Research article

Biopotential of microalgal extracts as a mosquito larvicide: An eco-friendly approach to control *Aedes aegypti*

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ABSTRACT

Introduction and Aim: The use of chemical pesticides has raised concerns about their safety and toxicological effects on the environment, people, and other species. Therefore, there is a demand for natural alternatives that are eco- friendly, biodegradable, and target specific. The purpose of this study was to test the larvicidal activity of three microalgae namely, *Chlorella* sp., *Chlorococcum* sp., and *Scenedesmus* sp., against *Aedes aegypti.*

Materials and Methods: The microalgal extracts were prepared with different concentrations after suitable pretreatment and extraction process. The effect of extracts was tested on *Aedes aegypti* larvae exposed to different concentrations after 24 and 48 hours.

Results: The ethanol extract of the *Scenedesmus* sp. showed the highest larvicidal activity. Whereas the larvicidal activity of methanol extract of the *Chlorococcum* sp. was the lowest. The phytochemical screening of algal extracts revealed the presence of phytochemicals present in the microalgal extracts namely flavonoids, terpenoids, alkaloids, quinones, saponins, and cellulose

Conclusion: This study demonstrated the biopotential of microalgae extracts for the control of the dengue vector, *Aedes aegypti.* Thus, the use of microalgae can be considered as an alternative to the conventional insecticides as it is more sustainable, non-toxic, and eco-friendly.

Keywords: Larvicidal activity; *Aedes aegypti;* microalgae; phytochemical screening; *Scenedesmus* sp.,

INTRODUCTION

Mosquito-borne diseases have become a major public health threat globally. Malaria, Dengue, Chikungunya, Filariasis, and Zika fever are some of the fatal diseases transmitted by mosquitoes. The World Health Organization states that, in many countries, dengue is fast emerging to be a pandemic-prone viral disease (1). The main vector of dengue fever is the *Aedes aegypti* mosquito. When an infected female *Aedes aegypti* bites a human during blood feeding, the dengue virus gets transmitted to the human.

Dengue fever, also called breakbone fever causing a severe flu-like illness, which is found throughout the world. Dengue is found to be endemic in at least 100 countries in Asia, the Pacific, the Americas, Africa, and the Caribbean. Dengue hemorrhagic fever has become a foremost cause of hospitalization and death among children and adults. The Center for Disease Control estimates that 400 million people are infected each year (2).

Mosquito control can be performed by following the Integrated Mosquito Management (IMM) concept. The mosquito control agencies use larviciding as a control method. This utilizes the application of insecticides targeted at the immature mosquitoes - the larvae or pupae.

The use of synthetic larvicide is said to be an effective way to kill mosquito larvae. The widespread use of synthetic larvicides such as carbamates, organophosphates and organochlorines has raised concerns about their safety and toxicological effects on the environment, people, and other species. The development of resistance in mosquitoes has been increased globally. Therefore, the search for natural alternatives that are eco- friendly, biodegradable, and target-specific is being studied.

Biological mosquito control with vertebrates mainly focuses on the role of larval-eating fish such as *Poecilia* (Poeciliidae) and *Gambusia* that consumes the mosquito's aquatic larval stage. However, these larval-eating fish pose a major threat to local aquatic fauna, which includes amphibians (3).

Control of mosquitoes using organisms that occur naturally are considered as one of the biological control strategies. Currently, the most used mosquito larvicide in many countries is *Bacillus thuringiensis* var. *israelensis* (Bti) (4,5). However, long-term use can lead to the development of resistance to the Bti toxin and using Bti in large urban mosquito breeding sites is logistically challenging. The entomopathogenic fungus

produces infectious spores (conidia) that attach and penetrate the mosquito cuticle and release toxins that kill the mosquito. The pathogenic effects on *Aedes aegypti* were demonstrated in several studies (6).

Similarly, microalgal species has also being studied for their larvicidal activity against *Aedes aegypti*. Microalgae are an important group of unicellular, photosynthetic microorganisms with great economic and ecologic impact (7). They are fast-growing photo-synthetic species, dwelling in different environments such as freshwater, marine water, or the surface of moist rocks. Algae are the major source of amino acids, terpenoids, phlorotannin, steroids, and phenolic compounds which are found to have a biological activity such as antibiotics, fungicidal, antivirals, antitumoral, etc., (8). In a study, mosquitoes fed with *Chlorococcum* UMACC 218 and *Scenedesmus* UMACC 220 were not able to survive, and the mortality was observed within 72 hours (9). The present study has been designed to determine the larvicidal effects of the extracts of three microalgal species namely *Chlorella* sp., *Chlorococcum* sp., and *Scenedesmus* sp., against the mosquito *Aedes aegypti*.

MATERIALS AND METHODS

Pretreatment of microalgae species

The microalgal cultures *Chlorella* sp., *Chlorococcum* sp., *and Scenedesmus* sp. were obtained from the Department of Microbiology, Madras Christian College (Fig. 1). The algal cultures were put under conditions of nutrient starvation, by introducing the grown cells into sterile tap water for a period of 4 to 5 days to increase the chance of secondary metabolite production. The cells were centrifuged and disrupted by ultrasonication using a bench-scale Branson 2000 Series ultrasonic system.

Preparation of extract

After the pretreatment process, the microalgal suspensions were treated with methanol, ethanol, and acetone for 24 hours and filtered with Whatman filter paper then evaporated using a rotary vacuum evaporator. The resulting solvent-free extract was stored in a freezer until used in larvicidal trials against mosquitoes. The stock solution of the extracts was prepared by dissolving in 100 ml of distilled water and kept as stock solution. The required concentrations of the extract were prepared using this stock solution.

Collection of Aedes larvae

The larvae of the *Aedes aegypti* mosquito were collected from stagnant water at various places within Chennai. The collected larvae were identified based on the morphology according to the guidelines (10) (Figs. 2 & 3).

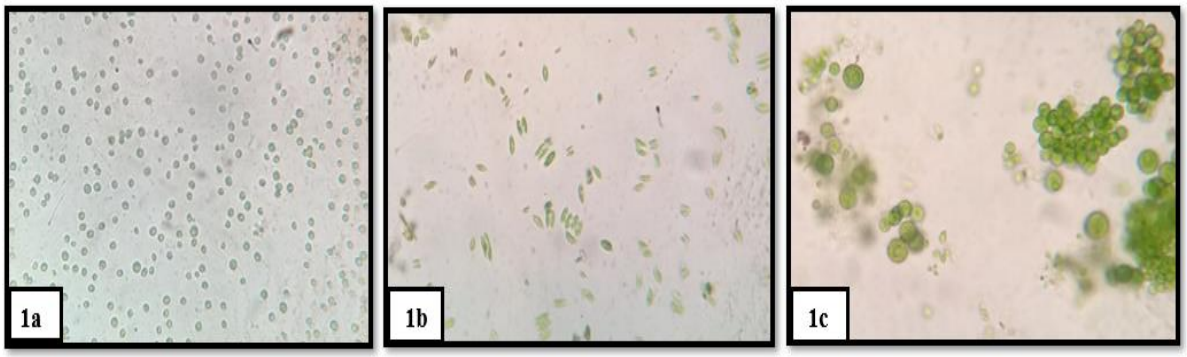

Fig. 1: Microalgae species used in the study.
Fig.1a: *Chlorella* sp., **Fig.1b:** *Scenedesmus* sp., **Fig. 1c:** *Chlorococcum* sp.

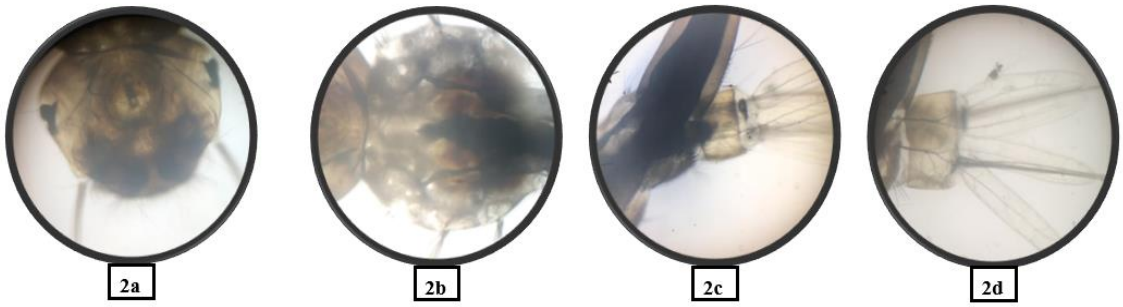

Fig. 2: The third instar stage of *Aedes aegypti* larvae is viewed under low power objective.
Fig. 2a: Dorsal view of head. **Fig. 2b:** Thorax. **Fig. 2c:** Siphon **Fig. 2d:** Anal papillae.

Fig. 3: The third instar stage of *Aedes aegypti* larva

Larvicidal bioassay

The larvicidal bioassay was performed according to World Health Organization protocol (11; Fig. 4). Batches of twenty-five numbers of larvae were introduced through a dropper to 200 ml paper cups. From the stock solution, varying concentrations were prepared and added to the test containers for four replicates. Several controls are set up simultaneously with tap water. The test containers were kept at 25-28°C temperature, and the photoperiod was maintained for 12 h light followed by 12 h dark (12L:12D). Resultants with 50% mortality within 48 hours were selected for further bioassays using narrow concentrations of 200, 400, 600, 800, and 1000 ppm to determine the concentrations required to kill 50% (LC) and 90% (LC) of the larvae.

Fig. 4: Experimental setup for larvicidal bioassay

Preliminary phytochemical screening

All the three microalgal extracts prepared using the solvents acetone, ethanol, and methanol were subjected to various preliminary phytochemical analyses such as tannins, flavonoids, terpenoids, saponins, alkaloids, and cellulose (12).

Statistical analysis

The probit analysis was used to calculate the lethal concentrations LC_{50} and LC_{90} and the chi-square values were also calculated with 95% confidence limits. The SPSS version 16.0 statistical software was used to perform the statistical analysis.

RESULTS

Larvicidal bioassay

The larvicidal activity of the ethanol, methanol and acetone extracts with varying concentrations of all the three microalgae species namely *Chlorella* sp., *Chlorococcum* sp., and *Scenedesmus* sp., against *Aedes aegypti*, were observed (Figs. 5-7; Tables 1 & 2). It was observed that the ethanol extract of the *Scenedesmus* sp. showed the highest larvicidal activity (LC_{50}= 514.240 and LC_{90}= 1053.029 ppm values at 24 hours and LC_{50}= 451.255 and LC_{90}= 927.572 ppm values were observed at 48 hours). Similarly, the lowest larvicidal activity was found in the *Chlorococcum* sp. methanol extract (LC_{50}= 1058.456 and LC_{90}= 1631.352 ppm values at 24 hours and LC_{50}= 902.754 and LC_{90}= 1567.994 ppm values at 48 hours respectively).

Among the three solvent extracts of *Scenedesmus* sp. the highest mortality was observed with the ethanol extract (LC_{50}= 514.240 and LC_{90}= 1053.029 ppm values at 24 hours and LC_{50}= 451.255 and LC_{90}= 927.572 ppm values at 48 hours). Similarly, among the three solvent extracts of *Chlorococcum* sp. the highest mortality was observed with the acetone extract (LC_{50}= 815.336 and LC_{90}= 1393.926 ppm values at 24 hours and LC_{50}= 770.265 and LC_{90}= 1243.061 ppm values at 48 hours).

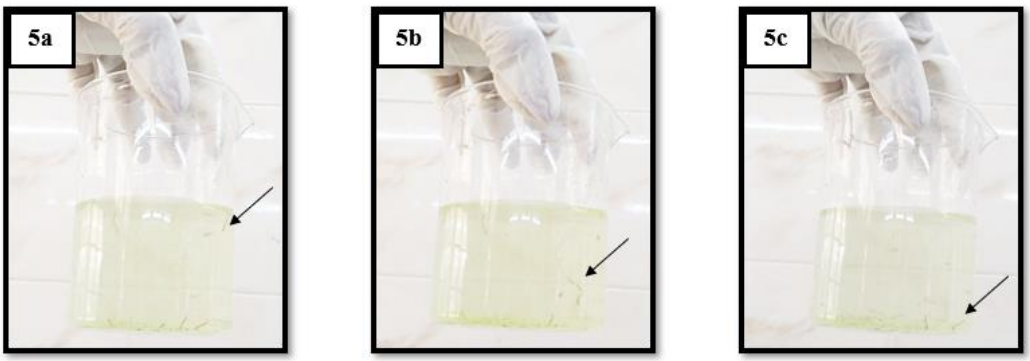

Fig. 5: Effects of ethanol extracts of microalgae on the third and fourth instar stage of *Aedes aegypti* larvae. Fig. 5a *Chlorella* sp. showing no mortality. Fig. 5b Larvae treated with *Chlorococcum* sp. showing morbidity. Fig. 5c Larvae treated with *Scenedesmus* sp. showing complete mortality.

Table 1: Mortality effects on *Aedes aegypti* larvae exposed to different concentrations of ethanol, methanol and acetone extracts of *Scenedesmus* sp., and *Chlorococcum* sp., after 24 and 48 hours.

Microalgae	Solvents	% Mortality (concentration in ppm)									
		200		400		600		800		1000	
		24h	48h	24h	48h	24h	48h	24h	48h	24h	48h
Scenedesmus.sp.,	Ethanol	30.4	34.2	46.0	52.8	64.2	69.4	76.4	78.4	80.1	90.0
	Acetone	14.2	24.6	34.4	35.8	42.8	53.0	52.0	60.2	64.4	78.0
	Methanol	21.8	26.8	32.0	33.4	43.6	40.0	56.0	55.4	62.4	64.6
Chlorococcum. sp.,	Ethanol	14.4	22.8	28.4	41.0	44.6	53.6	44.0	66.4	62.2	82.4
	Acetone	10.8	17.4	19.2	34.6	27.0	47.0	50.1	62.6	58.4	70.8
	Methanol	9.8	13.2	19.1	26.0	21.4	31.2	32.8	42.0	42.4	52.8

Table 2: The lethal concentrations LC_{50} and LC_{90} against the third and fourth instar larvae of *Aedes aegypti* for 24 and 48 hours were calculated

Microalgae	Solvents	24 hours			48 hours		
		LC_{50}	LC_{90}	$\chi 2$ (df=4)	LC_{50}	LC_{90}	$\chi 2$ (df=4)
Scenedesmus sp.	Ethanol	514.240	1053.029	4.842	451.255	927.572	0.037
	Acetone	746.438	1380.255	3.160	621.827	1195.868	0.470
	Methanol	735.353	1320.181	1.056	728.473	1344.256	0.138
Chlorococcum sp.	Ethanol	893.263	1548.319	1.602	782.685	1406.831	0.479
	Acetone	815.336	1393.926	0.294	770.265	1243.061	0.215
	Methanol	1058.456	1631.352	0.871	902.754	1567.994	0.753

df = Degree of freedom; $\chi 2$ = Chi-square value

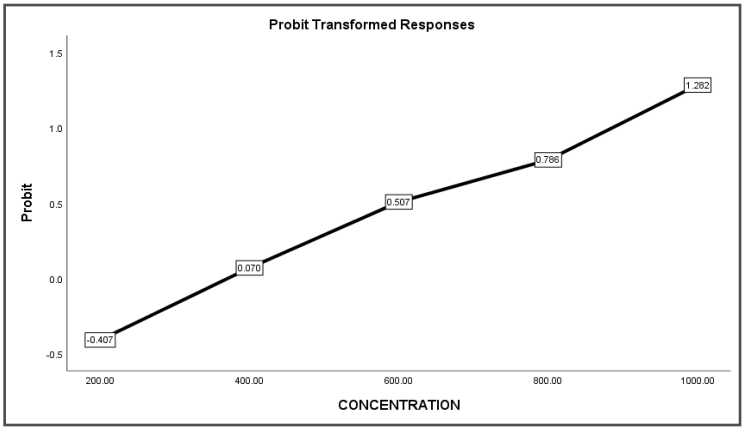

Fig. 6: The LC_{50} and LC_{90} of the ethanol extract of *Scenedesmus* sp. after 48 hours determined using the probit analysis graph

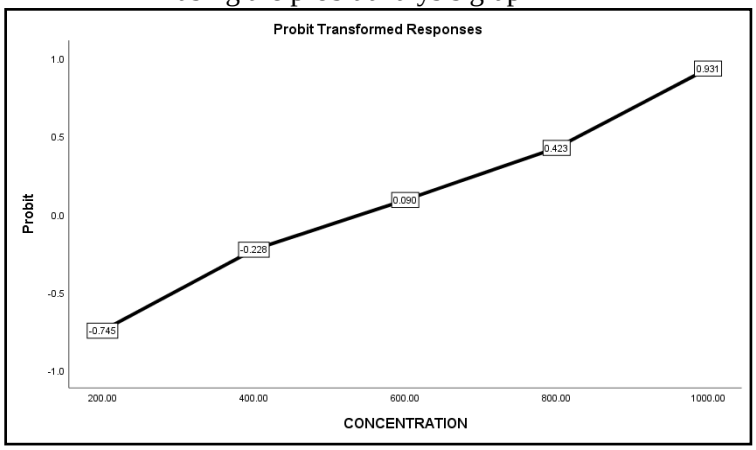

Fig. 7: The LC_{50} and LC_{90} of the ethanol extract of *Chlorococcum* sp. after 48 hours determined using the probit analysis graph

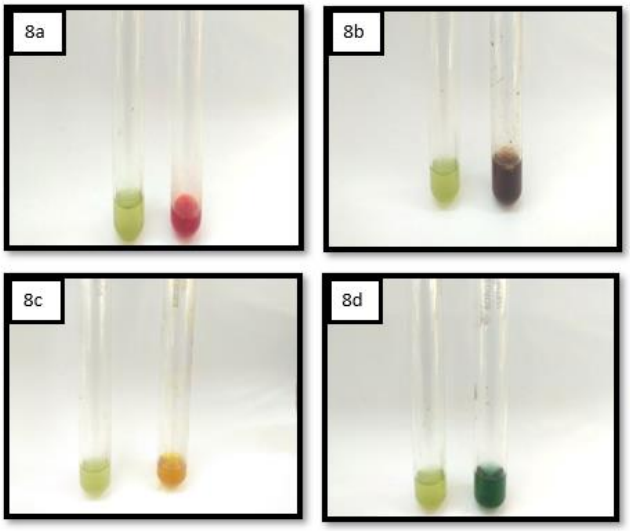

Fig. 8: Phytochemical tests were performed to detect the presence of secondary metabolites. Fig. 8a Color changes to red indicate the presence of cellulose. Fig. 8b Color changes to brown indicating the presence of alkaloids. Fig. 8c Color changes to yellow indicating the presence of flavonoids. Fig. 8d Color changes to blue green indicating the presence of anthocyanins

Table 3: Phytochemical analysis of acetone, ethanol and methanol extracts of *Scenedesmus* sp. and *Chlorococcum* sp.

Secondary metabolites	*Scenedesmus* sp.			*Chlorococcum* sp.		
	Ethanol	Acetone	Methanol	Ethanol	Acetone	Methanol
Anthocyanin	-	+	-	-	+	-
Flavonoids	+	+	+	+	+	+
Terpenoids	+	-	-	+	-	-
Saponins	+	-	+	+	-	+
Alkaloids	+	+	+	+	+	+
Quinone	+	-	-	+	-	-
Tannins	-	-	-	-	-	-
Cellulose	+	+	+	+	+	+

Phytochemical analysis

The phytochemicals present in the microalgal extracts were identified as flavonoids, terpenoids, alkaloids, quinones, saponins and cellulose were shown in Table 3. It was observed that flavonoids, alkaloids, and cellulose were present in all the extracts of both the microalgae. Tannin was absent in all the extracts and anthocyanin was present only in the acetone extracts of both the species. Quinones and terpenoids were present only in the ethanol extract of *Scenedesmus* and *Chlorococcum* sp. And saponins were present in the ethanol and methanol extracts of both the microalgae but absent in the extract of acetone (Fig. 8).

DISCUSSION

The present study was aimed at determining the larvicidal activity of three microalgal extracts that can be used as an alternative to the conventional chemical insecticides which causes a serious impact on environmental safety (13). Since microalgae have been reported to have several microbial and larvicidal activities (14), it was used to study the biopotential of their extracts to act as a larvicide against the mosquito *Aedes aegypti*.

In a study, 76 cyanobacterial isolates were screened of which several were found to contain compounds with mosquito larvicidal activity (15). Another study investigated the effect of ten microalgal chlorophytes isolated from mosquito breeding containers on the survival, larval development, and adult body size of the mosquito *Aedes aegypti* (9). But in all these studies employed, microalgae culture was directly used to perform larvicidal bioassay. In this study, we showed the larvicidal activities of microalgae extracts that have been pretreated using ultrasonication. The extracts were prepared using ethanol, acetone and methanol after pretreatment. The larvicidal bioassay was performed according to World Health Organization protocol (11). The probit analysis was then used to calculate the lethal concentrations LC_{50} and LC_{90}.

In the current study, it was observed that the ethanol extract of the *Scenedesmus* sp., showed the highest larvicidal activity. Whereas the larvicidal activity of methanol extract of the *Chlorococcum* sp. was the lowest. Similar work was done on the macroalgae *C. racemosa* in which its ethyl acetate extract showed potential larvicidal activity against *Aedes aegypti* (16).

A study performed the qualitative phytochemical screening of the ethanol extract of cashew shell wastes and found the presence of flavonoids, tannins, terpenoids and steroids (17). Similar phytochemical analysis of microalgae revealed the presence of terpenoids, quinones, alkaloids, and carbohydrates (18,19). In this study, the preliminary phytochemical analysis was performed for all the extracts of both *Scenedesmus* and *Chlorococcum* sp. And the results were found to be like the previous studies showing the presence of terpenoids, quinones, cellulose and alkaloids.

The larvicidal potential of the microalgae is due to the production of secondary metabolites secreted by them. A previous study states that quinones are the most important compound for larvicidal activity against *Aedes aegypti* (20). The presence of quinone in the ethanol extract could be the reason for higher larvicidal activity than the other extracts. Thus, in this study, the secondary metabolites of these algae were extracted using a suitable method and subjected to larvicidal bioassay. Among the three microalgae, *Scenedesmus* sp. showed to possess greater potential followed by *Chlorococcum* sp. While the Chlorella sp. showed no larvicidal activity. Further studies could be done to perform field trials and to determine the lethal concentrations required for field applications.

CONCLUSION

This study demonstrated the larvicidal properties of three microalgae namely *Scenedesmus* sp., *Chlorella* sp. and *Chlorococcum* sp. using their ethanol, methanol, and acetone extracts against *Aedes aegypti*. The statistical analysis revealed that the ethanol extract of the *Scenedesmus* sp. showed the highest larvicidal activity while the larvicidal activity of methanol extract of *Chlorococcum* sp. showed the lowest. This study showed the biopotential of microalgae extracts for the control of the dengue vector, *Aedes aegypti.* Thus, the use of microalgae can be considered as an alternative to the conventional insecticides as it is more sustainable, non-toxic, and eco-friendly.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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