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Research Article

IMPACT OF *HYGROPHILA AURICULATA*-GREEN MOSQUITOCIDAL ACTIVITY AGAINST MALARIA VECTOR, *ANOPHELES STEPHENSI* (DIPTERA: *CULICIDAE*)

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ABSTRACT

Objective: Mosquitoes are insect vectors responsible for the transmission of parasitic and viral infections to millions of people worldwide, with substantial morbidity and mortality. Insecticides of botanical origin may serve as suitable alternative bio-control techniques in the future.

Methods: The egg hatchability, growth regulatory, longevity, fecundity, and larvicidal activity of crude methanol leaf extract of *Hygrophila auriculata* were assayed for their toxicity tested against malarial vector mosquito, *Anopheles stephensi*.

Results: The eggs, larvae, larvae/pupae, and cumulative mortality were observed hatching rates for 100 ppm, 0–18 h (18 h exposed) at 24, 48, and 72 h, respectively, and mortality was 33.4, 44.6, 17.9, and 95.9% methanolic extract treatment, with the lethal concentration 50 (LC_{50})/LC_{90} values were 35.420 28/75.600 ppm. Effect of methanolic extract was larval, pupal, and adult duration and water extracts of caused longest delayed development from 16.6 days larvae, 7.5 days for pupae and longevity of adult female greatly reduced from 25.9 days and fecundity also reduced from 68.0 at 150 ppm. The larvicidal activity of methanol extract was decreased at 48 h as for instars larvae 63.44/271.95 ppm (I), 57.55/272.48 ppm (II); 62.49/301.22 ppm (III); 67.69/330.48 ppm (IV), and 76.99/343.82 ppm (pupae), respectively.

Conclusion: These results suggest that the methanol leaf extract has the potential to be used as an ideal eco-friendly approach for the control of mosquito vector. Therefore, this study provides on the mosquito eggs, larvae, and pupae activities of these plant methanol extract against *A. stephensi*.

Keywords: *Hygrophila auriculata*, *Anopheles stephensi* ovicidal, Growth regulatory, Larvicidal activity.

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INTRODUCTION

World Health Organization (WHO) is designing attention to a group of diseases that are spread by insects, the heavy health and economic burdens they impose, and what needs to be done to reduce these burdens. Mainly, vector-borne diseases (VBD) cause more than one million deaths each year. However, passing counts, though alarming, vastly underestimate the human misery and hardship caused by these diseases, as many people who survive infection are left permanently debilitated, disfigured, maimed or blind. WHO [1] estimated globally, the VBDs around 17% (700 million peoples) account for the global burden of infectious diseases annually, which are mainly highlighted in tropical and sub-tropical countries. More than 80% of the global population lives with the risk of these VBDs then remaining peoples are struggled with two or more VBDs. Those risks are particularly marked in urban areas (towns and cities) because these vectors are proliferating enormously in that favorable habitats, high abundance, and easy contact with human. Morbidity and mortality are often highly noticed in economically poor populations [2,3]. *Anopheles stephensi* is the principal vector of malaria and one of the most predominant diseases in the tropical world which disturbs 350–500 million peoples and kills more than 1 million infants and young children annually [4,5]. Malaria (major vector of *A. stephensi*) is one in all the grave scourges inflicted on human beings and causes human mortality alongside giant economic loss [6-8].

The genus, *Hygrophila auriculata* (Schumach.) Heine belongs to family *Acanthaceae* found in India, commonly known Kokilaksa, as in Sanskrit root and seeds used as tonic, for asthma and dysentery [9]. As per our tradition, roots, seeds, and aerial parts of the plant have been used in the treatment of jaundice, hepatic obstruction, rheumatism, inflammation, urinary infection, gout, and malaria [10]. The plant has been reported to contain flavonoids (apigenin 7-O-glucuronide, apigenin 7-O-glucoside) [11], alkaloids (asteracanthine and asteracanthicine) [12], aliphatic esters (25-oxo-hentricontyl acetate, methyl-8-hexyltetracosanoate) [13], minerals (Fe, Cu, Co) [14], sterols (stigmasterol) [15], and triterpenes (lupeol, hentriacontane, betulin, luteolin, and luteolin 7-O-rutinosides) [16]. Earlier scientific investigation showed that the crude extract of *H. auriculata* has anti-nociceptive [17], antitumor [18], antibacterial [19], and antioxidant [20]. The single oral dose of the *H. auriculata* leaves extract did not produce mortality or significant changes in the body weight, food, and water consumption. The internal organs weights of the control and test animals were normal. Apart from triglyceride, other biochemistry parameters demonstrated no significant changes as compared to the control [21].

In the present study, to evaluate the egg hatchability, growth regulatory, longevity, fecundity, and larvicidal properties of the plant extract of *H. auriculata* against medically important mosquitoes of malarial vector, *A. stephensi* as target species of the vector control management.

METHODS

Plants collection and extraction

The fresh plant, *H. auriculata* leaf (authentication number: AU/BOT/127) sampling was carried out during the growing season of January–March in and around Chidambaram, Cuddalore district, Tamil Nadu, India. Plant leaves were air-dried at room temperature 28±2°C, the sample was kept in dark place and sample was ground to a fine powder with the help of an electrical blender. The ground dried leaf powder was loaded in Soxhlet apparatus and extracted with high polarity methanol solvent. Later collecting liquid extract was allowed to condense by rotary vacuum evaporator. The condensed leafs extract was stored in below 4°C refrigerator until required for investigation for bioassays.

Ovicidal bioassay

The method of Su and Mulla (1998) [22] was slightly modified and used to test the ovicidal activity. Freshly, laid eggs were collected by providing ovitraps in mosquito cages from 10.00 to 16.00 h for collecting 0–6 h old eggs and 16.00 to 10.00 the next morning for collecting 0–18 h old eggs raft of *A. stephensi*. Ovidtraps were kept in the cages days after the female mosquitoes were given a blood meal. The eggs were laid on filter paper lining provided in the ovitrap. After scoring, the filter papers containing the eggs were exposed to graded does of different products methanol or control solution. A minimum of 100 eggs was used for each treatment, and the experiment was replicated 4 times. Eggs of the age group 0–6 were exposed to the test solution for 18 h, while eggs were sieved through muslin cloth, thoroughly rinsed with tap water, and left in enamel bowls filled with dechlorinated water for hatching. The hatching rate of eggs was assessed 3 days later. The percent egg mortality was calculated on the basis of non-hatching of eggs with unopened opercula.

Insect growth regulatory (IGR) bioassay

IGR activity was assessed using 3rd instar. An accurate initial count of the larvae was made to avoid recording-missing (as dead) larvae as a result of cannibalistic or scavenging behavior during the long exposure period, larvae provided with a small amount of food (finely ground dog biscuits and yeast) at a concentration of 10 mg/l at 2 day intervals. The food powder should be suspended in water and one or two drops added per cup until mortality counts were made. Mortality or survival is counted every day until the complete emergence of adults. At the end of the observation periods, the number of larvae that do not develop successfully into viable adults or larvae and pupae were dead as well as adult mosquitoes not completely separated from pupal cases were recorded and expressed as a percent of emergence inhibition (EI). The experiment was terminated when all larvae or pupae in the controls had died or emerged as adults. The total mean EIs were calculated on the basis of the number of third-stage larvae exposed and EI is calculated using the formula.

$$\mathrm{EI}(\%) = \frac{100 - [\mathrm{T} \times 100]}{\mathrm{C}} \quad (1)$$

Where T=% of emergence in treated cohorts and C=% of emergence in the control larval and pupal duration assay.

The effect of different products of *H. auriculata* on the length of the larval and pupal stages, the test solutions of sublethal concentration (LC) of different products of *H. auriculata* (methanol) were prepared in an enamel tray of 30 cm × 25 cm × 5 cm dimensions. Fifty eggs were released in treated water and allowed to hatch**,** and the total larval duration (days) was calculated form hatching to pupation. Pupae were placed in a small container closed with a transparent mesh. The pupal duration was calculated from the pupal molt to adult emergence.

Larvicidal bioassay

The larvicidal activity of *H. auriculata* methanol extract was evaluated as per the standard method [23]. The whole bioassay tests were analyzed between the doses ranged of 10–120 µg/ml and were tested only on early third instars larvae (0–6 h old) of the target mosquitoes. The phytocompound was dissolved in 1 ml dimethyl sulfoxide and taken in 249 ml of dechlorinated water. Each test species, 25 larvae used for single concentration and replicated 4 times. Percent mortality was rectified for control mortality was calculated using Abbott's [24] formula. The LC_{50} values, Chi-square values, and other statistical values were calculated using probit analysis described by Finney [25].

In case of the experiment for determining pupicidal activity, the mouth of each bowl containing pupae was covered with muslin cloth to prevent the escape of any emerged adult mosquitoes. Mortality in larvae and pupae was recorded at 24 and 48 h.

$$\text{Corrected mortality} = \frac{\text{Observed mortality treatment} - \text{Observed mortality control}}{100 - \text{Control mortality}} \quad (2)$$

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae / pupae}}{\text{Number of larvae / pupae introduced}} \times 100 \quad (3)$$

RESULTS AND DISCUSSION

Ovicidal activity

Malaria is the largest single component of disease burden; epidemic malaria, in particular, remains a major public health concern in tropical countries. In many developing countries, and especially in Africa, malaria exacts an enormous toll in lives, in medical costs, and in days of labor lost. The results, egg hatchability, growth regulatory, longevity, fecundity, and larvicidal activity of *H. auriculata* methanol leaf extract were tested against *A. stephensi*. Eggs, larval, and pupal/adult mortality were *A. stephensi* of the *H. auriculata* methanolic leaf extract, hatching rate of the group 0–6 h exposed for 18 h and 0–18 h eggs exposed for 6 h and 18 h in various concentrations of methanolic extract was assayed every 24 h up to 3 days and their survival rate until pupation. The hatching rates of eggs exposed to various concentrations of methanolic extract were significantly lower than those of control eggs. Treatment of eggs of 0–6 was more effective in inducing higher rates of mortality than for eggs of 0–18 h exposed for 6 h and 18 h. The 0–6 h eggs, larval, and pupal/adult mortality were exposed for 18 h had a hatching rate of 24, 48, and 72 h, respectively. The hatching rates for concentration (20 ppm) of 0–6 h laid eggs, larval, and pupal/adult mortality (6 h exposure) at 24, 48, and 72 h, respectively, were methanolic leaf extract treatment of 14.0, 6.7, and 2.1% and higher concentration (100 ppm) mortality of 35.2, 46.5, and 17.3%, respectively, with the LC_{50}/LC_{90} values were 38.73440/71.33925 ppm. Furthermore, hatching rates for concentration (20 ppm) of 18 h (18 h exposed) eggs, larval, pupal/adult mortality treatment were 9.8, 4.5, and 2.3%, and the highest concentration of 100 ppm was mortality 31.4, 48.7, and 16.3%, respectively, with the LC_{50}/LC_{90} values were 39.89316/76.36159 ppm. Moreover, the hatching rates for 20 ppm concentration of eggs, 0–18 h (18 h exposed) at 24, 48, and 72 h, respectively, were 13.4, 4.2, and 6.3%, and 100 ppm concentration of mortality was 33.4%, 44.6%, and 17.9% methanolic extract treatment, with the LC_{50}/LC_{90} values were 35.42028/75.60023 ppm. The treatment of eggs of 0–6 h was more effective in including higher rates of mortality than for eggs 0–18 h treated for 6 h and 18 h. Exposure of freshly laid eggs was more effective than of older eggs. As the concentration of methanolic extract increased, the hatching rates decreased: (Control) to 16.8, 1.3, 2.5, 24 h, 48, and at 72 h and exposed 18 h; from (control) 13.8, 2.3, and 5.0, and 15.7, 5.9, and 6.3 for 0–18 eggs exposed for 18 h. There was an inverse relationship between concentration and the magnitude of the hatching rate (Table 1). The present investigations are comparable with some of the other previous reports that the major phytochemical elements of citronella of *Morinda officinalis* essential oil had significant larvicidal (85.44 and 159.73 ppm) activity against malaria vector [26]. The observed mortality rate suggests that the above extract can be used as biopesticide. The LC_{50} of second-, third-, and fourth-instar larvae of *A. stephensi* were 0.276, 0.285, and 0.305%, respectively. Spinosad treatment reduced the larval and pupal properties of microbial insecticides development of growth control. The bacterial insecticide spinosad against the first- to fourth-instar larvae and pupae showed values of LC_{50}=51.76 61.87, 74.07, 82.18, and 93.44 ppm, respectively [27]. The phytochemicals and larvicidal activity with confirm the presence of various photochemical contained glycosidase, saponin, fixed oil and fats, protein, carbohydrates, and tannin. The most effective larvicidal activity of *Cassia tora* higher concentration gave more than 80% mortality noticed in the larvae of *A. stephensi* [28].

IGR bioassay

The IGR effect of the methanolic extract of *H. auriculata* on mortality and EI of 3rd instar larvae of *A. stephensi* after treated with different doses. After 8 days of treatment, 89.7% of the larvae treated 150 ppm failed to emerge whereas 25.46%, 23.40% of the larvae treated at 120, 90 ppm successfully emerged into adults. Mortality of the treated 4th instar larvae on pupation with partly detached larval skin was recorded to be relatively higher at 150 and 120 ppm, revealing growth regulatory effect of methanolic on development/molting of immature (Table 2). Application of plant-

Table 1: Egg, larval, and pupal/adult mortality of *Anopheles stephensi* when eggs of either 0–6 or 18 h were treated with leaves methanolic extract of *Hygrophila auriculata* for 6h and 18 h

Concentration ppm	Eggs 0–6 h				Eggs 0–18 h					
	Treated for 18 h				(Treated for 18 h)				(Treated for 18 h)	
	Egg mortality %	Larval mortality %	Pupal/adult mortality %	Cumulative mortality %	Egg mortality %	Larval mortality %	Pupal/adult mortality %	Cumulative mortality %	Egg mortality %	Larval mortality %	Pupal/adult mortality %	Cumulative mortality %
20	14.0	6.7	2.1	22.8	9.8	4.5	2.3	16.6	13.4	4.2	6.3	23.9
40	17.3	29.5	6.9	53.7	13.7	30.2	14.9	58.8	15.6	33.5	14.6	63.7
60	25.7	38.3	13.2	77.2	22.5	39.3	18.1	79.9	23.7	39.4	18.3	81.4
80	31.8	43.8	20.5	96.1	25.3	44.4	21.0	90.7	26.2	41.6	24.1	91.9
100	35.2	46.5	17.3	99.0	31.4	48.7	16.3	96.4	33.4	44.6	17.9	95.9
Control	16.8	1.3	2.5	20.6	13.8	2.3	5.0	21.1	15.7	5.9	6.3	27.7
LC_{50} and LC_{90} values (LFL-UFL)	38.73440 (34.67278–42.37146)				39.89316 (23.45801–50.93152)				35.42028 (17.02841–46.56776)			
	71.33925 (66.45155–77.63205)				76.36159 (63.52530–104.34442)				75.60023 (62.74972–103.04467)			
Chi-square	0.966				9.292				7.884			
Reg. coefficient	Y=–1.52248+0.03931x				Y=–1.40190+0.03514x				Y=–1.12974+0.03190x			

LC_{50}: Lethal concentration that kills 50% of the exposed larvae and pupae, LC_{90}: Lethal concentration that kills 90% of the exposed larvae and pupae, LFL: Lower fiducial limit, UFL: Upper fiducial limit, χ^2: Chi-square value, significant at $p<0.05$ level

derived IGR substances is known to be safe for man and environment in the integrated pest management programs and so far only azadirachin, an IGR derived from Indian neem (*Azadirachta indica*) seed kernel being commercialized for managing against pests rather than vectors of diseases [29]. However, such naturally occurring insecticide prototypes are in great demand at the global level [30]. Which can be comparable to IGR activity of *Annona squamosa* against *A. stephensi* caused 82% reduced emergence, prolonged total development period [31] and acetone extracts of *Ipomea carnea fistula* exhibited excellent IGR activity against *A. stephensi* with average larval, pupal, development periods, and growth index were 18.93, 6.36; 25.29 (days), and 0.94 compared to control 14.44, 3.58, 18.02 (days), and 5.54 [32]. In the field, IGRs have shown excellent activity against larvae of many species of mosquitoes and related groups [33].

Larvicidal bioassay

Effect of methanolic extract was larval, pupal, and adult duration against *A. stephensi*. Duration of larval instars and total developmental time was prolonged at all doses compared to that control, water extracts of caused longest delayed development from 8.7 (control) to 16.6 days at 150 ppm larvae; 2.6 (control) to 7.5 days for pupae, and longevity of adult female greatly reduced from 37.6 (control) to 25.9 days and fecundity also greatly reduced from 98.0 (control) to 68.0 at 150 ppm methanolic extract respectively (Table 3). The LC_{50}/LC_{90} and Chi-square of values were *H. auriculata* leaf methanolic extract for 1st instars larvae which were 135.93/361.87 ppm and 5.426; 139.69/375.38 ppm and 5.703 for 2nd instars larvae; 160.85/410.70 ppm and 7.568 for 3rd instars larvae; 168.66/430.45 ppm and 5.280 for 4th instars; and 188.52/457.37 ppm and 5.443 for pupae, respectively, at 24 h. Furthermore, decreased at 48 h as for 1st instars larvae 63.44/271.95 ppm and 3.958; 57.55/272.48 ppm and 3.824 for 2nd instars larvae; 62.49/301.22 ppm and 3.310 for 3rd instars larvae; 67.69/330.48 ppm and 3.610 for 4th instars larvae; and 76.99/343.82 ppm and 3.610 for pupae, respectively. Among the different larval stages, 1st instars (younger) larvae were more susceptible than the other instars of (older) larvae (Table 4). A large number of plant extracts have been reported to have mosquitocides or repellent activities against mosquito vectors, but very few plant products have shown practical utility for mosquito control [34-38]. Plant-borne compounds and the fractions were tested as larvicides, ovicides, and repellency against *A. stephensi*. The larvicides activity was tested by 11-octadecenoic acid, methyl ester compound against *A. stephensi* with LC_{50} values of 22.32 ppm, respectively [39]. The leaf extract of methanol *Orthosiphon thymiflorus* against the first- to fourth-instars larvae and pupae showed LC_{50} values of 3.02, 3.73, 4.47, 5.82, and 7.45%, respectively [40]. The highest larvicidal activity of important medicinal plants, *Sesamum indicum*, *Pongamia pinnata*, and *Croton bonplandianum* methanol extract with LC_{50} and LC_{95} values was 108.55 and 230.57 ppm, 143.59 and 305.52 ppm, and 154.51 and 319.01 ppm, respectively [37].

Mosquito immature third instar larval, *A. stephensi* has been exposed to different concentrations of 50–250 µg/ml. Larvae were death rate 98.2% (significant value 0.001^b) from methanol extract, and it is significant toxicity against larvae of *A. stephensi* with LC_{50}/LC_{99} values were 157.69/339.55 µg/ml, respectively [41]. The first- to fourth-instar larvae and pupae of *A. stephensi* had values of LC_{50}=309.16, 337.58, 390.42, 429.68, and 513.34 ppm, and *Aedes aegypti* had values of LC_{50}=334.78, 366.45, 422.97, 467.94, and 54.02 ppm, respectively [42]. For *Bacillus thuringiensis* against *A. stephensi*, the LC_{50} and LC_{90} values of first- to fourth-instar larvae and pupae are 37.24, 45.41, 57.82, 80.09, and 98.34 ppm while the LC_{90} values are 92.32, 112.99, 150.25, 181.42, and 185.56 ppm; for *A. aegypti*, the LC_{50} values are 42.38, 51.90, 71.02, 96.17, and 121.59 ppm while the LC_{90} values are 106.07, 122.42, 176.24, 210.53, and 234.94 ppm [40]. The toxicity of *Euphorbia milii* molluscicidal latex and niclosamide showed toxic affect to *Anopheles albitarsis*, *A. aegypti*, and *Aedes fluviatilis* larvae [43]. The leaf extract of *Acalypha alnifolia* plant was tested against 4th instars larvae, *A. stephensi* had values of LC_{50}=197.37, 178.75, 164.34, 149.90, and 125.73 ppm, respectively [44]. The adults mortality was found in ethanol extract of *Clonorchis sinensis* with the LC_{50} and LC_{90} values of 289.62 and 494.88 ppm, respectively [45]. Nathan *et al.* [46] considered pure

Table 2: Effect of methanol extract *Hygrophila auriculata* on growth development and emergence against of *Anopheles stephensi*

4th day					8th day				
Concentration (ppm)	Third instar	IV instar while pupation	Pupae	Total	Third instar	IV instar while pupation	Pupae	Total	Emergence inhibition
30	11.26^{e}	15.00^{e}	13.32^{de}	39.58^{e}	16.35^{e}	23.04^{e}	18.25^{e}	57.64^{e}	84.0^{e}
60	13.80^{d}	18.53^{d}	14.95^{d}	47.28^{d}	19.21^{cd}	25.16^{d}	21.90^{d}	66.27^{d}	86.1^{d}
90	15.75^{c}	20.13^{c}	17.26^{c}	53.14^{c}	20.58^{c}	27.88^{c}	23.40^{cd}	71.86^{c}	87.2b^{c}
120	18.01^{b}	22.45^{b}	19.55^{b}	60.01^{b}	23.45^{b}	29.13^{b}	25.46^{b}	78.04^{b}	88.2^{b}
150	20.12^{a}	24.80^{a}	22.86^{a}	67.78^{a}	25.00^{a}	35.22^{a}	29.65^{a}	89.87^{a}	89.7^{a}
Control	1.4^{f}	1.1^{f}	1.9^{f}	4.4^{f}	3.95^{f}	2.90^{f}	2.33^{a}	9.18^{f}	0^{f}

Within a column means followed by the same letter (s) are not significantly different by Duncan's multiple range test, Significant values=0.05

Table 3: Total larval, pupal and adult duration of *Anopheles stephensi* after treatment with methanolic extract of *Hygrophila auriculata*

Concentration (ppm)	Mean (±SE) total larval duration (days)	Mean (±SE) total pupal duration (days)	Mean (±SE) females longevity (days)	Fecundity (number of eggs laid by the females)
30	9.8±0.5^{e}	3.5±0.3^{e}	35.7±0.7^{de}	94±1^{e}
60	11.3±0.8^{cd}	4.3±0.9^{cd}	33.4±1.1^{cd}	91±3^{d}
90	12.1±1.1^{c}	5.0±1.0^{c}	31.5±0.2^{c}	87±2^{c}
120	14.0±0.9^{b}	6.4±0.5^{b}	28.4±1.9^{b}	79±1^{b}
150	16.6±1.8^{a}	7.5±1.4^{a}	25.9±0.6^{a}	68±3^{a}
Control	8.7±0.4^{f}	2.6±0.7^{f}	37.6±1.4^{f}	98±5^{f}

SE: Standard error. Means (±SE) followed by same letter (s) within rows indicate are not significantly different by Duncan's multiple range test, Significant values=0.05

Table 4: Lethal concentration values of leaves methanolic extract of *Hygrophila auriculata* against *Anopheles stephensi* treated with 24 and 48 h

Mosquito larval instars and pupae	Exposure hours	LC_{50} values (LFL-UFL) (ppm)	LC_{90} values (UFL-UFL) (ppm)	Regression equation	χ^2 (df=4)
1st instar	24	135.93 (32.57–191.28)	361.87 (301.42–486.26)	Y=−0.77107+0.00567x	5.426
	48	63.44 (9.42–99.20)	271.95 (243.96–309.84)	Y=−0.38995+0.00615x	3.958
2nd instar	24	139.69 (29.41–97.60)	375.38 (311.16–512.54)	Y=−0.75963+0.00544x	5.703
	48	57.55 (0.08–95.14)	272.48 (243.93–311.04)	Y=−0.34320+0.00596x	3.824
3rd instar	24	160.85 (28.77–226.51)	410.70 (333.20–606.95)	Y=−0.82507+0.00513x	7.568
	48	62.49 (3.23–101.83)	301.22 (270.35–343.37)	Y=−0.33551+0.00537x	3.310
4th instar	24	168.66 (132.62–197.11)	430.45 (92.72–483.12)	Y=−0.82567+0.00490x	5.280
	48	67.69 (5.90–108.88)	330.48 (296.845–377.39)	Y=−0.33015+0.00488x	4.247
Pupae	24	188.52 (94.97–245.72)	457.37 (381.02–626.19)	Y=−0.89863+0.00477x	5.443
	48	76.99 (17.83–117.08)	343.82 (309.50–391.76)	Y=−0.36980+0.00480x	3.610

LC_{50}: Lethal concentration that kills 50% of the exposed larvae and pupae, LC_{90}: Lethal concentration that kills 90% of the exposed larvae and pupae, LFL: Lower fiducial limit, UFL: upper fiducial limit, χ^2: Chi-square value, df: Degrees of freedom, Significant at p<0.05 level

limonoids of neem seed, testing for biological, larvicidal, pupicidal, adulticidal, and antiovipositional activity; *A. stephensi* and the larval mortality were dose-dependent with the highest dose of 1 ppm azadirachtin evoking almost 100% mortality, affecting pupicidal and adulticidal activity and significantly decreasing fecundity and longevity of *A. stephensi*. The larvicidal and adulticidal activities of ethanolic and water mixture (50:50) of plant extracts *Eucalyptus globulus*, *Cymbopogon citratus*, *Artemisia annua*, *Justicia gendarussa*, *Myristica fragrans*, *Annona squamosa*, and *Centella asiatica* were tested against *A. stephensi*, and the most effective between 80 and 100% was observed in all extracts [47].

CONCLUSION

H. auriculata leaf methanol extract has displayed toxicity on different larval instars of *A. stephensi*. The study showed an increase in mortality with the increase in concentration, and the early instars larvae are much susceptible than the later ones. The results suggested that a small volume of *H. auriculata* leaf methanol extract is sufficient and can be directly used in the dwelling habitats mosquito vector, *A. stephensi* for effective control. This is an environmentally safe and eco-friendly approach for the vector control programs.

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AUTHORS' CONTRIBUTION

Informed consent was obtained from both individual participants included in the study.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. World Health Organization. Malaria Fact Sheet, No. 9. Geneva: World Health Organization Press; 2015.
2. World Health Organization. Working to Overcome the Global Impact of Neglected Tropical Diseases: First WHO Report on Neglected Tropical Diseases. Geneva: Department of Reproductive Health and Research, World Health Organization; 2010. p. 172.
3. Golding N, Wilson AL, Moyes CL, Cano J, Pigott DM, Velayudhan R, *et al*. Integrating vector control across diseases. BMC Med 2015;13:249.
4. World Health Organization. Malaria Fact Sheet, No. 94. Geneva: World Health Organization; 2014.
5. Rathy MC, Sajith U, Harilal CC. Larvicidal efficacy of medicinal plant extracts against the vector mosquito *Aedes albopictus*. Int J Mosq Res 2015;2:80-2.
6. World Health Organization. World Malaria Report. Geneva: World Health Organization; 2016.
7. World Health Organization. World Malaria Report. Geneva: World Health Organization; 2018.
8. Baranitharan M, Tamizhazhagan V, Kovendan K. Medicinal plants as potent power for malaria control: A review. Entomol Appl Sci Lett 2019;5:28-44.
9. Bhuktar AS. In: Mungikar Am, Bhuktar AS, editor. Plant Resources Development. Aurangabad: Sarswati Printing Press; 2000. p. 146-52.
10. Jain SK. Dictionary of Indian Folk Medicine and Ethnobotany. New Delhi: Deep Publications; 1991. p. 105-6.
11. Bairaj P, Nagarajan S. Apigenin 7-O-glucuronide from the flowers of *Asteracantha longifolia* Nees. Indian Drug 1982;19:150-2.
12. Parashar VV, Harikishan S. Investigation of *Asteracantha longifolia* Nees. Indian J Pharmacol 1965;27:109-13.
13. Misra TN, Singh RS, Pandey HS, Pandey BK. Constituents of *Asteracantha longifolia* Nees. Fitoterapia 2001;72:194-6.
14. Choudhary BK, Bandyopdhyay NG. Important of mineral content and medicinal properties of *Moringa oleifera* and *Hygrophila auriculata*. Sachitra Ayurveda 1980;50:543-9.
15. Quasim C, Dutta NL. Reported the prescence of stigmasterol in the root of *Asteracantha longifolia* Nees. J Indian Chem Soc 1967;44:82-3.
16. Govindachari TR, Nagarajan K, Pai BR. Isolation of lupeol from the root of *Asteracantha longifolia* Nees. J Ind Sci Res 1957;16:72-7.
17. Shanmugasundaram P, Venkatraman S. Anti-nociceptive activity of *Hygrophila auriculata* (schum) Heine. Afr J Tradit Complement Altern Med 2005;2:62-9.
18. Mazumdar UK, Gupta M, Maiti S, Mukherjee D. Antitumor activity of *Hygrophila spinosa* on Ehrlich ascites carcinoma and sarcoma-180 induced mice. Indian J Exp Biol 1997;35:473-7.
19. Boily Y, Van Puyvelde L. Screening of medicinal plants of Rwanda (Central Africa) for antimicrobial activity. J Ethnopharmacol 1986;16:1-3.
20. Kumar KC, Müller K. Medicinal plants from Nepal; II. Evaluation as inhibitors of lipid peroxidation in biological membranes. J Ethnopharmacol 1999;64:135-9.
21. Vishnu SN, Sunil K, Ramdas P. Acute toxicity study of *Hygrophila auriculata* L. leaves methanolic extract in albino rats. J Pharm Chem Biol Sci 2015;3:388-95.
22. Su T, Mulla MS. Ovicidal activity of neem products (azadirachtin) against culex tarsalis and *Culex quinquefasciatus* (*Diptera*: *Culicidae*). J Am Mosq Control Assoc 1998;14:204-9.
23. World Health Organization. Guidelines for Laboratory and Field Testing of Mosquito Larvicides. Communicable Disease Control, Prevention and Eradication, WHO Pesticide Evaluation Scheme. Geneva: World Health Organization; 2005.
24. Abbott WS. A method of computing the effectiveness of an insecticide. J Eco Entomol 1925;18:265-7.
25. Finney DJ. Probit Analysis: A Ststistical Treatment of the Sigmoid Response Curve. London: Cambridge University Press; 1971. p. 633.
26. Baranitharan M, Dhanasekaran S, Murugan K, Kovendan K, Gokulakrishnan J. Chemical composition and laboratory investigation of *Melissa officinalis* essential oil against human malarial vector mosquito, *Anopheles stephensi* L. (*Diptera*: *Culicidae*). J Coast Life Med 2016;4:969-73.
27. Kovendan K, Murugan K, Kumar KP, Panneerselvam C, Kumar PM, Amerasan D, *et al*. Mosquitocidal properties of *Calotropis gigantea* (Family: *Asclepiadaceae*) leaf extract and bacterial insecticide *Bacillus thuringiensis* against the mosquito vectors. Parasitol Res 2012;111:531-44.
28. Swati S, Mansi P. Estimation of phytochemical components from *Cassia tora* and to study its larvicidal activity. Int J Pharm Sci Invent 1988;4:11-6.
29. Schmutterer H. Potential of Azdirachtin-containing pesticides for integrated pest control in developing and industrialized countries. J Insect Physiol 1988;34:713-9.
30. Dwijendra S, Sucheta SM, Nirmal KN, Yogendra NS, Mamta M. Now possible insect growth regulators from Catharanthus roseu. Cuur Sci 2003;84:1184-6.
31. Saxena SC, Yadav RS. A new plant extract to suppress the population of yellow fever and dengue vector *Aedes aegypti* (*Diptera*: *Culicidae*) Curr Sci 1983;52:713-5.
32. Saxena SC, Sumitra L. Laboratory evaluation of leaf extracts of new plant to suppress the population of malaria vector *Anopheles stephnensi* Liston (*Diptera*: *Culicidae*). J Am Mosq Control Assoc 1985;9:84-7.
33. Amalraj D, Vasugi V, Kalayanasundram M, Tay BK, Das PK. Laboratory and filed evaluation of three insect growth regulators against mosquito vectors. J Med Res 1988;87:24-31.
34. Thishimenan S, Baskaran J, Baranitharan M, Jeyasankar A. Laboratory investigation of *Terminalia arjuna* and *Trachyspermum roxburghianum* against groundnut pest, *Helicoverpa armigera*. Asian J Pharm Clin Res 2016;9:232-6.
35. Latif MS, Abbas S, Kormin F, Mustafa MK. Green synthesis of plant-mediated metal nanoparticles the role of polyphenols. Asian J Pharm Clin Res 2019;12:75-84.
36. Kovendan K, Chandramohan B, Dinesh D, Abirami D, Vijayan P, Vincent S, and Benelli G. Green-synthesized silver nanoparticles using *Psychotria nilgiriensis*: toxicity against the dengue vector *Aedes aegypti* (*Diptera*: *Culicidae*) and impact on the predatory efficiency of the non-target organism *Poecilia sphenops* (*Cyprinodontiformes*: *Poeciliidae*) J Asia Pacific Entomol 2016;19:1001-7.
37. Baranitharan M, Dhanasekaran S, Murugan K, Kovendan K, Gokulakrishnan J, Jeyasankar A. Experimental investigations of Nagapattinam indigenous medicinal plant extracts against dengue, malaria and filarial disease. Int J Zool Appl Biosci 2017;2:155-61.
38. Dhanasekaran S, Baranitharan M, Muthulingam M, Senthilmurugan S, Jeyasankar A, Sawicka B. Studies on the impact of medicinal plants in relation to malaria vector control against *Anopheles stephensi*. Innoriginal Int J Sci 2018;5:12-4.
39. Baranitharan M, Dhanasekaran S, Murugan K, Kovendan K, Gokulakrishnan J, Benelli G. *Coleus aromaticus* leaf extract fractions: A source ofnovel ovicides, larvicides and repellents against Anopheles, *Aedes* and *Culex* mosquito vectors? Proc Safe Environ Prot 2017;106:23-33.
40. Kovendan K, Murugan K, Vincent S. Mosquitocidal efficacy of *Orthosiphon thymiflorus* (Roth) sleesen. (Family: *Labiatae*) leaf extract and microbial insecticide, *Metarhizium anisopliae* against dengue vector, *Aedes aegypti* (*Diptera*: *Culicidae*). Divers Physiol Proc 2012;26:137-51.
41. Baranitharan M, Sawicka B, Gokulakrishnan J. Phytochemical profiling and larval control of *Erythrina variegata* methanol fraction against malarial and filarial vector. Adv Prev Med 2019;2019:2641959.
42. Kumar PM, Kovendan K, Murugan K. Integration of botanical and bacterial insecticide against *Aedes aegypti* and *Anopheles stephensi*. Parasitol Res 2013;112:761-71.
43. Oliveira-Filho EC, Paumgartten FJ. Toxicity of *Euphorbia milii* latex and niclosamide to snails and nontarget aquatic species. Ecotoxicol Environ Saf 2000;46:342-50.
44. Kovendan K, Murugan K, Vincent S. Evaluation of larvicidal activity of *Acalypha alnifolia* Klein ex Willd. (*Euphorbiaceae*) leaf extract against the malarial vector, *Anopheles stephensi*, dengue vector, *Aedes aegypti* and *Bancroftian filariasis* vector, *Culex quinquefasciatus* (*Diptera*: *Culicidae*). Parasitol Res 2012;110:571-81.
45. Murugan K, Kumar PM, Kovendan K, Amerasan D, Subrmaniam J, Hwang JS, *et al*. Larvicidal, pupicidal, repellent and adulticidal activity of *Citrus sinensis* orange peel extract against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (*Diptera*: *Culicidae*). Parasitol Res 2012;111:1757-69.
46. Nathan SS, Kalaivani K, Murugan K. Effects of neem limonoids on the malaria vector *Anopheles stephensi* Liston (*Diptera*: *Culicidae*). Acta Trop 2005;96:47-55.
47. Senthilkumar N, Varma P, Gurusubramanian G. Larvicidal and adulticidal activities of some medicinal plants against the malarial vector, *Anopheles stephensi* (Liston). Parasitol Res 2009;104:237-44.