European Review for Medical and Pharmacological Sciences 2011; 15: 787-794

Mosquito larvicidal and ovicidal activity of *Cardiospermum halicacabum* Linn. (Family: Sapindaceae) Leaf extract against *Culex quinquefasciatus* (say.) and *Aedes aegypti* (Linn.) (Diptera: Culicidae)

M. GOVINDARAJAN

Division of Vector Biology and Phytochemistry, Department of Zoology, Annamalai University, Annamalainagar (India)

Abstract. – *Objective:* **To investigate the larvicidal and ovicidal efficacy of different extracts of *Cardiospermum halicacabum* L. against *Culex quinquefasciatus* Say and *Aedes aegypti* L. (Diptera: Culicidae).**

Materials and Methods: **Larvicidal efficacy of the crude leaf extracts of *Cardiospermum halicacabum* with five different solvents like benzene, hexane, ethyl acetate, methanol and chloroform was tested against the early third instar larvae of *Culex (C.) quinquefasciatus* and *Aedes (A.) aegypti*. The ovicidal activity was determined against two mosquito species to various concentrations ranging from 100-600 ppm under the laboratory conditions.**

Results: **The benzene, hexane, ethyl acetate, methanol and chloroform leaf extract of *Cardiospermum halicacabum* was found to be more effective against *C. quinquefasciatus* than *A. aegypti*. The LC_{50} values were 174.24, 193.31, 183.36, 150.44, 154.95 ppm and 182.51, 200.02, 192.31, 156.80, 164.54 ppm respectively. Among five solvent tested the methanol and benzene crude extract was found to be most effective for ovicidal activity against two mosquito species. The extract of methanol and benzene exerted 100% mortality at 300 ppm against *C. quinquefasciatus*. *A. aegypti* attained the complete ovicidal activity at 400 ppm for the extract of methanol only.**

Conclusions: **From the results it can be concluded the crude extract of *Cardiospermum halicacabum* was a potential for controlling *C. quinquefasciatus* and *A. aegypti* mosquitoes.**

Key Words:

Cardiospermum halicacabum, Larvicidal activity, Ovicidal activity, *Culex quinquefasciatus, Aedes aegypti.*

Introduction

Mosquitoes are the major vector for the transmission of malaria, dengue fever, yellow fever, filariasis, schistosomiasis and Japanese encephalitis (JE)[1,2]. Mosquitoes also cause allergic responses in humans that include local skin and systemic reactions such as angioedema[3]. Lymphatic filariasis is a mosquito-borne disease caused by mosquito-transmitted filarial nematodes, including *Wuchereria (W.) bancrofti* and *Brugia malayi*. The infected people carry the nocturnally periodic *W. bancrofti*, which has *C. quinquefasciatus* as the main mosquito vector. *Culex (C.) quinquefasciatus* is a vector of lymphatic filariasis, which is a widely distributed tropical disease with around 120 million people infected worldwide, and 44 million people have common chronic manifestations[4]. *Aedes (A.) aegypti* (L.) is generally known as a vector for an arbovirus responsible for dengue fever, which is endemic to Southeast Asia, the Pacific island area, Africa, and the Americas. This mosquito is also the vector of yellow fever in Central and South America and West Africa. Dengue fever has become an important public health problem as the numbers 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 such as central nervous system involvement[5].

An obvious method for the control of mosquito-borne diseases is the use of insecticides, and many synthetic agents have been developed and

Corresponding Author: Marimuthu Govindarajan, M.Sc., M.Phil., Ph.D; e-mail: drgovind1979@rediffmail.com

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. The toxicity problem, together with the growing appearance of insect resistance, has called attention to the need for novel insecticides[6], and for more detailed studies of naturally-occurring insecticides[7]. These problems have highlighted the need for the development of new strategies for selective mosquito larval control. Extracts from plants may be alternative sources of mosquito egg and larval control agents, since they constitute a rich source of bioactive compounds that are biodegradable into non toxic 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[8-10].

Cardiospermum halicacabum (Linn), family Sapindaceae, is a deciduous, branching, herbaceous climber, which is distributed throughout the plains of India. The whole plant has been used for several centuries in the treatment of rheumatism, stiffness of limbs, snake bite[11]; its roots for nervous diseases, as a diaphoretic, diuretic, emetic, laxative, refrigerant, stomachic and sudorific ; its leaves and stalks are used in the treatment of diarrhoea, dysentery and headache[12] and as a poultice for swellings[11]; Phytochemical constituents such as flavones, aglycones, triterpenoids, glycosides and a range of fatty acids and volatile ester have been reported from the various extracts of this plant[13,14].

Recent studies stimulated the investigation of insecticidal properties of plant derived from materials or botanicals and concluded that they are environmentally safe, degradable and target specific[15-19]. The hexane crude extracts of *Spilanthes acmella*, *Spilanthes (S.) calva* and *Spilanthes (S.) paniculata*[20], the ethyl acetate partially purified extracts of leaves of *Vitex negundo*, *Nerium oleander*, and seeds of *Syzygium jambolanum*[21], the acetone extract of *Thuja orientelis*[22,] and *Murraya koenigii, Coriandrum sativum, Ferula asafetida, Trigonella foenum graceum*[23] have been tested against the larvae of *A. aegypti* and *C. quinquefasciatus*.

Murugan and Jeyabalan[24] reported that *Leucas aspera*, *A. indica*, *Allium sativum* and *Curcuma longa* had a strong larvicidal, antiemergence, adult repellency and antireproductive activity against *Anopheles stephensi*. In addition, *Pelargonium citrosa*[25], *Cymbopogan citrates*[26] and *Mentha piperita*[27] were shown to contain larvicidal and growth inhibitory activity against *Anophesel stephensi*. Rajkumar and Jebanesan[28] reported that the toxicity of the plant *Moschosma polystachyum* was evaluated against mosquito *C. quinquefasciatus*. 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 and ovicidal potential of the different solvent crude extracts from the medicinal plant *Cardiospermum halicacabum* against the medically important mosquito vectors, *C. quinquefasciatus* and *A. aegypti*.

Materials and Methods

Collection of Plants

Fully developed leaves of the *Cardiospermum halicacabum* were collected from different regions of Cuddalore District, Tamilnadu, India. It was authenticated by a plant taxonomist from the Department of Botany, Annamalai University. A voucher specimen is deposited at the Herbarium of Plant Phytochemistry Division, Department of Zoology, Annamalai University.

Extraction

The leaves were washed with tap water, shade dried and finely ground. The finely ground plant material (3.0 kg/solvent) was loaded in Soxhlet apparatus and was extracted with five different solvents namely benzene, hexane, ethyl acetate, methanol and chloroform individually[29]. The solvent from the extract was removed using a rotary vacuum evaporator to collect the crude extract. The crude residue of this plant varies with the solvents used. The *Cardiospermum halicacabum* with five different solvents yielded 96.20, 126.84, 102.34, 165.25 and 149.62 g of crude residue respectively. Standard stock solutions were prepared at 1% by dissolving the residues in acetone. From this stock solution, different concentrations were prepared and these solutions were used for larvicidal and ovicidal bioassays.

Test Organisms

The mosquitoes, *C.quinquefasciatus* and *A. aegypti* were reared in the vector control laboratory, Department of Zoology, Annamalai University. The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. Adults were pro-

vided with 10% sucrose solution and one week old chick for blood meal. Mosquitoes were held at 28 ± 2^0, 70-85% relative humidity (RH), with a photo period of 14 h light, 10 h dark

Larvicidal Bioassay

The larvicidal activity of the plant crude extracts was evaluated as per the method recommended by WHO[30]. Batches of 25 third instar larvae were transferred to a small disposable test cups, each containing 200 ml of water. The appropriate volume of dilution was added to 200 ml water in the cups to obtain the desired target dosage (concentration ranging from 70 to 375 ppm), starting with the lowest concentration. Six replicate were set up for each concentration and an equal number of control were set up simultaneously using tap water. To this 1 ml of appropriate solvent was added. The LC_{50} value was calculated after 24 h by probit analysis[31].

Ovicidal Activity

For ovicidal activity, slightly modified method of Su and Mulla[32] was performed. The egg raft/ eggs of *C. quinquefasciatus* and *A. aegypti* were collected from vector control Laboratory, Annamalai University. The different leaf extract diluted in the appropriate solvent to achieve various concentrations ranging from 100 to 600 ppm. Eggs of these mosquito species (100 nos.) were exposed to each concentrations of leaf extract until they hatched or died. After treatment the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under microscope. Each experiment was replicated six times along with appropriate control. The hatch rates were assessed 48 h post treatment by following formula.

$$\% \text{ of egg mortality} = \frac{\text{Number of hatched larvae}}{\text{Total no. of eggs/egg raft}} \times 100$$

Statistical Analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95% confidence limits of upper confidence limit and lower confidence limit, and chi-square values were calculated using the SPSS12.0 (Statistical Package of Social Sciences, Inc, Chicago, IL, USA) software. Results with $p<0.05$ were considered to be statistically significant.

Results

Plants are rich sources of bioactive compounds that can be used to develop environmentally safe vector and pest managing agents. The botanical extracts from the plant leaves, roots, seeds, flowers and bark in their crude form have been used as conventional insecticides for centuries. The activity of crude plant extracts is often attributed to the complex mixture of active compounds. In the present investigation, the toxicity of different solvent extract of *Cardiospermum halicacabum* was tested against *C. quinquefasciatus* and *A. aegypti* (Tables I, II). The data were recorded and statistical data regarding LC_{50}, LC_{90}, LCL, UCL and chi-square values were calculated. The LC_{50} value of benzene, hexane, ethyl acetate, methanol and chloroform extract of *Cardiospermum halicacabum* against early third instar larvae of *C. quinquefasciatus* were 174.24, 193.31, 183.36, 150.44 and 154.95 ppm and against *A. aegypti* value were 182.51, 200.02, 192.31, 156.80 and 164.54 ppm, respectively. No mortality was observed in control. Chi-square values were significant at $p<0.05$ level. Table III shows the mean per cent hatchability of *C. quinquefasciatus* and *A. aegypti*. The methanol and benzene extract found to be more effective than the other extract against *C. quinquefasciatus* eggs, the 100% mortality at 300 ppm. *A. aegypti* attained the complete ovicidal activity at 400 ppm for the extract of methanol followed by chloroform and benzene (500 ppm); ethyl acetate and hexane (600 ppm).

Discussion

Different parts of plants contain a complex of chemicals with unique biological activity[33] which is thought to be due to toxins and secondary metabolites, which act as attractants or deterrents[34]. Our results showed that different solvent extract of *Cardiospermum halicacabum* have significant larvicidal as well as ovicidal activity against filarial and dengue vector mosquitoes. This result is also comparable to earlier reports of Vasudevan et al[35] who observed the larvicidal effect of crude extracts of dried ripened fruids of *Piper nigrum* against *C. quinquefasciatus* larval instars. LC_{50} and LC_{90} values as observed for early IV larval instar of *C. quinquefasciatus* were 29.11 and 62.37 mg/l and 63.82

Table I. Larvicidal activity of different solvent extracts of *Cardiospermum halicacabum* against *Culex quinquefasciatus.*

Name of the extract	Concentration (ppm)	% of mortality ± SD	LC_{50} (ppm) (LCL-UCL)	LC_{90} (ppm) (LCL-UCL)	χ^2
Benzene	Control	0.0 ± 0.0	174.24	320.94	17.127*
	75	28.2 ± 1.2	(128.38-217.82)	(267.42-426.47)	
	150	45.0 ± 1.8			
	225	67.2 ± 1.6			
	300	79.8 ± 0.8			
	375	98.0 ± 1.2			
Hexane	Control	0.0 ± 0.0	193.31	336.52	13.706*
	75	21.6 ± 1.2	(154.97-231.99)	(287.38-426.04)	
	150	35.6 ± 1.8			
	225	62.3 ± 2.2			
	300	75.9 ± 1.8			
	375	97.6 ± 1.4			
Ethyl acetate	Control	0.0 ± 0.0	183.36	332.77	18.061*
	75	25.9 ± 2.2	(136.19-229.45)	(276.36-477.55)	
	150	42.2 ± 1.4			
	225	64.4 ± 1.6			
	300	76.0 ± 1.2			
	375	97.9 ± 1.8			
Methanol	Control	0.0 ± 0.0	150.44	295.81	22.599*
	70	37.8 ± 0.8	(94.93-199.52)	(238.00-426.55)	
	140	46.2 ± 1.4			
	210	71.6 ± 1.2			
	280	83.0 ± 1.2			
	350	96.2 ± 1.6			
Chloroform	Control	0.0 ± 0.0	154.95	295.13	16.965*
	70	31.9 ± 1.2	(110.74-195.65)	(244.94-393.86)	
	140	48.0 ± 1.4			
	210	70.0 ± 14			
	280	82.6 ± 1.8			
	350	96.6 ± 1.6			

*Significant at $P<0.05$. SD = Standard Deviation; LCL = Lower Confidence Limits; UCL = Upper Confidence Limits; χ^2 = Chi square

and 108.90 mg/l for aqueous and ethanol extracts respectively. The methanol leaf extracts of *Vitex(V.) negundo*, *V. trifolia*, *V. peduncularis*, and *V. altissima* were used for larvicidal assay with LC_{50} values of 212.57, 41.41, 76.28, and 128.04 ppm, respectively, against the early fourth-instar larvae of *C. quinquefasciatus*[36]. The water extract of citrus-seed extract showed LC_{50} values of 135,319.40 and 127,411.88 ppm against the larvae of *A. aegypti* and *C. quinquefasciatus*[37]. The aqueous extract of *Piper retrofractum* showed LC_{50} values of 135 and 79 (mg/l) against *C. quinquefasciatus* and *A. aegypti*, respectively[38]. The chloroform extracts of *Euphorbia tirucalli* latex and stem bark were evaluated for larvicidal activity against laboratory-reared larvae of *C. quinquefasciatus* with LC_{50} values of 200.76 mg/L) and 343.51 mg/L, respectively[39].

Mullai and Jebanesan[40] have reported that the methanol leaf extracts of *Cucurbita(C.) colocynthis* and *C. maxima* showed the LC_{50} values were 117.73 and 171.64 ppm, respectively, against *C. quinquefasciatus* larvae. Larvicidal efficacies of methanol extracts of *Momordica charantia*, *Trichosanthes anguina*, *Luffa acutangula*, *Benincasa cerifera*, and *Citrullus vulgaris* tested with LC_{50} values were 465.85, 567.81, 839.81, 1,189.30, and 1,636.04 ppm respectively, against the late third larval age group of *C. quinquefasciatus*[41]. Dua et al[42] have reported that the lethal concentration values of the aqueous extract from the roots of *H. abelmoschus* against the larvae of *Anopheles culicifacies*, *Anopheles stephensi*, and *C. quinquefasciatus* were 52.3, 52.6, and 43.8 ppm, respectively. A piperidine alkaloid from *Piper longum* fruit was found to be active against mosquito larvae of *Culex pipiens*[43]. The ovicidal efficacy compared

Table II. Larvicidal activity of different solvent extracts of *Cardiospermum halicacabum* against *Aedes aegypti*.

Name of the extract	Concentration (ppm)	% of mortality ± SD	LC_{50} (ppm) (LCL-UCL)	LC_{90} (ppm) (LCL-UCL)	χ^2
Benzene	Control	0.0 ± 0.0	182.51	332.68	16.286*
	75	26.4 ± 1.8	(137.92-225.96)	(278.51-438.58)	
	150	42.0 ± 2.2			
	225	64.6 ± 1.8			
	300	77.2 ± 1.4			
	375	97.2 ± 1.6			
Hexane	Control	0.0 ± 0.0	200.02	341.99	17.524*
	75	18.6 ± 1.2	(156.50-244.97)	(287.55-451.27)	
	150	36.3 ± 1.6			
	225	58.0 ± 1.4			
	300	72.0 ± 1.4			
	375	99.0 ± 1.8			
Ethyl acetate	Ccontrol	0.0 ± 0.0	192.31	341.96	12.802*
	75	22.4 ± 1.2	(154.17-230.56)	(292.27-431.45)	
	150	39.2 ± 1.6			
	225	61.4 ± 1.8			
	300	75.6 ± 1.4			
	375	96.2 ± 1.6			
Methanol	Control	0.0 ± 1.8	156.80	300.44	17.064*
	70	31.2 ± 1.6	(111.66-198.35)	(248.93-402.85)	
	140	49.2 ± 1.6			
	210	68.9 ± 1.4			
	280	81.4 ± 1.2			
	350	95.9 ± 2.2			
Chloroform	Control	0.0 ± 0.0	164.54	313.45	17.234*
	70	29.9 ± 1.4	(118.68-207.85)	(259.29-423.67)	
	140	47.2 ± 1.6			
	210	65.0 ± 1.8			
	280	78.4 ± 1.4			
	350	95.2 ± 1.2			

*Significant at $P<0.05$. SD = Standard Deviation; LCL = Lower Confidence Limits; UCL = Upper Confidence Limits; χ^2 = Chi square.

with an earlier report; the bioactive compound Azadirachtin (*Azadirachta indica*) showed complete ovicidal activity in eggs of *Culex tarsalis* and *C. quinquefasciatus* exposed to10ppm concentration[32]. The seed extract of *Atriplex canescens* showed complete ovicidal at 1,000 ppm concentration in eggs of *C. quinquefasciatus*[44].

In our previous study, we have reported the methanol extract of *Cassia fistula* exhibited LC_{50} values of 17.97 and 20.57 mg/l against *Anopheles stephensi* and *C. quinquefasciatus*, respectively[15]. The crude leaf extract of *Acalypha indica* with different solvents, viz. benzene, chloroform, ethyl acetate and methanol were tested for larvicidal activity against *Anopheles stephensi*. The LC_{50} values were 19.25, 27.76, 23.26 and 15.03 ppm, respectively[16]. The LC_{50} of leaf extract of *Cassia fistula* with different solvents, viz. methanol, benzene and acetone against *A. aegypti* were 10.69, 18.27 and 23.95 mg/l respectively[17]. Larvicidal efficacy of the crude leaf extracts of *Ficus benghalensis* with three different solvents like methanol, benzene and acetone was tested against the early second, third, fourth instar larvae of *C. quinquefasciatus, A. aegypti* and *An. stephensi*. Among the three solvents the maximum efficacy was observed in methanol. The lethal concentration (LC_{50}) values of *Ficus benghalensis* against early second, third and fourth larvae of *C. quinquefasciatus, A. aegypti* and *An. stephensi* were 41.43, 58.21 and 74.32 ppm, 56.54, 70.29 and 80.85 ppm and 60.44, 76.41 and 89.55 ppm respectively[18]. The LC_{50} and LC_{90} values of crude methanol extract of leaves of *Ervatamia coronaria* on *C. quinquefasciatus, A. aegypti* and *An. stephensi* larvae in 24 h were 72.41, 65.67, 62.08 and 136.55, 127.24 and 120.86 mg/l, respectively[10]. Larvicidal activ-

Table III. Ovicidal activity of *Cardiospermum halicacabum* plant extracts against *Culex quinquefasciatus* and *Aedes aegypti*

Mosquito	Name of the solvent	Percentage of egg hatch ability Concentration (ppm)						
		Control	100	200	300	400	500	600
Culex quinquefasciatus	Benzene	100.0 ± 0.0^{a}	59.6 ± 0.8^{b}	37.8 ± 1.2^{c}	NH	NH	NH	NH
	Hexane	100.0 ± 0.0^{a}	69.9 ± 1.2^{b}	49.8 ± 0.8^{c}	29.4 ± 1.8^{d}	16.2 ± 1.2^{e}	NH	NH
	Ethyl acetate	100.0 ± 0.0^{a}	64.8 ± 1.8^{b}	42.5 ± 1.6^{c}	25.2 ± 1.4^{d}	12.6 ± 1.8^{e}	NH	NH
	Methanol	100.0 ± 0.0^{a}	44.6 ± 2.2^{b}	28.4 ± 1.4^{c}	NH	NH	NH	NH
	Chloroform	99.6 ± 1.2^{a}	53.9 ± 1.4^{b}	36.2 ± 1.8^{c}	21.6 ± 1.6^{d}	NH	NH	NH
Aedes aegypti	Benzene	100.0 ± 0.0^{a}	72.4 ± 1.6^{b}	56.6 ± 1.2^{c}	43.8 ± 1.4^{d}	29.4 ± 1.4^{e}	NH	NH
	Hexane	100.0 ± 0.0^{a}	88.6 ± 1.4^{b}	74.9 ± 0.8^{c}	63.4 ± 1.2^{d}	45.8 ± 1.8^{e}	32.6 ± 1.2^{f}	NH
	Ethyl acetate	97.2 ± 1.6^{a}	79.3 ± 1.8^{b}	62.7 ± 1.4^{c}	51.8 ± 1.8^{d}	39.6 ± 1.4^{e}	27.2 ± 0.8^{f}	NH
	Methanol	100.0 ± 0.0^{a}	56.8 ± 1.6^{b}	39.2 ± 2.2^{c}	23.6 ± 2.2^{d}	NH	NH	NH
	Chloroform	99.2 ± 1.4^{a}	63.7 ± 1.4^{b}	47.6 ± 1.8^{c}	36.8 ± 1.4^{d}	24.2 ± 0.8^{e}	NH	NH

Values in a row with a different superscript are significantly different at $p < 0.05\%$ level (DMRT test). Each value ($\bar{X} \pm SD$) represents the mean of six values. NH = No hatchability (100% mortality).

ity of crude extract of *Sida acuta* against *C. quinquefasciatus, A. aegypti* and *An. stephensi* with LC_{50} values ranging between 38 to 48 mg/l[19]. The current investigation revealed that the different solvent crude leaf extract of *Cardiospermum halicacabum* possesses remarkable larvicidal and ovicidal activities against the tested mosquito species. Further purification and characterization of the bioactive fraction of *Cardiospermum halicacabum* are underway in our laboratory.

Acknowledgements

The Author is thankful to the Department of Science and Technology (DST) (SERC-Fast Track Young Scientist Project), New Delhi, India for providing financial assistance for the present investigation. The Author is grateful to the Dr. (Mrs) Selvi Sabhanayakam, Professor and Head, Department of Zoology, Annamalai University for the laboratory facilities provided. Furthermore, the Author acknowledges the staff members of the VCRC (ICMR), Pondicherry for their cooperation.

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