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Larvicidal, ovicidal and pupicidal activities of *Gliricidia sepium* (Jacq.) (Leguminosae) against the malarial vector, *Anopheles stephensi* Liston (Culicidae: Diptera)

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ABSTRACT

Objective: To investigate the potentiality of mosquitocidal activity of *Gliricidia sepium* (*G. sepium*) (Jacq.) (Leguminosae). **Methods:** Twenty five early third instar larvae of *Anopheles stephensi* (*An. stephensi*) were exposed to various concentrations (50-250 ppm) and the 24 h LC_{50} values of the *G. sepium* extract was determined by probit analysis. The ovicidal activity was determined against *An. stephensi* to various concentrations ranging from 25-100 ppm under laboratory conditions. The eggs hatchability was assessed 48 h post treatment. The pupicidal activity was determined against *An. stephensi* to various concentrations ranging from 25-100 ppm. Mortality of each pupa was recorded after 24 h of exposure to the extract. **Results:** Results pertaining to the experiment clearly revealed that ethanol extract showed significant larvicidal, ovicidal and pupicidal activity against the *An. stephensi*. Larvicidal activity of ethanol extracts of *G. sepium* showed maximum mortality in 250 ppm concentration (96.0±2.4)%. Furthermore, the LC_{50} was found to be 121.79 and the LC_{90} value was recorded to be 231.98 ppm. Ovicidal activity of ethanol extract was assessed by assessing the egg hatchability. Highest concentration of both solvent extracts exhibited 100% ovicidal activity. Similarly, pupae exposed to different concentrations of ethanol extract were found dead with 58.10% adult emergence when it was treated with 25 ppm concentration. Similarly, 18.36 (n=30; 61.20%); 21.28(70.93) and 27.33(91.10) pupal mortality was recorded from the experimental pupae treated with 50, 75 and 100 ppm concentration of extracts. Three fractions have been tested for their larvicidal activity of which the Fraction 3 showed the LC_{50} and LC_{90} values of 23.23 and 40.39 ppm. With regard to the ovicidal effect fraction 3 showed highest ovicidal activities than the other two fractions. Furthermore, there were no hatchability was recorded above 50 ppm (100% egg mortality) in the experimental group. Statistically significant pupicidal activity was recorded from 75 ppm concentration. **Conclusions:** From the results it can be concluded the crude extract of *G. sepium* is an excellent potential for controlling *An. stephensi* mosquito. It is apparent that, fraction 3 possess a novel and active principle which could be responsible for those biological activities.

1. Introduction

Ecologically, mosquitoes are important components of aquatic and terrestrial food chains as they serve as food for a number of animals, such as fish and birds. With respect to the human well-being, mosquitoes are of great economic impact because their bites are annoying and may cause skin allergies, and they are vectors for a number of diseases, such as malaria, yellow fever, dengue, filariasis, and certain types of encephalitis such as West Nile Fever[1,2]. Malaria and filariasis rank amongst the world most prevalent tropical infectious diseases. An estimated 300-500 million people are infected with malaria annually, resulting in 1.5-3.0 million deaths[3]. Malaria remains a major health problem in Sudan. Accordingly about 20%-40% of outpatient clinic visits and approximately 30% of total hospital admissions are due to malaria[4]. Lymphatic filariases (LF) is probably the fastest spreading insect-borne disease of human in the tropic, about 30% (394 million) of the global population are estimated to be in the LF-endemic countries

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of the African region[5]. *Anopheles stephensi* (*An. stephensi*) (Liston) is the primary vector of malaria in India and other West Asian countries, and improved methods of control are urgently needed[6]. Insect vectors, especially mosquitoes are responsible for spreading serious human diseases like malaria[7]. The distribution and abundance of these diseases is strongly influenced by the presence of humans and by the level of poverty of the population[8]. Malaria is by far the most important insect transmitted disease[9], remaining a major health problem in many parts of the world and is responsible for high childhood mortality and morbidity in Africa and Asia[10-12]. *An. stephensi* have, therefore, become a challenging problem to public health worldwide, and it has a serious social and economical impact especially in tropical and subtropical countries[13-15]. The control of mosquito at the larval stage is necessary and efficient in integrated mosquitoes management. During the immature stage, mosquitoes are relatively immobile; remaining more concentrated than they are in the adult stage[16]. 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 non-target organisms, and fostered environmental and human health concerns[17]. Thus, the effort towards mosquito control continues to be an important strategy in preventing the mosquito borne diseases[18]. Over the past 50 years, more than 2 000 plant species belonging to different families and genera have been reported to contain toxic principles, which are effective against insects[19,20]. In India, there are various plants known for their insecticidal property and are popular as pesticides. Plant derived compounds (phytopesticides) in general have been recognized as an important natural resource of insecticides[21]. Several phytochemicals have been reported to exhibit detrimental effect on mosquitoes[22-24]. In addition to application as general toxicant against mosquito larvae, botanical insecticides also have potential uses as growth and reproduction inhibitors, repellents, ovicidal and oviposition deterrents[25-27]. Furthermore, a mosquitocidal property of *Gliricidia sepium* (*G. sepium*) has not yet reported. Therefore, the present study was carried out to determine the larvicidal, ovicidal and pupicidal efficacy of *G. sepium* against *An. stephensi*.

2. Materials and methods

2.1. Collection and processing of plants

Plant sampling was carried out during the growing season (March-April) of 2010 from different places of Cuddalore districts of the Tamilnadu. Bulk samples were air-dried in the shade and after drying each sample was ground to a fine powder. At the time of collection, two pressed voucher herbarium specimens were prepared per species and identified with the help of plant taxonomist, Department of Botany, Annamalai University, whenever possible, flowering or fruiting specimens were collected to facilitate taxonomic identification.

2.2. Extraction method

The dried leaf (100 g) were powdered mechanically using commercial electrical stainless steel blender and extracted sequentially with ethyl acetate and ethanol (500 mL, Ranchem), in a Soxhlet apparatus separately until exhaustion. The extract was concentrated under reduced pressure 22-26 mmHg at 45 ℃ by 'Rotavapour' and the residue obtained was stored at 4 ℃.

2.3. Mosquito rearing

Eggs of *An. stephensi* were collected from ICMR centre, Virudachalam. The egg rafts were then brought to the laboratory. The eggs were placed in enamel trays (30 cm×24 cm×5 cm) each containing 2 L of tap water and kept at room temperature (28 ± 2) ℃ with a photoperiod of 16:8 h (L:D) for larval hatching. The larvae of each mosquito species were maintained in separate trays under the same laboratory conditions and fed with a powdered feed containing a mixture of dog biscuit and baker's yeast (3:1 ratio). The trays with pupae of each mosquito species were maintained in separate mosquito cages at (26±2) ℃ and relative humidity of (85±3)% under a photoperiod of 16:8 h (L:D) for adult emergence. Cotton soaked in 10% aqueous sucrose solution in a Petri dish to feed adult mosquitoes was also placed in each mosquito cage. An immobilized young chick was placed for 3 h inside the cage in order to provide blood meal especially for female mosquitoes. A plastic tray (11 cm× 10 cm×4 cm) filled with tap water with a lining of partially immersed filter paper was then placed inside each cage to enable the female mosquitoes to lay their eggs. The eggs obtained from the laboratory-reared mosquitoes were immediately used for toxicity assays or allowed to hatch out under the controlled laboratory conditions described above. Only the newly hatched larvae/pupae of *An. stephensi* were used in all bioassays.

2.4. Larvicidal activity

The larvicidal activity of plant crude extract was assessed by using the standard method as prescribed by[28]. From the stock solution, five different test concentrations (*viz.*, 50, 100, 150, 200 and 250 ppm were prepared and they were tested against the freshly moulted (0-6 h) third instar larvae of *An. stephensi*. The larvae of test species (25) were introduced in 500 mL plastic cups containing 250 mL of aqueous medium (249 mL of dechlorinated water + 1 mL of emulsifier) and the required amount of plant extract was added. The larval mortality was observed and recorded after 24 h of post treatment. For each experiment, five replicates were maintained at a time. The LC_{50} value was calculated by using Probit analysis[29].

2.5. Ovicidal activity

The method of Su and Mulla[30], was slightly modified and used to test the ovicidal activity. The various concentrations

as stated in the previous experiments were prepared from the stock solution. Before treatment, the eggs of *An. stephensi* were counted individually with the help of hand lens. Freshly hatched eggs (100) were exposed to each concentration of leaf extract until they hatched or died. Eggs exposed to DMSO in water served as control. After treatment, the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under a microscope. Each test was replicated five times. The hatchability was assessed 48 h post treatment.

2.6. Pupicidal activity

Batches of ten pupae were introduced into 500 mL of the test medium containing particular concentration of the crude extract in a plastic cups in five replications. In control, the same number of pupae was maintained in 500 mL of dechlorinated water containing appropriate volume of DMSO. All containers were maintained at room temperature (28±2) ℃ with naturally prevailing photoperiod (12:12h/L:D) in the laboratory. Any pupa was considered to be dead if did not move when prodded repeatedly with a soft brush. Mortality of each pupa was recorded after 24 h of exposure to the extract following the Abbott formula[31].

2.7. Determination of lethal concentrations

Lethal concentration (LC_{50}) represents the concentration of the test material that caused 50% mortality of the test (target and non target) organisms within the specified period of exposure, and it was determined by exposing various developmental stages of the mosquitoes to different concentrations of the extract. Based on the mortality of the test organisms recorded in these bioassays, LC_{50} was calculated along with their fiducial limits at 95% confidence level by probit analysis using SPSS software package.

2.8. Identification of fractions

The effective plant extract was used for further analysis to identify the number of compounds responsible for their effectiveness. The extract was run on pre coated Thin Layer Chromatography sheets (ALUCHROSEP SILICA GEL 60/ UV product no 25425 S.D. fine Chem. Ltd., Mumbai). The solvent mixture consisting of ethylacetate : ethanol 0.5:9.5 ratio. Then the maximum number of fractions (4 fractions) was obtained with the same solvent system in Column Chromatography.

3. Results

3.1. Larvicidal activity of plant extracts

Larvicidal activity of *G. sepium* ethyl acetate and ethanol extracts were tested against third instar larvae of *An. stephensi*. Data pertaining to the results clearly revealed that minimum larval mortality was observed in ethyl acetate extract of *G. sepium* with 21.2±1.6 at 50 ppm concentration and the maximum mortality was observed from the same extract with (92.2±1.24)%. Furthermore, the LC_{50} and LC_{90} value for ethyl acetate extract was found to be 144.25 (LCL=115.65; UCL=175.87) and 260.01 (LCL=218.21; UCL=343.30) ppm respectively with the chi square value of 13.707. Similarly larvae exposed to 50 ppm concentration of ethanol extract showed less susceptibility whereas, experimental larvae exposed to 250 ppm concentration showed more susceptibility to the same extract. Furthermore, the LC_{50} was found to be 121.79(LCL=88.91 and UCL=154.01) and the LC_{90} value was recorded to be 231.981 (LCL=191.28 and UCL=16.65 ppm with the chi square value of 17.15. The recorded data were found statistically significant (Table 1; DMRT, $P<0.05$).

3.2. Ovicidal activity of plant extracts

Ovicidal activity of ethyl acetate and ethanol extract was assessed by assessing the egg hatchability. It was noted that 100% hatchability was noted from the control groups, which means 0% ovicidal activity. Highest concentration of both solvent extracts exhibited 100% ovicidal activity as it was evident from the Table 2. Further, as the concentration increased the mortality of the eggs were also increased with decreased hatching percentage. The data obtained in the experiments were statistically significant over the control.

Table 1
Larvicidal activity of *G. sepium* crude extracts against freshly moulted (0–6 h old) third instar larvae of *An. stephensi*.

Crude extract tested	Concentration (ppm)	Mortality (%)*
Ethyl acetate extract	Control	1.4 ± 0.8[a]
	50	21.2 ± 1.6[b]
	100	35.6 ± 0.6[c]
	150	49.4 ± 1.6[d]
	200	64.6 ± 1.6[e]
	250	92.2 ± 1.2[f]
Ethanol extract	Control	1.6 ± 0.6[a]
	50	31.2 ± 0.6[b]
	100	42.8 ± 1.6[c]
	150	56.4 ± 1.8[d]
	200	78.2 ± 1.6[e]
	250	96.0 ± 2.4[f]

Value represents mean ± SD of five replications. *Mortality of the larvae observed after 24 h of exposure period. Different alphabets in the column are statistically significant at $P<0.05$ level DMRT test.

Table 2
Ovicidal activity of *G. sepium* crude extracts against eggs (0–6 h old) of *An. stephensi*.

Concentrations (ppm)	Crude extracts tested	
	Ethyl acetate extract	Ethanol extract
Control	100.00 ± 0.00[a]	100.00 ± 0.00[a]
25	87.64 ± 1.82[b]	81.34 ± 1.62[b]
50	63.63 ± 1.64[c]	52.25 ± 1.83[c]
75	48.42 ± 1.28[d]	35.36 ± 1.44[d]
100	0.00 ± 0.00[e]	0.00 ± 0.00[e]

Values represent mean ± SD of five replications. Different alphabets in the column are *statistically significant at $P<0.05$ level DMRT test. Eggs in control groups were sprayed with no phytochemicals.

3.3. Pupicidal activity of plant extracts

Effect of ethyl acetate and ethanol crude extract of the *G. sepium* tested on the pupae of *An. stephensi*, data obtained from the experiment is presented in Table 3. About 13.62 pupae were found dead with 54.60% adult emergence when it was treated with 25 ppm concentration of ethyl acetate extract of *G. sepium*. Similarly, 17.43±1.38 (*n*=30; 58.10%); (20.33±1.69)(67.76%) and (25.48±2.33)(84.93%) pupal mortality was recorded from the experimental pupae treated with 50, 75 and 100 ppm concentration of extracts. Pupae exposed to different concentrations of ethanol extract were found dead with 58.10% adult emergence when it was treated with 25 ppm concentration. Similarly, 18.36 (*n*=30; 61.20%); 21.28 (70.93%) and 27.33 (91.10%) pupal mortality was recorded from the experimental pupae treated with 50, 75 and 100 ppm concentration of extracts.

3.4. Fractionation of ethanol extract

In the above results it is evident that ethanol extract of *G. sepium* exhibited strong activity against the three mosquito species. Hence, it was fractioned using TLC with varying solvent systems. Ethyl acetate: ethanol (2:8) gave 1 fraction, two fractions have been obtained in ethyl acetate: ethanol (1.5:8.5), two fractions have been obtained in ethyl acetate: ethanol (1:9) and the maximum of three fractions (Fraction 1, Fraction 2 and Fraction 3) have been obtained in ethyl acetate: ethanol (0.5:9.5). Further, the three fractions were checked for their bioefficacy against the selected mosquito species.

3.5. Bioassay of fractions

Three fractions have been tested for their larvicidal activity against the larvae of *An. stephensi*, of which the Fraction 3 showed the LC_{50} and LC_{90} values of 23.23 and 40.39 ppm against *An. stephensi* (Table 4). With regard to the ovicidal effect of obtained fractions, the number of eggs hatched in the experimental batches treated with 25 and 50 ppm concentration of the fraction 3 showed highest ovicidal activity than the other two fractions. Furthermore, there were no hatchability was recorded above 50 ppm (100% egg mortality) in the experimental group (Table 5). Pupicidal activity of three fractions were tested at 25, 50 and 75 ppm concentrations against *An. stephensi*, data pertaining to the experiment revealed that least pupicidal activity was (12.45±1.23) observed at 25 ppm concentration whereas, at 50 moderate pupicidal activity was recorded (24.62±1.64). Besides, the 100% pupicidal activity was noticed from 75 ppm concentration (Table 6). It is apparent that, fraction 3 possess a novel and active principle which could be responsible for those biological activities. Hence, a detailed spectral analysis is to be made to identify the compound(s).

Table 3
Pupicidal activity of *G. sepium* crude extracts against the pupae of *An. stephensi*.

Concentration (ppm)		Mortality*		Adult emergence	
		Pupal mortality	% mortality	Adult	% emergence
Ethyl acetate extract	25	13.62 ± 1.56^{b}	45.40	16.38 ± 1.34^{d}	54.60
	50	17.43 ± 1.38^{c}	58.10	12.57 ± 1.65^{c}	41.90
	75	20.33 ± 1.69^{d}	67.76	9.67 ± 0.87^{b}	32.23
	100	25.48 ± 2.33^{e}	84.93	4.52 ± 1.23^{a}	15.06
	Control	3.45 ± 0.98^{a}	11.50	26.55 ± 2.33^{e}	88.50
Ethanol extract	25	12.57 ± 1.25^{b}	41.90	17.43 ± 1.22^{d}	58.10
	50	18.36 ± 1.38^{c}	61.20	11.64 ± 1.36^{c}	38.80
	75	21.28 ± 1.29^{d}	70.93	8.72 ± 1.45^{b}	29.06
	100	27.33 ± 1.36^{e}	91.10	2.67 ± 0.32^{a}	8.90
	Control	1.83 ± 1.87^{a}	6.10	28.17 ± 1.37^{e}	93.90

Value represents mean ± SD of five replications. *Mortality of the pupae observed after 24 h of exposure period. Values in the column with a different superscript alphabet are significantly different at $P<0.05$ level DMRT test.

Table 4
Larvicidal activity of ethanol extract of *G. sepium* fractions against freshly moulted (0–6 h old) third instar larvae of *An. stephensi*.

Fractions tested	LC_{50} (ppm)	LCL	UCL	LC_{90} (ppm)	LCL	UCL	*df*	χ^2 value
Fraction 1	30.50	25.81	35.72	31.59	44.55–	64.22	4	10.841
Fraction 2	39.45	37.08	42.20	62.71	57.98–	60.06	4	7.189
Fraction 3	23.23	18.41	27.91	40.39	34.58–	50.69	4	13.465

LC_{50}=Lethal concentration for 50% larval mortality and LC_{90} = Lethal concentration for 90% larval mortality. LCL = Lower confidence limit; UCL = Upper confidence limit.

Table 5
Ovicidal activity of ethanol extract of *G. sepium* fractions against eggs (0–6 h old) of *An. stephensi.*

Concentrations tested	25 ppm	50 ppm
Control	100.00 ± 0.00[a]	100.00 ± 0.00[a]
Fraction 1	46.32 ± 2.36[c]	12.38 ± 1.22[c]
Fraction 2	48.66 ± 2.44[b]	14.72 ± 1.42[b]
Fraction 3	18.08 ± 1.34[d]	0.00 ± 0.00[d]

Values represent mean ± SD of five replications. Different alphabets in the column are *statistically significant at $P<0.05$ level DMRT test. Eggs in control groups were sprayed with no phytochemicals.

Table 6
Pupicidal activity of ethanol extract of *G. sepium* fractions against the pupae of selected *An. stephensi*.

Concentration (ppm)	Pupal Mortality*	Adult emergence (%)	
Fraction 1	25	6.54 ± 0.82[b]	23.46 ± 1.36[c]
	50	10.66 ± 1.29[c]	19.34 ± 1.68[b]
	75	26.18 ± 1.56[d]	3.82 ± 1.23[a]
	Control	1.24 ± 0.26[a]	28.76 ± 1.33[d]
Fraction 2	25	8.64 ± 1.33[b]	21.36 ± 1.84[c]
	50	19.28 ± 1.16[c]	10.72 ± 1.82[b]
	75	23.22 ± 0.00[d]	6.78 ± 1.44[a]
	Control	1.46 ± 0.26[a]	28.54 ± 2.36[d]
Fraction 3	25	12.45 ± 1.23[b]	17.55 ± 1.36[c]
	50	24.62 ± 1.64[c]	5.38 ± 1.23[b]
	75	30.00 ± 0.00[d]	0.00 ± 0.00[a]
	Control	1.33 ± 0.26[a]	28.67± 2.33[d]

Value represents mean ± SD of five replications. *Mortality of the pupae observed after 7 d of exposure period). Values in the column with a different superscript alphabet are significantly different at $P<0.05$ level DMRT test.

4. Discussion

Chavasse and Yap[32] reported that with increasing legislative restrictions being implemented concerning the use of pesticides, safe, but efficient alternatives and application techniques must be developed to allow the least-toxic but most efficient means of integrated vector control, especially during emergency situations. Venkatachalam and Jebanesan[33] they have been reported that methanolic extracts of few plants exhibited larvicidal activity against *Culex quinquefasciatus* (*Cx. quinquefasciatus*). Rajkumar and Jebanesan[34] reported that increase in the concentration of leaf extract of *Solanum aerianthum* induced the oviposition attractant activity in *Cx. quinquefasciatus*. Recently Mathivanan *et al*[35–45] reported that the methanol extract of *Ervatamia coronaria* (*E. coronaria*) showed promising larvicidal and ovicidal activity agains *An. stephensi*. The direct and indirect contributions of such effects to treatment efficacy through reduced larval feeding and fitness need to be properly understood in order to improve the use of botanical insecticides for management of *An. stephensi*. Wandscheer *et al*[46] reported the naturally occurring insecticides may play a more prominent role in mosquito control programs in the future. Abdalla *et al*[47] reported that the larvicidal activity was monitored against 2nd, 3rd and 4th instar larvae of each mosquito species 24 h post-treatment. Adult emergence inhibition activity was tested by exposing 3rd instar larvae of each mosquito species to different concentrations of extracts (200, 400, 600, 800 and 1 000 ppm for *Anopheles arabiensis* and 100, 200, 300, 400, 500 and 600 ppm for *Cx. quinquefasciatus*. The oviposition deterrent activity was tested by using three different concentrations of extracts (1 000, 500 and 200 for *Anopheles arabiensis*, and 1 000, 500 and 100 for *Cx. quinquefasciatus* that caused high, moderate and low larval mortality in the larvicidal experiment against 3rd instar larvae. It was found that, LC_{50}–LC_{90} values calculated were 273.53–783.43, 366.44–1018.59 and 454.99–1224.62 ppm for 2nd, 3rd and 4th larval instars, respectively, of *Anopheles arabiensis* and 187.93–433.51, 218.27–538.27 and 264.85–769.13 ppm for 2nd, 3rd and 4th larval instars, respectively, of *Cx. quinquefasciatus*. Kweka *et al*[48] they have been reported that necessitated the search and development of environmentally safe, biodegradable, low-cost, and indigenous methods for vector control, which can be used without risk of harm to individuals and communities. The efficacy shown by *Schinus terebinthifolia* (*S. terebinthifolia*) for knockdown time and 100% mortality after 24 h to adult mosquitoes from wild resistant population warrants further investigation of these compounds for IRS small scale whether singly or in blends. This essential oil may be of great value in complementing other compounds which are losing efficacy[49]. This is

because larval habitat treatment is more localized in time and space resulting in effective control. In tropical countries, plants are known to possess larvicidal, ovicidal and adulticidal activities[50].

Recently Eliningaya *et al*[51] have been reported the mortality of *Cx. quinquefasciatus* ranged from 0.50% to 96.75% while for *Anopheles gambiae* s.s it was from 13.75% to 97.91%. In the semi- field experiments, the mortality rates observed varied for both species with time and concentrations. The LC_{50} and LC_{95} value in the laboratory was similar for both species while in the semi- field they were different for each. In wild, adult mosquitoes, the KT_{50} for *S. terebinthifolia* was 11.29 min while for alphacypermethrin was 19.34 min. The 24 h mortality was found to be 100.0% for *S. terebinthifolia* and 75.0% for alphacypermethrin which was statistically significant ($P<0.001$). Govindarajan *et al*[52] reported that the crude extract of *Ervatamia coronaria* exerted zero hatchability (100% mortality) at 250, 200 and 150 ppm for *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*, respectively. The crude extract of *Caesalpinia pulcherrima* zero hatchability (100% mortality) at 375, 3 000 and 225 ppm for *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*, respectively. The methanol extract of *E. coronaria* found to be more repellenct than *C. pulcherrima* extract. A higher concentration of 5.0 mg/cm^2 provided 100% protection up to 150, 180 and 210 min against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*, respectively. Since there is no previous record of literature available about the mosquitocidal activity of the selected plant *G. sepium* these present investigations serve as first hand information. The finding of the present investigation revealed that the leaf extract of *G. sepium* possessed remarkable larvicidal, ovicidal activity and pupicidal activity against the malarial vector *An. stephensi*.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Jang Y, Skim MK, Ahn YS, Lee HS. Larvicidal activity of Brazilian plant against *Ades aegypti* and *Culex pipiens* (Dipteral:Culicidae). *Agric Chem Biotechnol* 2002; **4**: 131-134.

[2] WHO. *Pesticides and their application for the control of vectors and pests of public health importance. World Health Organization. WHO/CDS/WHOPES/GCDPP/*. Geneva: WHO; 2006.

[3] WHO. *World Health Organization. Expert committee on malaria. Technical report series 892*. Geneva: WHO; 2000.

[4] WHO and UNICEF. *World malaria report 2005, WHO/HTM/MAL/2005*. Roll Back Malaria: World Health Organization and UNICEF; 2005.

[5] WHO. *Global programme to eliminate lymphatic filariasis*. Geneva: WHO; 2006.

[6] Burfield T, Reekie SL. Mosquitoes, malaria and essential oils. *Int J Aroma* 2005; **15**(3): 30-41.

[7] Halstead SB. Global perspective on dengue research. *Dengue Bull* 2000; **24**: 77-82.

[8] Mendonca FAC, Silva KFS, Santos KK, Ribeiro KAL, Sant Ana AEG. Activities of some brazilian plants against larvae of the mosquito *Aedes aegypti*. *Fitoterapia* 2005; **76**: 629-636.

[9] Halstead SB. Global perspective on dengue research. In: Gilles HM, Warrell DA. *Bruce-Chwatt's essential malariology*. 3rd ed. London: Edward Arnold; 1993, p. 77-82.

[10] Kleinschmidt I, Bagayoko M, Clarke GPY, Craig M, Sueur DL. A spatial statistical approach to malaria mapping. *Int J Epidemiol* 2000; **29**: 355-361.

[11] Pates H, Curtis C. Mosquito behavior and vector control. *Annu Rev Entomol* 2005; **50**: 53-70.

[12] Senthil Nathan S, Savitha G, Dency KG, Narmadha A, Suganya L, Chung PG. Efficacy of *Melia azedarach* L. extract on the malarial vector *Anopheles stephensi* Liston (Diptera: Culicidae). *Bioresour Technol* 2005; **5**: 118-123.

[13] Borovsky D. Trypsin-modulating oostatic factor. A potential new larvicide for mosquito control. *J Exp Biol* 2003; **206**: 3869-3875.

[14] Spielman A. Research approaches in the development of interventions against vector-borne infection. *J Exp Biol* 2003; **206**: 3727-3734.

[15] Bossche V, Coetzer JA. Climate change and animal health in Africa. *Rev Sci Tech* 2008; **27**: 551-562.

[16] Rutledge CR, Clarke F, Curtis A, Sackett S. Larval mosquito control. *Technical Bull Florida Mosq Control Assoc* 2003; **4**: 16-19.

[17] Lee SE, Kim JE, Lee HS. Insecticide resistance in increasing interest. *Agric Chem Biotechnol* 2001; **44**: 105-112.

[18] Billingsley PF, Foy B, Rasgon JL. Mosquitocidal vaccines: a neglected addition to malaria and dengue control strategies. *Trends Parasitol* 2008; **24**: 396-400.

[19] Govindarajan M, Jebanesan A, Pushpanathan T. Larvicidal and ovicidal activity of *Cassia fistula* Linn. Leaf extract against filarial and malarial vector mosquitoes. *Parasitol Res* 2008; **102**: 289-292.

[20] Sivagnaname N, kalyanasundaram M. Laboratory evolution of methanolic extract of atlantia monophylla (Family: Rutaceae) against immature stages of mosquitoes and non-torget organisms. *Mem Inst Oswaldo Cruz Rio De Jenerio* 2004; **99**(1): 115-118.

[21] Gbolade AA, Oyedele AO, Sosan MB, Adewayin FB, Soyela OL. Mosquito repellent activities of essential oils from two Nigerian *Ocimum* species. *J Trop Med Plants* 2000; **1**: 146-148.

[22] Kuo PM, Chu FH, Chang ST, Hsiao WF, Wang ST. Insecticidal

activity of essential oil from *Chamaecyparis formosensis*. *Holzforschung* 2007; **61**: 595–599.

[23]Ghosh A, Chowdhury N, Chandra G. Laboratory evaluation of a phytosteroid compound of mature leaves of Day Jasmine (Solanaceae: Solanales) against larvae of *Culex quinquefasciatus* (Diptera: Culicidae) and nontarget organisms. *Parasit Res* 2008; **103**: 271–277.

[24]Rahuman AA, Bagavan A, Kamaraj C, Saravanan E, Zahir AA, Elango G. Efficacy of larvicidal botanical extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae). *Parasitol Res* 2009; **104**: 1365–1372.

[25]Prajapati V, Tripathi AK, Aggarwal KK, Khanuja SP. Insecticidal, repellent and oviposition-deterrent activity of selected essential oils against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. *Bioresource Technol* 2005; **96** (16): 1749–1757.

[26]Rajkumar S, Jebanesan A. Larvicidal and adult emergence inhibition effect of *Centella asiatica* Brahmi (Umbelliferae) against mosquito *Culex quinquefasciatus* Say (Diptera: Culcidae). *Afr J Biomed Res* 2005; **8**: 31–33.

[27]Pushpanathan T, Jebanesan A, Govindarajan M. Larvicidal, ovicidal and repellent activities of *Cymbopogan citratus* Stapf. (Graminae) essential oil against the filarial mosquito *Culex quinquefasciatus* (Say) (Diptera: Culicidae). *Trop Biomed* 2006; **23** (2): 208–212.

[28]World Health Organization. *Guidelines for laboratory and field testing of mosquito larvicides. WHO/CDS/WHOPES/GCDPP/2005.13*. Geneva: World Health Organization; 2005.

[29]Finney DJ. *Probit analysis*. London: Cambridge University Press; 1979, p. 68–72.

[30]Su T, Mulla MS. Ovicidal activity of neem products (azadirachtin) against *Culex tarsalis* and *Culex quinquefasciatus* (Diptera: Culicidae). *J Am Mosq Cont Asso* 1998; **14**(2): 204–209.

[31]Abbott WS. A method for computing the effectiveness of an insecticide. *J Econ Entomol* 1925; **18**: 265–267.

[32]Chavasse DC, Yap HH. *Chemical methods for the control of vectors and pests of public health importance. WHO/CTD/WHOPES/97.2*. Geneva: World Health Organization; 1997.

[33]Venkatachalam MR, Jebanesan A. Larvicidal activity of *Hydrocotyl javanica* Thumb. (Apiaceae) extract against *Culex quinquefasciatus*. *J Exp Zool India* 2001; **4**(1): 99–101.

[34]Rajkumar S, Jebanesan A. Oviposition attractancy of *Solanum aerianthum* leaf extract for *Culex quinquefasciatus*. *J Exp Zool India* 2002; **5**(2): 221–224.

[35]Mathivanan T, Govindarajana M, Elumalai K, Krishnappa K, Ananthan A. Mosquito larvicidal and phytochemical properties of *Ervatamia coronaria* Stapf. (Family: Apocynaceae). *J Vector Borne Dis* 2010; **47**: 178–180.

[36]Prabhu K, Murugan K, Nareshkumar A, Ramasubramanian N, Bragadeeswaran S. Larvicidal and repellent potential of *Moringa oleifera* against malarial vector, *Anopheles stephensi* Liston (Insecta: Diptera: Culicidae). *Asian Pac J Trop Biomed* 2011; **1**(2): 124–129.

[37]Phasomkusolsil S, Soonwera M. Comparative mosquito repellency of essential oils against *Aedes aegypti* (Linn.), *Anopheles dirus* (Peyton and Harrison) and *Culex quinquefasciatus* (Say). *Asian Pac J Trop Biomed* 2011; **1**(Suppl 1): S113–S118.

[38]Sritabutra D, Soonwera M, Waltanachanobon S, Poungjai S. Evaluation of herbal essential oil as repellents against *Aedes aegypti* (L.) and *Anopheles dirus* Peyton & Harrion. *Asian Pac J Trop Biomed* 2011; **1**(Suppl 1): S124–S128.

[39]Arivoli S, Samuel T. Larvicidal efficacy of *Cleistanthus collinus* (Roxb.) (Euphorbiaceae) leaf extracts against vector mosquitoes (Diptera: Culicidae). *Asian Pac J Trop Biomed* 2011; **1**(Suppl 2): S281–S283.

[40]Beula JM, Ravikumar S, Ali MS. Mosquito larvicidal efficacy of seaweed extracts against dengue vector of *Aedes aegypti*. *Asian Pac J Trop Biomed* 2011; **1**(Suppl 2): S143–S146.

[41]Singha S, Banerjee S, Chandra G. Synergistic effect of *Croton caudatus* (fruits) and *Tiliacora acuminata* (flowers) extracts against filarial vector *Culex quinquefasciatus*. *Asian Pac J Trop Biomed* 2011; **1**(Suppl 2): S159–S164.

[42]Aarthi N, Murugan K. Effect of *Vetiveria zizanioides* L. root extracts on the malarial vector, *Anopheles stephensi* Liston. *Asian Pac J Trop Dis* 2012; **2**(2): 154–158.

[43]Rana IS, Rana AS. Efficacy of essential oils of aromatic plants as larvicide for the management of filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae) with special reference to *Foeniculum vulgare*. *Asian Pac J Trop Dis* 2012; **2**(3): 184–189.

[44]Ravindran J, Samuel T, Alex E, William J. Adulticidal activity of *Ageratum houstonianum* Mill. (Asteraceae) leaf extracts against three vector mosquito species (Diptera: Culicidae). *Asian Pac J Trop Dis* 2012; **2**(3): 177–179.

[45]Rawani A, Banerjee A, Chandra G. Mosquito larvicidal and biting deterrency activity of bud of *Polianthes tuberosa* plants extract against *Anopheles stephensi* and *Culex quinquefasciatus*. *Asian Pac J Trop Dis* 2012; **2**(3): 200–204.

[46]Wandscheer CB, Duque JE, da Silva MAN, Fukuyama Y, Wohlke JL, Adelmann J, et al. Larvicidal action of ethanolic extracts from fruit endocarps of *Melia azedarach* and *Azadirachta indica* against the dengue mosquito *Aedes aegypti*. *Toxicon* 2004; **44**: 829–835.

[47]Abdalla M Elimam, Khitma H Elmalik, Faysal S Ali. Efficacy of leaves extract of *Calotropis procera* Ait. (Asclepiadaceae) in controlling *Anopheles arabiensis* and *Culex quinquefasciatus* mosquitoes. *Saudi J Biol Sci* 2009; **16**: 95–100.

[48]Kweka EJ, Mosha FW, Lowassa A, Mahande AM, Mahande MJ, Massenga CP, et al. Longitudinal evaluation of *Ocimum* and other plants effects on the feeding behavioral response of mosquitoes (Diptera: Culicidae) in the field in Tanzania. *Parasit vectors* 2008; **1**: 42.

[49]Tren R, Roberts D. DDT and malaria prevention. *Environ Health Perspect* 2010; **118**: 14–15.

[50]Trudel RE, Bomblies A. Larvicidal effects of Chinaberry (Melia azederach) powder on *Anopheles arabiensis* in Ethiopia. *Parasit Vectors* 2011; **4**: 72.

[51]Eliningaya J Kweka1, Mramba Nyindo, Franklin Mosha, Ary G Silva. Insecticidal activity of the essential oil from fruits and seeds of *Schinus terebinthifolia* Raddi against African malaria vectors. *Parasit Vectors* 2011; **4**: 129.

[52]Govindarajan M, Mathivanan T, Elumalai K, Krishnappa K, Anandan A. Ovicidal and repellent activities of botanical extracts against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* (Diptera: Culicidae). *Asian Pac J Trop Biomed* 2011; **1**: 43–48.