Pharmacogn J. 2018; 10(6): 1137-1142
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com | www.journalonweb.com/pj | www.phcog.net

Original Article

Larvicidal and Pupicidal activity of *Clerodendrum philippinum* Schauer Leaf Extracts against *Anopheles stephensi* and *Aedes aegypti*

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History

- Submission Date: 23-04-2018;
- Review completed: 28-06-2018;
- Accepted Date: 23-07-2018

DOI : 10.5530/pj.2018.6.194

Article Available online
http://www.phcogj.com/v10/i6

ABSTRACT

Objective: The purpose of this study is to investigate the larvicidal and pupicidal activity of *Clerodendrum philippinum* leaf extracts against disease causing vectors *Anopheles stephensi* and *Aedes aegypti*. **Methods**: Five different concentrations (200, 300, 400, 500 and 600 ppm) of leaves were prepared by using aqueous (distilled water), ethanol, methanol, n-hexane, chloroform and tested for both the activity. The LC_{50} and LC_{90} values of leaf extracts were determined with the help of probit analysis. **Results**: Among the different extracts of leaf screened, the ethanol extract of *C. philippinum* was recorded the highest larvicidal and pupicidal activity of 100 ± 1.9 % (1st instar) and 58 ± 0.8 % at 600 ppm concentration for controlling *An. stephensi*, respectively. Similarly for *Ae. aegypti*, 97 ± 1.2 (1st instar) and 51 ± 0.5 (pupa) percentage of inhibition were achieved for the same solvent extracts of the leaf which were maximum than others. Moreover, the values of LC_{50} and LC_{90} clearly indicate that the activity of both larvicidal and pupicidal are not only solvent extracts dependant but also depend on their concentration. **Conclusion**: The obtained data highlight the potential role of ethanolic extracts of *C. philippinum* for controlling *An. stephensi* and *Ae. aegypti* mosquitoes at their larval and/ pupal stages of development.

Key words: Larvicidal activity, Pupicidal activity, *Clerodendrum philippinum, Anopheles stephensi, Aedes aegypti.*

INTRODUCTION

Mosquitoes are serious human disease causing insects which transmit many dreadful diseases and therefore they are considered as 'public enemy number one'.[1-2] They are contributing as major public health issues by transmitting many life threatening diseases like malaria, dengue, chikungunya, filariasis, encephalitis, yellow fever and West Nile fever in almost all developed and developing countries of world.[3-4] *Anopheles stephensi* is a primary vector for malaria whereas, *Aedes aegypti* is responsible for causing dengue as well as chikungunya mainly in Asian countries including India.[5-6] Malaria caused by *An. stephensi* has become a major contribution in India and are particularly transmitting in the urban and industrial area.[7-8] In other hands, more than 70,000 and 18,000 cases of dengue and chikungunya, respectively are reported in India caused by *Ae. aegypti*.[9]

Many control majors (environmental sanitation, epidemiological, surveillance, laboratory and research support and education) are available to check against the vectors, those are responsible for spreading the different life threatening disease.[10] Moreover, synthetic insecticides are applied to control the agents but have resistant and that might be due to repeated use of such products. It has also caused the environmental threat by destroying non-target organisms and raised adverse effects on both environmental quality and food chain.[4,11] For which, Integrated Mosquito Management (IMM) emphasizes the application of alternative strategies to control the larva or pupa stages of mosquito. Under such conditions, plants or plant based products showed their effectiveness as controlling agents of mosquitoes which are safer from environment point of view and target specific. They possess rich source of secondary metabolites which having insecticidal activity (antibacterial, antifungal and larvicidal potential) and is possible due to presence of active phenols, alkaloids, terpenoids, coumarins, polysaccharides and flavonoids etc.[12-13] In fact, many studies have been conducted to find out the effectiveness of different plant extracts against larvicidal and pupicidal activity of mosquito,[4,12,14-17] but there is no such report against *Clerodendrum philippinum*.

Cite this article: Dhal P, Rout JR, Dash PK, Panda S, Pati P, Rath CC, Pradhan C, Sahoo SL. Larvicidal and Pupicidal activity of *Clerodendrum philippinum* Schauer Leaf Extracts against *Anopheles stephensi* and *Aedes aegypti*. Pharmacog J. 2018;10(6):1137-42.

Clerodendrum philippinum Schauer is an important medicinal plant, which belongs to the family Verbenaceae. The plant is also called Peanut Butter plant as the leaf spreads after peeling a peanut butter scent.[18-19] It is a semi-woody perennial shrub found in Southern part of Asia and in India, abundantly available in various states like Karnataka, Kerala, Tamil Nadu and Odisha. It grows up to a height of 10 ft tall. Leaves are broad, up to 1 ft cm long and wide, margins toothed, somewhat lobed. Flowers are found in tight clusters, white with pink or red tinge and highly fragranced.[20-21] Different parts of the plant have been used against various ailments like inflammatory disorders, cancer, diabetic, rheumatism, asthma, constipation, colic pain, jaundice, syphilis, typhoid etc.[18,20,22-23] It is also reported that the plant has antibacterial and antifungal properties, however no evidence is found regarding its antimalarial/ larvicidal activity.[24-25] Hence, the present study is undertaken to evaluate the larvicidal and pupicidal activity of leaf extracts as well as flower essential oil against two vectors, *Anopheles stephensi* and *Aedes aegypti.*

MATERIALS AND METHODS

Collection and preparation of leaf extracts

Healthy leaves of *C. philippinum* were collected from the botanical garden of Post Graduate Department of Botany, Utkal University and were washed and air dried under the environmental temperature of 27°C–37°C. The dried leaves were powdered by using a mortar and pestle. The powdered sample was eluted using a Soxhlet apparatus with different solvents [aqueous, ethanol, methanol, n-hexane, chloroform) sequentially in a ratio of 1:10 for a period of 72 h each and filtered. The diluted extracts were concentrated (200, 300, 400, 500 and 600 ppm) at low temperature using a rotary evaporator and stored at -4°C for further analysis.

Collection of eggs and larval culture

The eggs of *An. stephensi* and *Ae. aegypti* were provided by Department of Entomology, Regional Medical Research Centre, Bhubaneswar, Odisha, India. The eggs were brought to the laboratory and kept in plastic and enamel tray containing normal tap water for hatching. The larvae were fed on a diet having fine a mixture of dog biscuits and dry yeast in a ratio of 3:1, till the pupal stage.

Maintenance of pupae and adults

Developed pupae were relocated in to separate plastic cup containing tap water and were kept under periodical check-up for emerging into adults. Larvae/ pupae were maintained throughout at 27 ± 2°C and 75–85 % of relative humidity with a photoperiod of 14 h/ 10 h light and dark cycles. After pupation, they were allowed inside a mosquito cage for becoming adults. Prior to blood feeding, 10 % sucrose solution was provided for 3 days and finally, adult female mosquitoes were maintained by providing a blood meal from rabbit (exposed in dorsal side).

Larvicidal and pupicidal activity test

The bioassay was done as per the standard method of World Health Organization by taking different solvent extracts of leaf.[26] Five different concentrations (200, 300, 400, 500 and 600 ppm) of each extract were tested against freshly moulted first (1st), second (2nd), third (3rd) and fourth (4th) instar larvae and pupae of *An. stephensi* and *Ae. aegypti.* Twenty numbers of first to fourth instar larvae and pupae were introduced into 500 ml thermocol glass containing 249 ml dechlorinated water and 1 ml of desired concentrations of plant extract (dilution was done by dechlorinated water). At each tested concentration, three trials were made and each trial consisted of five replicates. The control was set up by providing 250 ml of dechlorinated water however the larval food was added to all tested and control cups. The larval mortality was observed and recorded after 24 h of post treatment. The mortalities were recorded by using Abbott's formula (Abbott, 1925).

Statistical analysis

Results of larval and pupal mortality were the mean of three independent experimental replicates (n = 5) and were subjected to probit analysis[27] for calculating LC_{50}, LC_{90} and other statistics at 95 % fiducial limits of upper fiducial limit (UFL) and lower fiducial limit (LFL) and chi-square values were calculated using the SPSS 16.0 version (software package). Values with $P<0.05$ were considered to be statistically significant.

RESULTS

Larvicidal and pupicidal activity against *An. stephensi*

Larvicidal and pupicidal mortality responses from various leaf extracts (aqueous (distilled water), ethanol, methanol, n-hexane, chloroform) of *C. philippinum,* against malaria causing vector *An. stephensi* are presented in Tables 1-5. The different concentrations (200, 300, 400, 500 and 600 ppm) of each extract were tested against 1st, 2nd, 3rd, 4th instar and pupal stages of *An. stephensi.* Increasing trends of mortality were found against both larval and pupal stages of *An. stephensi* with raising concentrations (200 to 600 ppm) of each leaf extracts. A significant mortality was noticed in all applied solvent systems however, best mortality was observed in ethanolic extracts. At 600 ppm, the ethanolic extract of leaf exhibited 100 % of results in 1st instar stage of larva and subsequently the percentage of mortality was decreased to 97 ± 0.8 %, 85 ± 0.7 %, 73 ± 0.9 % (2nd, 3rd and 4th instar, respectively). A similar trend has been noticed for the pupal stage that the maximum mortality (58 ± 0.8 %) was achieved in 600 ppm of etanolic extract (Table 2). Furthermore, the LC_{50} and LC_{90} values for ethanol extract of 1st instar and pupa were found to be 257.14, 811.11 and 582.04, 1185.71 ppm respectively with the chi square values of 1.92 and 0.85. The values of LC_{50} and LC_{90} of other extracts against instars and pupae are shown in Tables 1-5, where the chi square values were statistically significant at $P<0.05$ level.

Larvicidal and pupicidal activity against *Ae. aegypti*

The same set up was tried against dengue and chikungunya causing mosquito, *Ae. aegypti* and the results of larvicidal and pupicidal activity of different leaf extracts of *C. philippinum* were tabulated (Tables 6-10). Similar findings were detected as occurred in *An. stephensi* that the ethanol extract of leaves gave maximum mortality in comparison to others. 97 ± 1.2 and 51 ± 0.5 % of larval and pupal mortality, respectively were recorded at 600 ppm of ethanol extract, which was maximum to others (Table 6). When the values of larvicidal activity were noticed in all solvent extracts, the effective results were obtained in 1st instar and then followed by 2nd, 3rd and 4th against individual concentration. Moreover from the solvent efficacy point of view, the hierarchy of mortality (both larval and pupal) was found to be ethanol > methanol > chloroform > n-hexane > aqueous. The LC_{50} and LC_{90} values for different extracts against larval and pupal mortality of *An. stephensi* are represented in Tables 6-10. The tables indicating chi square values were significant at $P<0.05$ (Tables 6-10).

DISCUSSION

In the present study, probit analysis reveals that LC_{50} and LC_{90} of leaf extracts of *C. philippinum* were effective against *An. stephensi* and *Ae. aegypti* during the larval and pupal stage. In general, the mortality rate from 1st to 4th instar were cumulatively decreasing which indicate that the extracts created a more toxic environment during the very beginning stages of mosquitoes (that have tried). Leaf extracts produced enhanced mortality with increasing concentrations of extracts and further, it has been noticed that ethanol extracts possessed strong activity than others

Table 1: Larval and pupal toxicity effect of aqueous extracts of *C. philippinum* against *An. stephensi*.

Mosquito life stage	% of larval and pupal mortality ± SD					LC_{50}	LC_{90}	95 % confidence limit (LFL-UFL)		χ^2 (df=4)
	200 ppm	300 ppm	400 ppm	500 ppm	600 ppm			LC_{50}	LC_{90}	
1st instar	32 ± 0.8	42 ± 0.9	53 ± 0.9	74 ± 2.1	85 ±1.2	372.72	662.50	284.72-460.72	622.50-702.50	1.74*
2nd instar	30 ±0.9	33 ± 2.1	46 ±1.4	62 ±0.9	81 ± 0.8	431.25	799.99	210.25-652.25	763.99-835.99	1.70*
3rd instar	27 ± 0.7	27 ± 0.9	44 ±1.1	56 ± 0.5	68 ±0.5	441.66	866.66	369.66-513.60	830.66-902.66	0.70*
4th instar	23 ± 1.9	28 ±0.7	38 ±0.8	52 ±0.8	59 ±0.5	485.71	1075.02	317.71-653.71	969.87-1145.09	0.25*
Pupa	10 ± 1.5	18 ± 1.9	28 ± 0.9	33 ± 0.4	47 ± 0.8	635.71	1187.50	537.71-721.04	1067.50-1307.50	0.74*

Control: Nil mortality; LFL: Lower fiducial limit; (UFL): Upper fiducial limit; χ2: Chi square value; d*f*: Degrees of freedom; *: Significant at P<0.05 level.

Table 2: Larval and pupal toxicity effect of ethanol extracts of *C. philippinum* against *An. stephensi*.

Mosquito life stage	% of larval and pupal mortality ± SD					LC_{50}	LC_{90}	95 % confidence limit (LFL-UFL)		χ^2 (df=4)
	200 ppm	300 ppm	400 ppm	500 ppm	600 ppm			LC_{50}	LC_{90}	
1st instar	46 ± 1.1	53 ± 0.9	59 ± 0.5	84 ± 0.7	100 ± 1.9	257.14	811.11	229.14-285.14	711.11-911.11	1.92*
2nd instar	40 ±0.9	43 ± 0.6	58 ±0.8	73 ±0.8	97 ± 0.8	346.66	833.33	241.66-451.66	737.33-929.33	1.80*
3rd instar	36 ± 1.2	36 ± 0.9	51 ±0.7	64 ± 1.1	85 ± 0.7	393.33	953.84	183.33-603.33	953.84-1033.84	0.90*
4th instar	29 ± 1.0	30 ±1.1	48 ±0.6	59 ±0.8	73 ± 0.9	445.45	1012.50	423.45-467.45	984.50-1040.50	0.31*
Pupa	21 ± 0.7	28 ± 0.9	34 ± 0.7	41 ± 0.6	58 ± 0.8	582.04	1185.71	467.41-682.41	1133.71-1237.71	0.85*

Control: Nil mortality; LFL: Lower fiducial limit; (UFL): Upper fiducial limit; χ2: Chi square value; d*f*: Degrees of freedom; *: Significant at P<0.05 level

Table 3: Larval and pupal toxicity effect of methanol extracts of *C. philippinum* against *An. stephensi*.

Mosquito life stage	% of larval and pupal mortality ± SD					LC_{50}	LC_{90}	95 % confidence limit (LFL-UFL)		χ^2 (df=4)
	200 ppm	300 ppm	400 ppm	500 ppm	600 ppm			LC_{50}	LC_{90}	
1st instar	41 ± 1.3	48 ± 0.4	57 ± 0.7	79 ± 1.9	92 ± 0.9	355.55	625.07	292.55-418.55	549.55-701.55	1.89*
2nd instar	38 ±0.7	39 ± 0.3	54 ±1.1	68 ±0.6	87 ± 0.4	435.71	777.79	270.71-600.71	701.79-853.79	1.76*
3rd instar	31 ± 0.9	31 ± 0.7	48 ±1.5	60 ± 0.9	78 ± 0.4	441.66	877.77	417.66-465.66	841.77-913.77	0.83*
4th instar	27 ± 1.2	27 ± 0.9	44 ±0.9	56 ±0.7	67 ± 0.3	545.55	975.43	473.55-617.55	927.05-1023.15	0.27*
Pupa	17 ± 0.5	24 ± 0.6	38 ± 0.6	37 ± 0.9	55 ± 0.3	627.77	1020.23	393.77-861.77	980.20-1060.01	0.81*

Control: Nil mortality; LFL: Lower fiducial limit; (UFL): Upper fiducial limit; χ2: Chi square value; d*f*: Degrees of freedom; *: Significant at P<0.05 level.

Table 4: Larval and pupal toxicity effect of n-hexane extracts of *C. philippinum* against *An. stephensi*.

Mosquito life stage	% of larval and pupal mortality ± SD					LC_{50}	LC_{90}	95 % confidence limit (LFL-UFL)		χ^2 (df=4)
	200 ppm	300 ppm	400 ppm	500 ppm	600 ppm			LC_{50}	LC_{90}	
1st instar	35 ± 0.9	44 ± 1.5	54 ± 0.8	75 ± 1.1	86 ± 0.8	360.00	628.25	300.03-420.01	584.25-672.25	1.80*
2nd instar	31 ±1.1	35 ± 1.0	49 ±1.9	64 ± 1.9	82 ± 0.4	407.33	766.66	323.33-543.33	718.66-814.66	1.69*
3rd instar	28 ± 0.9	27 ± 0.7	45 ± 0.7	57 ± 0.7	69 ± 0.8	441.66	800.33	381.66-501.66	760.23-840.01	0.75*
4th instar	24 ± 0.7	23 ± 0.9	39 ± 0.1	53 ± 0.4	61 ± 0.9	478.57	900.00	394.57-632.57	860.01-940.43	0.27*
Pupa	11 ± 0.5	21 ± 0.9	30 ± 0.6	31 ± 0.1	49 ± 1.1	627.77	1031.25	495.77-769.27	971.25-1091.25	0.78*

Control: Nil mortality; LFL: Lower fiducial limit; (UFL): Upper fiducial limit; χ2: Chi square value; d*f*: Degrees of freedom; *: Significant at P<0.05 level.

Table 5: Larval and pupal toxicity effect of chloroform extracts of *C. philippinum* against *An stephensi*.

Mosquito life stage	% of larval and pupal mortality ± SD					LC_{50}	LC_{90}	95 % confidence limit (LFL-UFL)		χ^2 (df=4)
	200 ppm	300 ppm	400 ppm	500 ppm	600 ppm			LC_{50}	LC_{90}	
1stinstar	37 ± 1.1	46 ± 0.5	56 ± 0.6	76 ± 1.0	88 ± 1.0	350.00	689.04	310-390	578.03-765.89	1.85*
2nd instar	33 ±0.9	37 ± 0.9	51 ± 1.2	65 ± 1.2	85 ± 0.3	392.85	842.5	346.85-494.85	806.50-878.50	1.72*
3rd instar	29 ± 0.7	28 ± 0.3	46 ± 1.0	58 ± 0.4	70 ± 0.7	441.66	883.65	409.66-473.66	799.65-967.65	0.80*
4th instar	25 ± 0.5	24 ± 0.5	41 ± 0.4	54 ± 0.5	63 ± 0.5	469.23	986.34	352.23-586.23	908.23-1145.89	0.24*
Pupa	13 ± 0.9	22 ± 0.2	32 ± 0.3	33 ± 0.6	50 ± 0.7	600.00	1070.25	583.00-617.00	1015.23-1198.32	0.77*

Control: Nil mortality; LFL: Lower fiducial limit; (UFL): Upper fiducial limit; χ2: Chi square value; d*f*: Degrees of freedom; *: Significant at P<0.05 level.

Table 6: Larval and pupal toxicity effect of aqueous extracts of *C. philippinum* against *Ae. aegypti*.

Mosquito life stage	% of larval and pupal mortality ± SD					LC_{50}	LC_{90}	95 % confidence limit (LFL-UFL)		χ^2 (df=4)
	200 ppm	300 ppm	400 ppm	500 ppm	600 ppm			LC_{50}	LC_{90}	
1st instar	27 ± 0.9	31 ± 0.9	43 ± 0.8	58 ± 3.1	81 ± 0.5	433.33	768.45	328.33-583.33	695.32-840.56	1.70*
2nd instar	22 ±0.4	29 ± 0.5	34 ±2.1	51 ±1.1	73 ± 0.5	494.11	850	386.78-566.11	814.04-886.53	1.67*
3rd instar	19 ± 1.6	22 ± 1.1	32 ±0.8	43 ± 0.6	66 ± 0.3	521.73	900.68	444.73-598.73	825.13-975.98	0.68*
4th instar	11 ± 0.6	18 ±0.5	26 ±0.3	41 ±1.1	53 ± 1.2	575.03	1085.35	479.00-671.04	925.00-1234.09	0.19*
Pupa	7 ± 0.2	11 ± 0.7	22 ± 0.4	30 ± 2.2	41 ± 0.9	645.45	1133.33	546.45-744.45	997.33-1269.33	0.61*

Control: Nil mortality; LFL: Lower fiducial limit; (UFL): Upper fiducial limit; χ2: Chi square value; d*f*: Degrees of freedom; *: Significant at P<0.05 level.

Table 7: Larval and pupal toxicity effect of ethanol extracts of *C. philippinum* against *Ae. aegypti*.

Mosquito life stage	% of larval and pupal mortality ± SD					LC_{50}	LC_{90}	95 % confidence limit (LFL-UFL)		χ^2 (df=4)
	200 ppm	300 ppm	400 ppm	500 ppm	600 ppm			LC_{50}	LC_{90}	
1st instar	35 ± 0.7	42 ± 0.9	51 ± 0.9	69 ± 0.6	97 ± 1.2	388.88	700.11	294.88-460.88	564.12-836.09	1.87*
2nd instar	31 ± 0.6	38 ± 0.2	46 ± 0.5	62 ± 0.7	92 ± 0.6	431.25	733.33	399.25-463.25	697.33-769.33	1.76*
3rd instar	27 ± 0.3	28 ± 0.7	41 ± 0.2	52 ± 0.4	87 ± 0.5	481.81	768.75	382.81-580.81	628.75-908.75	0.85*
4th instar	21 ± 0.9	26 ± 0.9	37 ± 0.7	48 ± 0.9	65 ± 0.4	529.41	883.65	507.41-551.41	847.65-919.65	0.27*
Pupa	17 ± 0.19	22 ± 0.8	32 ± 0.9	39 ± 0.6	51 ± 0.5	591.66	1183.33	519.66-673.66	1147.33-1219.33	0.73*

Control: Nil mortality; LFL: Lower fiducial limit; (UFL): Upper fiducial limit; χ2: Chi square value; d*f*: Degrees of freedom; *: Significant at P<0.05 level.

Table 8: Larval and pupal toxicity effect of methanol extracts of *C. philippinum* against *Ae. aegypti*.

Mosquito life stage	% of larval and pupal mortality ± SD					LC_{50}	LC_{90}	95 % confidence limit (LFL-UFL)		χ^2 (df=4)
	200 ppm	300 ppm	400 ppm	500 ppm	600 ppm			LC_{50}	LC_{90}	
1st instar	33 ± 0.6	38 ± 1.9	49 ± 0.8	67 ± 0.4	86 ±1.4	404.90	618.57	397.02-428.77	547.57-698.57	1.85*
2nd instar	29 ± 0.8	33 ± 0.8	44 ± 0.9	57 ± 1.2	78 ± 0.8	538.46	663.63	460.46-616.46	563.63-763.63	1.71*
3rd instar	25 ± 0.9	28 ± 0.4	39 ± 1.2	49 ± 0.6	70 ± 0.6	510.01	790.64	500.09-520.09	746.64-834.64	0.79*
4th instar	19 ± 0.4	23 ± 0.6	35 ± 0.5	46 ± 0.4	60 ± 0.4	536.66	868.66	492.66-580.66	812.66-921.66	0.24*
Pupa	14 ± 0.2	19 ± 0.2	28 ± 0.6	36 ± 0.5	46 ± 0.8	650.02	979.32	610.11-690.09	861.32-1097.35	0.70*

Control: Nil mortality; LFL: Lower fiducial limit; (UFL): Upper fiducial limit; χ2: Chi square value; d*f*: Degrees of freedom; *: Significant at P<0.05 level.

Table 9: Larval and pupal toxicity effect of n-hexane extracts of *C. philippinum* against *Ae. aegypti*.

Mosquito life stage	% of larval and pupal mortality ± SD					LC_{50}	LC_{90}	95 % confidence limit (LFL-UFL)		χ^2 (df=4)
	200 ppm	300 ppm	400 ppm	500 ppm	600 ppm			LC_{50}	LC_{90}	
1st instar	29 ± 1.0	33 ± 1.3	45 ± 1.2	61 ± 1.1	83 ± 0.9	431.25	663.63	371.25-491.25	619.75-707.75	1.72*
2nd instar	24 ± 0.7	30 ± 0.1	37 ± 0.9	53 ± 1.0	74 ± 0.3	481.25	812.50	501.25-689.25	713.50-772.50	1.61*
3rd instar	21 ± 0.4	24 ± 0.9	34 ± 0.3	45 ± 0.6	67 ± 0.4	525.45	842.85	464.05-618.95	814.85-870.85	0.65*
4th instar	15 ± 0.2	19 ± 0.2	29 ± 0.7	42 ± 0.4	55 ± 0.9	561.53	953.84	457.53-665.53	897.46-1125.07	0.17*
Pupa	8 ± 0.6	13 ± 0.21	24 ± 1.1	28 ± 0.9	43 ± 0.6	620.33	1068.76	503.33-701.33	1004.76-1132.76	0.68*

Control: Nil mortality; LFL: Lower fiducial limit; (UFL): Upper fiducial limit; χ2: Chi square value; d*f*: Degrees of freedom; *: Significant at P<0.05 level.

Table 10: Larval and pupal toxicity effect of chloroform extracts of *C. philippinum* against *Ae. aegypti*.

Mosquito life stage	% of larval and pupal mortality ± SD					LC_{50}	LC_{90}	95 % confidence limit (LFL-UFL)		χ^2 (df=4)
	200 ppm	300 ppm	400 ppm	500 ppm	600 ppm			LC_{50}	LC_{90}	
1st instar	31 ± 0.8	35 ± 1.1	47 ± 0.6	65 ± 0.9	84 ± 2.1	427.27	637.65	391.27-463.27	561.65-713.65	1.76*
2nd instar	27 ±0.5	32 ± 1.0	39 ± 0.5	54 ± 1.9	76 ± 1.3	476.13	658.33	428.33-558.33	570.33-746.33	1.64*
3rd instar	23 ± 0.2	26 ± 0.7	36 ± 0.9	47 ± 0.9	68 ± 1.2	515.80	735.75	484.80-546.17	672.80-834.11	0.70*
4th instar	17 ± 0.7	21 ± 0.5	31 ± 0.9	44 ± 0.9	57 ± 0.5	546.15	876.74	468.15-624.15	826.46-928.46	0.19*
Pupa	10 ± 0.5	15 ± 0.6	26 ± 0.2	35 ± 1.5	44 ± 0.2	655.55	982.14	601.55-709.55	943.14-1018.14	0.65*

Control: Nil mortality; LFL: Lower fiducial limit; (UFL): Upper fiducial limit; χ2: Chi square value; d*f*: Degrees of freedom; *: Significant at P<0.05 level.

(Tables 1-10). Globally, the people are always searching the eco-friendly alternative options to control the mosquitoes. For that, exploring of floral biodiversity is preferred, as they contain vast repository secondary metabolites. The tested plant, *C. philippinum* has larvicidal and pupicidal activity against *An. stephensi* which may be due to the presence of active biological compounds including terpenoids, flavonoid, alkaloids and phenolics etc.[28-29] The above mentioned compounds may jointly or independently contribute to impact on larvicidal and pupicidal activity against *An. stephensi* and a similar report was given by Subarani *et al*. in which aqueous and solvent leaf extracts of *Catharanthus roseus* is able to impact on *An. stephensi*.[4] The other effective results for malarial vector *An. stephensi* were also reported against different plant extracts such as *Euphorbia hirta,*[11] *Leucas aspera,*[30] *Citrus sinensis,*[31] *Gliricidia sepium*.[32] However, the ethanol extracts showed potential mortality in leaf and peel extract of *Citrus sinensis* and *Gliricidia sepium*, respectively as suggested from LC_{50} and LC_{90} values.[31-32]

The immature stages of mosquitoes are more susceptible to control as not only evidenced by *An. stephensi* but also from *Ae. Aegypti* against *leaf extracts of C. philippinum* (Tables 6-10). The synthetic toxins have been proved to be effective, but they pose some hazardous substances. Due to aquatic condition, they cause adverse effects on the environment and human health[33] and hence, this finding not only helps to control the spreading of mosquito but also biodegradable and easily available in low cost.[34] Plants possessing bioactive compounds are main the culprit against mortality of *Ae. Aegypti* which was suggested by Gandhi *et al*. after experimented on roots of *Rubia cordifolia* with LC_{50} and LC_{90} values of 3.86 and 8.28 ppm for larvae, and 3.92 and 8.05 ppm for pupae of *A. aegypti*.[35] Further, these group isolated alizarin from roots of *R. cordifolia,* which is the main source to destroy the larva/ pupa of mosquito. Larvicidal and pupicidal actions of methanol leaf extract of *Tephrosia purpurea* was also observed against *A. aegypti* and found that the LC_{50} values of 1st instar to 4th larval instars were 139.24, 176.24, 219.28, 256.27, and 326.29 ppm, respectively whereas LC_{50} value of pupa was 326.29 ppm.[15] The results so obtained also having similarities to Amir *et al*. who have taken the direct leaf and stem extracts of *Parthenium hysterophorus* against *A. aegypti* and confirmed as potential natural larvicidal agent.[12]

In our present study, the ethanol and methanol extracts of *C. philippinum* possessed higher larvicidal and pupicidal activities against *An. stephensi* and *Ae. Aegypti* if compare to aqueous, n-hexane and chloroform. This variation was attributed with dissolving properties of the bioactive compounds in respect to solvent system or the polarity of the solvents.[36] However, the mode of action of plant extracts on mosquito larvae are still unknown and up to some extent, it is postulated that the active phytochemicals interfering with the proper functioning of mitochondria more specifically at the proton transferring sites.[37] Moreover, some secondary metabolites are inhibiting the growth of mosquito larvae by interacting with the midgut epithelium, gastric caeca and the malpighian tubules.[38-40]

CONCLUSION

The finding of the current investigation revealed that the leaf extracts of *C. philippinum* possess potential mosquito larvicidal and pupicidal activity against *An. stephensi* and *Ae. aegypti*. Since there is no availability of previous larvicidal and pupicidal activity of the selected plant species, this investigation serves as first-hand information. However, these results need corroboration further by characterizing, size estimating and determining the mode of action of individual bioactive compounds from leaf against both larval and pupal stages of development. In the future, this attempt may be recommended as an alternative tool to control the actions of *An. stephensi* and *Ae. aegypti* at their early life cycle processes.

ACKNOWLEDGEMENT

The authors are grateful to the Head, Post Graduate Department of Botany, Utkal University for providing necessary laboratory facilities.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

LC_{50}: Lethal Concentration 50; **LC_{90}:** Lethal Concentration 90; **PPM:** Parts Per Million.

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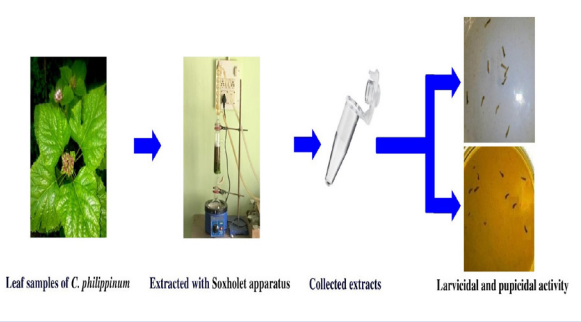
GRAPHICAL ABSTRACT



SUMMARY

- The present investigation provides sufficient evidence to control the spreading of *Anopheles stephensi* and *Aedes aegypti* during at their larval and pupal stages by application of leaf extracts of *Clerodendrum philippinum* Schauer.

Cite this article: Dhal P, Rout JR, Dash PK, Panda S, Pati P, Rath CC, Pradhan C, Sahoo SL. Larvicidal and Pupicidal activity of *Clerodendrum philippinum* Schauer Leaf Extracts against *Anopheles stephensi* and *Aedes aegypti*. Pharmacog J. 2018;10(6):1137-42.