. indica* lam against the dengue vector, *A. aegypti*

Larval stage	Slope	LC50(LC90) (ppm)	95%Confidence Limit				X^2 (df=3)
			LC50		LC90		
			LCL	UCL	LCL	UCL	
I	0.3964	116.82(214.27)	106.16	123.68	214.27	233.40	3.216
II	0.3464	97.24(203.53)	84.22	108.42	188.09	244.02	2.721
III	0.3484	144.50(233.82)	101.40	126.15	215.58	258.52	1.241
IV	0.3464	169.79(297.12)	157.81	182.66	272.86	330.99	0.421

LCL - lower confidence limit, UCL - upper confidence limit, χ2 - chi square value, df - degrees of freedom

Table 3. Larvicidal activity of Chloroform solvent extracts of *C. indica* lam against the dengue vector, *A. aegypti*

Larval stage	Slope	LC50(LC90) (ppm)	95%Confidence Limit				X^2 (df=3)
			LC50		LC90		
			LCL	UCL	LCL	UCL	
I	0.3892	115.70(215.46)	104.76	125.75	200.42	235.12	3.420
II	0.3388	96.09(204.98)	61.15	120.15	174.31	264.80	5.432
III	0.3484	144.50(233.82)	101.40	126.15	215.58	258.52	1.241
IV	0.3508	174.28(298.02)	162.53	187.05	274.23	331.06	0.749

LCL - lower confidence limit, UCL - upper confidence limit, χ2 - chi square value, df - degrees of freedom

RESULTS

The Ethanolic, Hexane, Chloroform and Petroleum ether extracts of the leave of the plant *Cadaba indica* lam were studied for use as eco-friendly insecticides instead. Results on the larvicidal activities of leaf extracts obtained in this study confirm their potential for the control of larval population of mosquito vector *A. aegypti*. Ethanolic, Hexane, Chloroform and Petroleum ether resulted in moderate mortality, however instead of polarity based four solvent the ethanolic extract (Tables 1) had higher mortality with the values of LC50= 115.70, 96.09, 144.50, and 143.75 ppm

and LC90= 215.46, 204.98, 233.82 and 260.86 ppm was observed. The Hexane extract (Tables 2) observed in *A. aegypti* had values of LC50=116.82, 97.24, 144.50 and 169.7955 ppm and LC90=214.27, 203.53, 233.82 and 297.12 ppm, respectively. The Chloroform extract (Tables 3) observed in had values of LC50=115.70, 96.09, 144.50 and 174.28 ppm and LC90=215.46, 204.98, 233.82 and 298.02 ppm, respectively. The Petroleum ether extract (Tables 4) observed in had values of LC50=137.32, 156.52, 181.06 and 202.37 ppm and LC90=263.81, 303.44, 346.62 and 348.83 ppm, respectively. The $\chi 2$ values are significant at $P<0.05$ level. The 95% confidence limits LC50 (LCL–UCL) and LC90 (LCL–UCL) were also calculated. Larval mortality was observed after 24 h exposure. No mortality was observed in the control group. The results of larvicidal activity clearly indicate that the percentage of mortality being directly proportional to the concentration of the extract. Solvents of the plant extract of *C. indica* lam were used at different concentrations, ranging from 50 to 250 ppm, respectively.

Table 4. Larvicidal activity of Petroleum ether solvent extracts of *C. indica* lam against the dengue vector, *A. aegypti*

Larval stage	Slope	LC50(LC90) (ppm)	95%Confidence Limit				X^2 (df=3)
			LC50		LC90		
			LCL	UCL	LCL	UCL	
I	0.3508	137.32(263.81)	124.91	149.225	242.54	293.09	3.362
II	0.3140	156.52(303.44)	142.94	170.52	275.23	344.36	0.2100
III	0.2780	181.06(346.62)	165.80	198.93	309.27	403.89	1.425
IV	0.2892	202.37(348.83)	187.64	220.67	314.374	400.24	0.666

LCL - lower confidence limit, UCL - upper confidence limit, χ2 - chi square value, df - degrees of freedom

DISCUSSION

Mosquito-borne diseases, such as filariasis, malaria, dengue, yellow fever, and Japanese encephalitis, contribute significantly to disease burden, death, poverty, and social debility in tropical countries [12]. Lymphatic filariasis caused by *W. bancrofti* and transmitted by mosquito *C. quinquefasciatus* is found to be more endemic in the Indian subcontinent. It is reported that *C. quinquefasciatus* infects more than 100 million individuals worldwide annually [23]. The larvicidal activity of the essential oil aqueous solutions of the stalks and leaves of *Croton argyrophylloides*, *Croton nepetaefolius*, *Croton sonderianus*, and *Croton zehntneri* showed 100% mortality at 50 ml against *A. aegypti* [15], [17] also reported that the main components methyleugenol and alpha-copaene for *C. nepetaefolius* (LC50 of 84 ppm); alpha-pinene and beta-pinene for *C. argyrophylloides* (LC50 of 102 ppm); and alpha-pinene, beta-phelandrene, and transcaryophyllene for *C. sonderianus* (LC50 of 104 ppm) and *C. zehntneri* exhibited higher larvicidal activity with an LC50 of 28 ppm against *A. aegypti*. [9] Reported that the highest larval mortality was found in benzene extract of Ervatamia coronaria against the larvae of *C. quinquefasciatus*, with LC50 and LC90 values of 96.15 and 174.10 ppm. The corresponding LC50 value of leaf acetone, absolute alcohol, petroleum ether, chloroform/methanol (1:1, v/v), benzene and ethyl acetate extracts of *Solanum nigrum* were 72.91, 59.81, 54.11, 32.69, 27.95 and 17.04 ppm, respectively, after 24 h of exposure period against *C. quinquefasciatus* [22]. In the present study the ethanolic extract had higher mortality with the values of LC50= 115.70, 96.09, 144.50, and 143.75 ppm and LC90= 215.46, 204.98, 233.82 and 260.86 ppm was observed against *A. agypti* followed by the Hexane extract observed in *A. aegypti* had values of LC50=116.82, 97.24, 144.50 and 169.7955 ppm and LC90=214.27, 203.53, 233.82 and 297.12 ppm, respectively. The Chloroform extract observed in had values of LC50=115.70, 96.09, 144.50 and 174.28 ppm and LC90=215.46, 204.98, 233.82 and 298.02 ppm, respectively. The Petroleum ether extract observed in had values of LC50=137.32, 156.52, 181.06 and 202.37 ppm and LC90=263.81, 303.44, 346.62 and 348.83 ppm, respectively. [20], [21] have reported that the highest larval mortality was found in leaf acetone, chloroform, methanol and petroleum ether of *Canna indica* (LC50=29.62, 59.18, 40.77 and 44.38 ppm; LC90=148.55, 267.87, 165.00 and 171.91 ppm) against second-instar larvae (LC50=121.88, 118.25, 69.76 and 56.31 ppm; LC90=624.35, 573.93, 304.27 and 248.24 ppm) and against fourth-instar larvae and acetone, hot water, methanol and petroleum ether extracts of *Ipomoea carnea* (LC50=61.17, 41.07, 41.82 and 39.32 ppm; LC90 = 252.91, 142.67, 423.76 and 176.39 ppm) against second-instar larvae (LC50=145.37, 58.00, 163.81 and 41.75 ppm; LC90=573.30, 181.10, 627.38 and 162.63 ppm) and fourth-instar larvae of *C. quinquefasciatus*, respectively.

The present investigation revealed that the crude extract of *C. indica* lam possesses remarkable larvicidal activities against dengue vector, *A. aegypti* as target species. This study is the first to report on the mosquito larvicidal activity of *C. indica* lam leaf extracts of plant showed that it has good and effective mosquito control properties and also can

act as an eco-friendly biopesticide for further vector control programs. Further purification and characterization of the bioactive fraction of *C. indica* lam are underway in our laboratory.

REFERENCES

[1] Abbott, W.S., J *Econ Entomol*, **1925,** 18, 267-269.
[2] Abubakar, A.A., Salka, M.N., Hassan, F.B., *Asian J. Plant Sci. Rec.*, 2011, 1, 1, 95
[3] Amer, A., Mehlhorn, H., *Parasitol Res*, **2006a,** 99, 473–477.
[4] Amer, A., Mehlhorn, H., *Parasitol Res*, **2006b,** 99, 466–472.
[5] Ansari, M.A., Razdan, R.K., Tandon, M., Vasudevan, P., *Bioresour Technol*, **2000,** *73*, 207–211.
[6] Babu, R., Murugan, K., Larvicidal effect of resinous exudate from the tender leaves of *Azadirachta indica*. Neem Newsletter, **2000,** 17(3).
[7] Finney, D.J., Probit Analysis. Cambridge University. London, **1971**, pp 68-78.
[8] Fradin, M.S., Day, J.F., *N Engl J Med*, **2002**, 347, 13–18.
[9] Govindarajan, M., Mathivanan, T., Elumalai, K., Krishnappa, K., Anandan, A., *Parasitol Res*, **2011**, 109(2), 353-67.
[10] Gubler, D.J., *Emerg Infect Dis*, **1998,** 4, 442–450.
[11] James, A.A., *Science*, **1992,** 257, 37–38.
[12] Jang, Y.S., Kim, M.K., Ahn, Y.J., Lee, H.S., *Agric Chem Biotechnol*, **2002**, 45(3), 131–134.
[13] Kovendan, K., Murugan, K., Vincent, S., Kamalakannan, S., *Parasitol Res*, **2011,** 109(5), 1251-7.
[14] Lee, S.E., Kim, J.E., Lee, H.S., Agric Chem Biotechnol, **2001,** 44, 105–112.
[15] Lima, M.G., Maia, I.C., Sousa, B.D., Morais, S.M., Freitas, S.M., *Rev Inst Med Trop Sao Paulo* 2006, 48(4), 211–214.
[16] Macedo, M.E., Consoli, R.A.G.B., Grandi, T.S.M., Anjos, A.M.G., Oliveira, A.B., Mendes, N.M., Queiroz, R.O., Zani, C.L., *Mem Inst Oswaldo Cruz*, **1997,** 92, 565–570.
[17] Morais, S.M., Cavalcanti, E.S., Bertini, L.M., Oliveira, C.L., Rodrigues, J.R., Cardoso, J.H., *J Am Mosq Control Assoc*, **2006,** 22(1), 161-164.
[18] Murugan, K., Jeyabalan, D., *Curr Sci*, **1999,** 76, 631–633.
[19] Prabakaran, M., Chandrakala, N., Panneerselvam, A., *Asian J. Plant Science and Resear*., **2011**, 1 (2), 18-25.
[20] Rahuman, A.A., Bagavan, A., Kamaraj, C., Saravanan, E., Zahir, A.A, Elango, G., *Parasitol Res*, **2009a,** 104(6), 1365–1372.
[21] Rahuman, A.A., Bagavan, A., Kamaraj, C., Vadivelu, M., Zahir, A.A., Elango, G., Pandiyan, G., *Parasitol Res*, **2009b,** 104(3), 637–643.
[22] Rahuman, A.A., Venkatesan, P., Parasitol Res, **2008,** 103, 133–139.
[23] Rajasekariah, G.R., Parab, P.B., Chandrashekar, R., Deshpande, L., Subrahmanyan, D., *Ann Trop Med Parasitol*, **1991,** 85(6), 663–665.
[24] Samarasekera, J.K., Khambay, B.P., Hemalal, K.P, *Nat Prod Res*, **2004,** 18(2), 117–122.
[25] Sathya, A., Ambikapathy, V., *Asian J. Plant Sci. Rec.*, 2012, 2(4), 486-489
[26] Shailesh, M.K., *Asian J. Plant Sci. Rec.*, 2012, 2(4), 421-427
[27] Sharma, P., Mohan, L., Srivastava., C.N, *Bioresour Technol*, **2006**, 97(14), 1599–1604.
[28] Singh, R.N., Saratchandra, B., *Caspian J Env Sci*, **2005,** 3(1), 1–8.
[29] Wink, M, Production and application of pytochemicals from an agricultural perspective. In: Van Beek TA, Breteler H (eds) Phytochemistry and agriculture. Clerendon, Oxford, **1993,** pp 171–213. 27