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Research Article

Larvicidal, Ovicidal, and Repellent Activities of Marine Sponge *Cliona celata* (Grant) Extracts against *Culex quinquefasciatus* Say and *Aedes aegypti* L. (Diptera: Culicidae)

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Solvent extracts of marine sponge *Cliona celata* (Grant) were screened for larvicidal, ovicidal, and repellent properties against the filarial vector *Culex quinquefasciatus* Say and dengue vector *Aedes aegypti* L. Larvicidal and ovicidal activities of hexane, ethyl acetate, and methanol extracts were tested in four different concentrations ranging as 62.5, 125, 250, and 500 ppm. Among the three solvent extracts of *C. celata*, methanol extract showed the highest larvicidal activity at 500 ppm against both mosquito species. The LC_{50} and LC_{90} values of *C. celata* methanol extract were recorded as 95.63 and 242.16 ppm against *C. quinquefasciatus* larvae and 158.40 and 780.16 ppm against *A. aegypti* larvae, respectively. Ovicidal activity was high in methanol extract, in which 100% ovicidal activity was recorded in *C. quinquefasciatus*, and 72% ovicidal activity was recorded in *A. aegypti* at 500 ppm. The hexane extract was found to be the most effective protectant against the adult mosquitoes of both species. The mean protection time recorded in hexane extract was up to 273 and 165 min at 5 mg/cm^2 dosage against *C. quinquefasciatus* and *A. aegypti*, respectively. Considering these bioactivities, *C. celata* could be used to obtain some novel pesticidal molecules.

1. Introduction

Mosquitoes are the major arthropod vectors and the most dangerous human health pests. The war against mosquitoes using chemical pesticides has failed due to the resistance developed by mosquitoes [1, 2]. Chemical control of mosquitoes is also causing many unwanted effects on human health and nontarget animals. By understanding these side effects of chemicals, people are now showing interest towards biopesticides and botanical formulations, which are considered as eco-friendly. Nature is providing numerous bioactive products against vector mosquitoes in the form of plant products, marine products, microbial products, and other biological derivatives. *Culex quinquefasciatus* is a major vector of *Wuchereria* species causing lymphatic filariasis; it is widely distributed in tropical regions with around 120 million people getting infected and 44 million people under clinical manifestation [3]. India alone contributes around 40% of global filariasis burden, and the estimated annual economic loss is about 720 crore Indian rupees [4]. The mosquito *Aedes aegypti* is more widely distributed in tropical and subtropical regions of the world. It is a major vector of arboviruses causing chikungunya and dengue fever. Natural insecticides are generally pest specific, biodegradable, usually nonallergic to human as well as nontarget organisms [5], and they may possess novel compounds with a wide range of activities [6, 7]. In recent years, plant products and phytochemicals have been studied for the control of mosquitoes [8–12]. Likewise, some investigators have reported that the secondary metabolites of marine sponges possessed insecticidal activities [13–16].

Sponges (Phylum: Porifera) are the oldest metazoan organisms and have been recognized as a rich source of biologically active compounds that are of potential interest to mankind [17, 18] and are good alternatives for synthetic pesticides [19]. They are a potential source of novel antimicrobial agents [20]. Till date, nearly 8000 species of sponges

have been described throughout the world, and 108 species of sponges have been identified in Gulf of Mannar, India [21]. Till date, practically very few species of sponges have been studied for their mosquito larvicidal activity [22–25]. However, studies on mosquitocidal properties of marine sponges from Indian waters are limited, and the larvicidal, ovicidal and repellent efficacy of the marine sponge *Cliona celata* (Porifera: Hadromerida: Clionidae) has not been studied previously. Hence, the present study was undertaken to screen the crude extracts of marine sponge *C. celata* from the Gulf of Mannar for their larvicidal, ovicidal, and repellent activities against two vector mosquitoes *C. quinquefasciatus* and *A. aegypti*.

2. Materials and Methods

2.1. Collection of Sponges. Marine sponge *C. celata* was collected from the Gulf of Mannar (between 8°47′ to 9°05′N Latitude and 78°12′ to 79°07′E Longitude, India) at depths varying from 15 to 25 feet by scubadiving. Sponges were gently removed from the substratum and transferred to laboratory within 48 h; then, the specimen was identified and deposited (deposition number: MBRC/ZSI-S.225) in National Zoological Collection of Marine Bio Resource Centre, Zoological Survey of India (13°04′N Latitude and 80°17′E Longitude, India).

2.2. Preparation of Crude Extracts. The Marine sponge *C. celata* was thoroughly washed with distilled water to remove the sand particles and cut into small pieces before shade drying for 48 h. Then the sponges were homogenized and extracted sequentially with hexane, ethyl acetate, and methanol. Initially the powder of the *C. celata* (500 g) was soaked in 1 litre hexane for 72 h and filtered through filter paper. The residue was dried and then extracted sequentially with ethyl acetate and methanol solvents after 72 h of soaking in each solvent separately. The extracts were condensed separately under reduced pressure by using vacuum evaporator, and the solvent free crude extracts were collected in glass vials and stored in 4°C until use. Stock solutions (10% in acetone) of all the three solvent extracts were prepared and then subjected to bioassay screening.

2.3. Test Insect. Eggs, larvae, and adults of *C. quinquefasciatus* and *A. aegypti* were obtained from the stock culture maintained at Entomology Research Institute, which were free of exposure to pathogens, insecticides, or repellents. Laboratory rearing was done at a temperature of 27 ± 2°C, 75–85% relative humidity, and a photoperiod of 11 ± 0.5 h.

2.4. Larvicidal Bioassay. Larvicidal activity was evaluated by following the methods of WHO [26] with slight modifications. Twenty numbers of early fourth instar larvae of *C. quinquefasciatus* and *A. aegypti* were introduced into the test containers. The extracts taken in four concentrations were 500, 250, 125, and 62.5 ppm. Normal control and solvent control (Acetone in water) were maintained separately. Mortality rate were registered after 24 h exposure period. The moribund and dead larvae were collected, and larval mortality was calculated for each concentration. The bioassays were performed at a room temperature of 27 ± 1°C with five replicates for each concentration. Mortality was converted into percent mortality (a), and corrected mortality was calculated using Abbot's formula (b) [27].

(a) Percentage of mortality is as follows:

$$\frac{\text{no. of dead larvae}}{\text{no. of larvae introduced}} \times 100. \quad (1)$$

(b) Corrected percentage of mortality is as follows:

$$\frac{1 - n \text{ in T after treatment}}{N \text{ in C after treatment}} \times 100, \quad (2)$$

where n is the number of larvae, T is the number of larvae survived in treated, and C is the number of larvae survived in control.

The corrected percentage mortality value of each concentration was considered to estimate LC_{50} and LC_{90} values using SPSS Probit analysis statistical pack, version 11.5.

2.5. Ovicidal Bioassay. Ovicidal activity was evaluated by following the method of Elango et al. [28] with slight modification. Twenty five freshly laid eggs of *C. quinquefasciatus* and *A. aegypti* were treated separately with the *C. celata* extracts at 62.5, 125, 250, and 500 ppm concentrations. Normal control was kept separately. Acetone in water was served as solvent control. Each treatment was replicated five times. The ovicidal activity was assessed up to 120 h posttreatment and thereafter control; treated eggs were observed under the microscope and photographed using stereo zooming microscope (Wild M7S TYP 308700, Switzerland). The nonhatched eggs with unopened opercula were counted in each treatment, and the percent mortality was calculated using the following formula and analysed in Graph Pad Prism version 3.0 for Windows, Graph Pad Software, San Diego, CA, USA:

$$\%\ \text{of egg mortality} = \frac{\text{No. of unhatched eggs}}{\text{Total No. of eggs}} \times 100. \quad (3)$$

2.6. Repellent Bioassay. For repellent experiment, 3 to 6 days old, hundred laboratory reared blood-starved adult female mosquitoes were introduced into separate laboratory cages (45 × 45 × 40 cm). *C. quinquefasciatus* was tested during the night time, while *A. aegypti* was tested during the day time. Before each test, the forearms of a human subject were washed with unscented neutral soap, thoroughly rinsed, and allowed to dry before the application of the extract at 5, 2.5, and 1 mg/cm^2 concentrations. The *C. celata* extracts being tested were applied on the right upper forearm and remaining regions were covered with gloves. The arm was left undisturbed. The left arm served as control. N-N Diethyl benzamide (12%, w/w) was used as negative control. The mosquito bites were observed for three full minutes of every fifteen minutes. Protection time was recorded as the time that elapsed between extract application and the observation period immediately preceding that in which a confirmed bite

Table 1: Lethal concentration (in ppm) of *C. celata* extracts against the larvae of *C.*

Mosquito species	Treatment	LC_{50} (ppm)	95% confidence limit		LC_{90} (ppm)	95% confidence limit		Slope ± SE	Intercept ± SE	χ^2
			LL	UL		LL	UL			
Culex quinquefasciatus	Hexane	268.28	165.88	441.84	1008.42	559.85	6552.24	2.2 ± 0.6	−0.4 ± 1.5	0.06*
	Ethyl acetate	218.71	129.78	359.38	970.53	518.88	6501.77	1.9 ± 0.5	0.3 ± 1.2	0.3*
	Methanol	95.63	61.22	127.45	242.16	176.09	460.04	3.1 ± 0.7	−1.2 ± 1.5	0.3*
Aedes aegypti	Hexane	364.71	239.50	585.41	897.53	567.82	8000.22	3.2 ± 1.1	−3.3 ± 2.9	0.05*
	Ethyl acetate	285.65	176.91	475.96	1033.36	575.13	7376.91	2.2 ± 0.6	−0.6 ± 1.6	0.2*
	Methanol	158.40	84.60	249.72	780.16	423.58	4793.81	1.8 ± 0.5	0.9 ± 1.1	0.09*

Culex quinquefasciatus and *A. aegypti*.
LC_{50} lethal concentration that kills 50% of the exposed larvae, LC_{90} lethal concentration that kills 90% of the exposed larvae, LL lower limit (95% confidence limit), and UL upper limit (95% confidence limit).
*$P \leq 0.05$, level of significance of chi-square values.

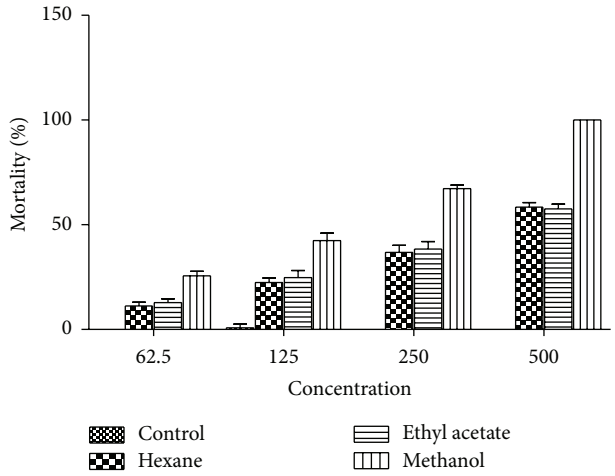


Figure 1: Percent ovicidal activity of marine sponge *C. celata* extracts against the eggs of *C. quinquefasciatus*.

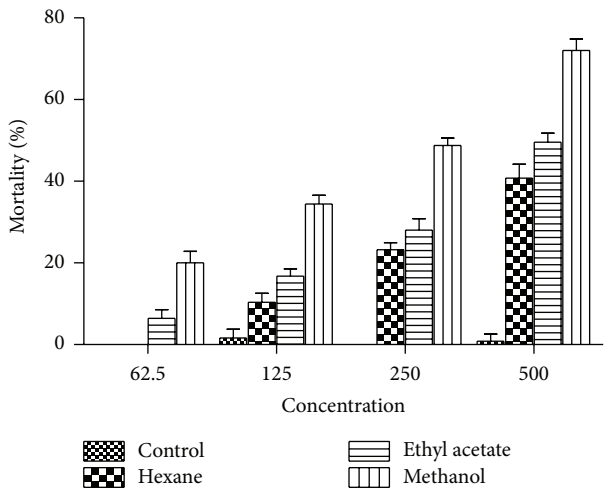


Figure 2: Percent ovicidal activity of marine sponge *C. celata* extracts against the eggs of *A. aegypti*.

was obtained. The experiments were replicated five times in separate cages, and in each replicate different volunteer was used to nullify any effect of skin differences on repellence. The protection time of each extract was calculated using previously established methods [29, 30].

2.7. Statistical Analysis. The results were presented as mean ± SD. Statistical analyses of all the data obtained in larvicidal activity were evaluated using Probit analysis (SPSS Program; Version 11.5). The differences were considered as significant at $P \leq 0.05$.

3. Results

3.1. Larvicidal Activity and Lethal Doses. All the three extracts of the sponge *C. celata* showed larvicidal activity. The larvicidal activity varied between the solvent extracts and the mosquito species. Table 1 shows the results on effective lethal concentration (LC_{50} and LC_{90}) values of hexane, ethyl acetate, and methanol extracts of *C. celata* after 24 h treatment period. It was clear from the results that the methanol extract recorded the maximum larvicidal activity in both *C. quinquefasciatus* and *A. aegypti*. The LC_{50} and LC_{90} values of methanol extract recorded in *C. quinquefasciatus* were 95.63 and 242.16 ppm, respectively (Table 1). *A. aegypti* was less susceptible than *C. quinquefasciatus*, and the LC_{50} and LC_{90} values of methanol extract for *A. aegypti* were 158.40 and 780.16 ppm, respectively. Significant chi-square values were recorded in all the extracts (Table 1). In normal control and solvent control, all the larvae were active and exhibited normal movement. But in the treated larvae, restless movement was observed. After 1 h, tremor and convulsion were observed in all the treated larvae and dead larvae settled down as reported earlier [31].

3.2. Ovicidal Activity. The ovicidal activities of *C. celata* extracts on *C. quinquefasciatus* and *A. aegypti* eggs are given in Figures 1 and 2. Methanol extract was found to be highly lethal to the eggs of both species. In *C. quinquefasciatus*, methanol extract presented 100% ovicidal activity at 500 ppm concentration after 120 h posttreatment period. The lowest concentration (62.5 ppm) of methanol extract caused 25.6% egg mortality in *C. quinquefasciatus*. *A. aegypti* eggs were found to be more tolerant to all the three extracts of *C. celata*

Table 2: Complete protection time of three solvent extracts of *C. celata* against *C. quinquefasciatus*.

Extract	Concentration mg/cm^2	Complete protection time (min)	
		Control	Treated
Hexane	1.0	1.4 ± 0.54	105 ± 3.16
	2.5	2.2 ± 0.83	150 ± 2.91
	5.0	2.0 ± 1.22	273 ± 2.54
Ethyl acetate	1.0	1.6 ± 0.54	44 ± 2.73
	2.5	1.1 ± 0.19	99 ± 3.16
	5.0	1.4 ± 0.54	178 ± 1.58
Methanol	1.0	1.2 ± 0.44	44 ± 2.44
	2.5	1.6 ± 1.34	76 ± 2.73
	5.0	1.8 ± 0.44	120 ± 2.73
N-N Diethyl benzamide 12%	1.0	2.4 ± 0.89	110 ± 1.0
	2.5	1.8 ± 0.83	194 ± 1.58
	5.0	2.0 ± 0.70	323 ± 2.0

Table 3: Complete protection time of three solvent extracts of *C. celata* against *A. aegypti*.

Extract	Concentration mg/cm^2	Complete protection time (min)	
		Control	Treated
Hexane	1.0	0.33 ± 1.11	44 ± 1.58
	2.5	0.30 ± 0.08	87 ± 2.54
	5.0	0.52 ± 0.30	165 ± 1.0
Ethyl acetate	1.0	0.51 ± 0.30	28 ± 1.58
	2.5	1.16 ± 0.11	64 ± 3.16
	5.0	0.35 ± 0.10	118 ± 1.87
Methanol	1.0	1.2 ± 0.44	25 ± 2.12
	2.5	0.51 ± 0.05	51 ± 1.58
	5.0	0.40 ± 0.10	75 ± 2.91
N-N Diethyl benzamide 12%	1.0	1.2 ± 0.70	55 ± 1.0
	2.5	1.4 ± 0.54	95 ± 1.41
	5.0	1.2 ± 0.44	182 ± 0.70

at all concentrations. At the highest concentration (500 ppm) of methanol extract, 72% ovicidal activity was noticed in *A. aegypti* after 120 h posttreatment period. In both species, the ovicidal effect of all the three extracts was directly proportional to the concentration. In methanol extract treated *C. quinquefasciatus* eggs, most of them did not hatch and some were hatched in an abnormal way at 500 ppm and the larvae died before completion of eclosion (Figure 3(b)). The methanol extract-treated eggs of *A. aegypti* were shrunken and most did not hatch at 500 ppm concentration (Figure 4(b)). In normal control and solvent control, hatchability of eggs of both species was normal (Figures 3(a) and 4(a)).

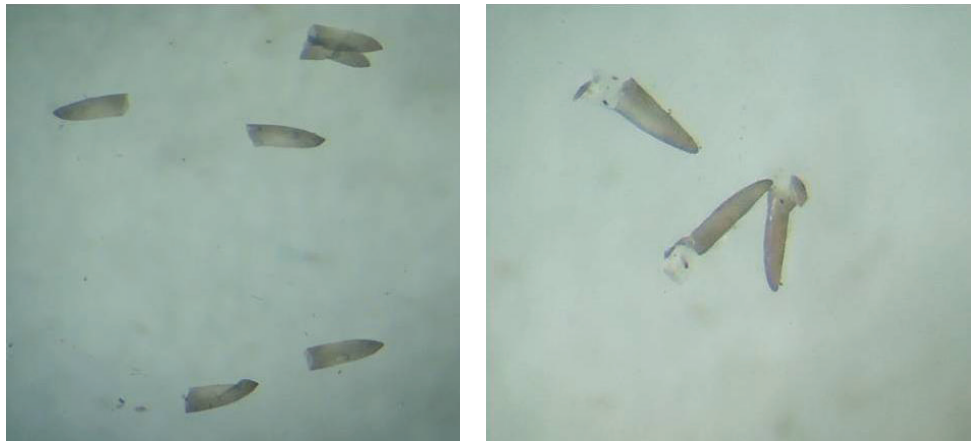
3.3. Repellent Activity. The complete protection times for all the three extracts of *C. celata* against both the species of mosquitoes were recorded by standard skin repellence experiments, and the results are given in Tables 2 and 3. The repellence was directly proportional to the dose and protection time (min) for each extract showed variations between two species. In general hexane extract gave maximum protection time against both mosquito species compared to ethyl acetate and methanol extracts. Furthermore, it was noted that all the three extracts were found to be more effective against *C. quinquefasciatus* than *A. aegypti* mosquitoes. Hexane extract gave a maximum protection time of 273 min against *C. quinquefasciatus* at 5 mg/cm^2 dosage (Table 2). The same treatment recorded the highest protection time of 165 min against *A. aegypti* at 5 mg/cm^2 dosage (Table 3). These results were comparable with negative control (N-N Diethyl benzamide 12%, w/w), which showed maximum of 323 and 182 min protection at 5 mg/cm^2 dosage against *C. quinquefasciatus* and *A. aegypti* mosquitoes, respectively.

4. Discussion

Searching for eco-friendly pesticide molecules among natural sources has become an important research in these days, and it is promoted for mosquito control than chemical insecticides [32]. Nature is providing innumerable bioactive molecules for the well-being of mankind. Plant kingdom and marine organisms provide majority of the beneficial biomolecules or products that are used by people in their daily life. Marine sponges are known to produce toxins and other compounds to repel and deter predators [33, 34]. These compounds of marine sponges are reported to be of the bioactive compounds which can be used in the treatment of many diseases [35–39].

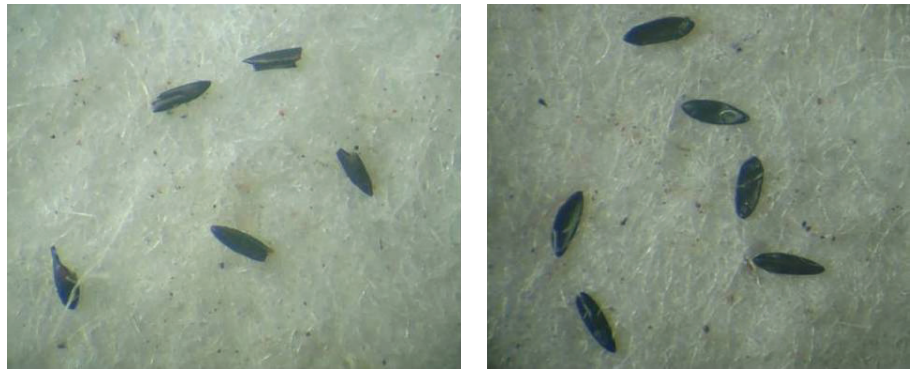
In recent years, researchers are concentrating on marine organisms to study their biological activities; especially, marine sponges (Porifera) have attracted significant attention from various scientific disciplines [40]. The literature also indicates that the marine products possess maximum percentage of bioactive substances with novel biological properties than the molecules originating from terrestrial origin [24, 41]. Recently, marine sponge extracts and their compounds have been screened for antimicrobial [42, 43], antiplasmodicidal [44], antifilarial [45, 46], and antihelminthic activity [47]. Marine sponge extracts have also been screened against agricultural pests. Edrada et al. [48] have reported an insecticidal and growth regulating compound from the philippine marine sponge *Xestospongia ashmorica* against *Spodoptera littoralis*, an important polyphagous pest. Supriyono et al. [49] have reported that two guanidine alkaloids, namely, hymenialdisine and debromohymenialdisine from the tropical marine sponge *Axinella carteri* exhibited insecticidal activity with LD_{50} value of 88 and 125 ppm, respectively, against the neonate larvae of *S. littoralis* in feeding bioassay experiments.

In this regard, very few researchers have studied the mosquitocidal potential of marine sponge extracts. Venkateswara Rao et al. [23] have screened the methanol-dichloromethane (1:1) extracts of 18 different sponges collected from Palk bay and Gulf of Mannar waters against *A. aegypti* and houseflies (*Musca domestica*). They found that *Psammaplysilla purpurea* (LC_{50} = 25.9 ppm) and *Haliclona*



(a) (b)

Figure 3: Hatched eggs of *C. quinquefasciatus* in control (a). The larvae of *C. quinquefasciatus* died in the egg case before completion of eclosion in methanol extract treated *C. celata* after 120 h posttreatment (b).



(a) (b)

Figure 4: Hatched eggs of *A. aegypti* in control (a). Dead and shrunken eggs of *A. aegypti* in methanol extract treated *C. celata* after 120 h posttreatment (b).

cribricutis (LC_{50} = 31.46 ppm) were more effective against *A. aegypti* mosquito larvae. In our study, we observed that the methanol extract of *C. celata* was the most effective in larvicide, and the LC_{50} of methanol extract against *C. quinquefasciatus* was found to be higher (95.63 ppm) than *A. aegypti* (158.40 ppm) values. An interesting finding in this study was that the low polar solvent extract; that is, hexane extract showed repellent activity and the high polar solvent (methanol) extract showed larvicidal and ovicidal activities. This result was comparable with the earlier report of Sonia and Lipton [25] who have screened the methanol extracts of five marine sponges, namely, *Acanthella elongata, Echinodictyum gorgonoides, Axinella donnani, Callyspongia subarmigera*, and *Callyspongia diffusa* for larvicidal activity against *Culex* sp. They found that *Acanthella elongata* extract was the most effective with the LC_{50} value of 0.066 mg/mL than other extracts.

In the present study, all the three solvent extract treatments were not equally effective against *C. quinquefasciatus* and *A. aegypti* larvae, eggs, and adults. *C. quinquefasciatus* life stages were found to be more susceptible to the treatments than *A. aegypti*. A similar result was reported by Martínez et al. [22]; they have screened the ethanol extracts of five marine sponges, namely, *Amphimedon compressa, Topsentia ophiraphidites, Svenzea zeai, Ircinia campana*, and *Agelas sventres* against fourth instar larvae of *A. aegypti* and *C. quinquefasciatus*. They found that *Ircinia campana* extract was the most effective against the larvae of two mosquitoes and the activity was higher in *A. aegypti* than *C. quinquefasciatus*.

Earlier, Rey et al. [50] and David et al. [51] proved that the plant extracts primarily affect the midgut epithelium, and this is based on the concentration, duration of the treatment, and mosquito species used. Further, Daniel Reegan et al. [52] proved that the orange peel extract primarily damages the midgut epithelial columnar cells, gastric caeca (GC), and brush border cells (BB) in the treated larvae of *A. stephensi*. Acetylcholinesterase (AChE) is the molecular target for mosquito repellents [53]. Possibly, these extracts of marine sponge *C. celata* may have impact on midgut cells of the treated larvae and on AChE.

5. Conclusion

In Conclusion, the present study reports for the first time repellent, larvicidal, and ovicidal activities of marine sponge *C. celata* against *C. quinquefasciatus* and *A. aegypti*. The screening results suggest that the hexane and methanol extracts of *C. celata* are promising in mosquito control and repellent. This is an eco-friendly alternative to chemical insecticide which is promising in mosquito control.

Conflict of Interests

The authors declare that they have no conflict of interests.

Authors' Contribution

Appadurai Daniel Reegan and Arokia Valan Kinsalin contributed equally to this paper.

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