



# Efficacy of Mosquito Repellent and Adulticidal Activities of *Halophila Ovalis* Extract Against Filaria Vectors

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## Abstract

The repellent and adulticidal activities were analyzed for ethanol extract of *Halophila ovalis* against *Culex quinquefasciatus* (*Cx. quinquefasciatus*). The extraction was done in soxhlet apparatus using ethanol as solvent. The repellent activity of the *Halophila ovalis* was determined against *Culex quinquefasciatus* in different concentration of the extract at 100, 150, 200, 250 and 300  $\mu\text{L}/\text{cm}^2$ . Adulticidal activity of the *Halophila ovalis* extract was tested against four to five day old female adults of *Culex quinquefasciatus*. The adult mortality was observed 24h under the laboratory conditions. Each experiment was conducted with the three replicates and a concurrent control group. As per the WHO guidelines, the experiments were conducted. At 250  $\mu\text{L}$  of concentration extracts of *Halophila ovalis* showed maximum repellency percentage of 95% against *Culex quinquefasciatus*. The highest adulticidal activity was observed in 100  $\mu\text{L}$  concentration against *Culex quinquefasciatus* with  $\text{LC}_{50}$  were  $(50.2 \pm 0.7) \mu\text{L}/\text{ml}$  and  $(51.2 \pm 0.9) \mu\text{L}/\text{ml}$ . The GC-MS reveals unique chemical compounds obtained from the extract of *Halophila ovalis*. The present study identifies the active insecticidal compounds from *Halophila ovalis* by GC-MS and from the results it can be concluded the extract of *Halophila ovalis* can be novel efficient biocontrol source against filariasis mosquitoes.

**Keywords:** *Halophila ovalis*; *Culex quinquefasciatus*; Mosquito; Repellent

## Introduction

Mosquito vectors are essential for understanding vector-borne disease transmission dynamics among human populations because patterns of genetic structure and pathogen transfer through vector populations [1, 2]. In India alone 25 million people suffer from filariasis [3]. Dengue is prevalent in more than 100 countries and threatens the health of approximately 2.5 billion people. Around 80 million people are infected annually at an attack rate of 4% worldwide [4]. The mosquito control continues to be an important strategy in preventing the mosquito-borne diseases [5]. Diseases that are healthcare associated transmission of viruses to human from mosquitoes are an expanding problem in tropical and subtropical regions [6]. Currently, most insecticides are non-selective and can be harmful to other organisms and to the environment [7]. The activity of crude plant extracts is often attributed to the complex mixture of active compounds [8]. Repeated use of synthetic insecticides for mosquito control has disrupted natural biological control systems and led to resurgences in mosquito populations [9]. In view of the residue problems in the environment and development of insect resistance to synthetic insecticides like DDT and chlorinated hydrocarbons [10]. Development of resistance to commercial acaricides by parasites has stimulated the search for new control strategies [11]. Plant parts have been provided as a good source of novel drug compounds [12]. However, mosquitoes have successfully adapted to most plant based insecticides by becoming physiologically or behaviorally resistant to them [13]. Marine biodiversity provides important sources of chemical compounds, which have many therapeutic applications such as antimicrobial, infertility and anticancer activities [14]. Seagrasses are one of the most important marine resources of the world and being used as animal feed and raw material for many industries. For centuries, seagrass has been of botanical and pharmaceutical interest [15]. Various surveys have shown that seaweeds are an excellent source of constituents such as polysaccharides, tannins, flavonoids, phenolic acids, bromophenols, carotenoids and exhibited different biological activities [16]. The objective of this present study was to evaluate larvicidal, repellent and adulticidal activities of *Halophila ovalis* extracts against *Culex quinquefasciatus*.

## Materials and Methods

### Plant materials

Fresh sample of *Halophila ovalis* was collected from Mandapam, Ramanathapuram District, Tamil Nadu of south east coast of India (Latitude 9° 45' N and longitude 79° 13' E). The collected samples were washed in seawater to remove sand, mud and all epiphytes, thrice with tap water and twice with distilled water to remove the adhering salts. The samples were dried at room temperature and were ground separately into powder using a miller before extraction of the crude seaweed extract.

### Extract preparation

The seagrass powder was boiled in ethanol and distilled water mixture (7:3 v/v) at 55°C for 2 h using a soxhlet apparatus under reduced pressure. The filtrate was condensed by evaporating to a minimal volume at 45°C and then freeze-dried (-80°C). The extract obtained was referred to as crude seaweed extract. The percentage of extraction was calculated by using the following formula,

$$\text{Percent of extraction} = \frac{\text{Weight of the extract}}{\text{Weight of the plant material}} \times 100$$

The extract preparation was done by following the method of Ali et al., [12] with slight modification.

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## Mosquito larval culture

Mosquito larva of *Culex quinquefasciatus* were collected from our college (Mohamed sathak college of arts and science, sholinganallur, Chennai) sewage water, placed in dechlorinated water in separate plastic trays. They were reared indoors at  $(28 \pm 2)^\circ\text{C}$  and 75-85 % relative humidity under 14:10 light and dark period cycle. The larvae were fed with powdered mix of dog biscuits and brewer's yeast powder in 3:1 ratio. Pupae were transferred from the trays to a cup containing tap water and were maintained in cages (45×45×40 cm) where adults emerged. After five days emergence, female mosquitoes were moved into a mosquito cage where the emerging adults were fed with a 10% sucrose solution in air-tight cylindrical glass container with a cotton wick. Glass Petri dishes with 50 ml of tap water lined with filter paper were kept inside the cage for oviposition. The Mosquito larval culture was done by following with slightly modified the method of Ali et al. [14].

## Repellent activity

The repellent activity was conducted with slightly modified as per the method of Syed Ali et al., [10] and was determined by the percentage protection time in relation to dose method WHO [17]. Repellency bioassays were carried out in a 10×10×3 m room at 27-35°C and 60-80% RH. The target *Culex quinquefasciatus*, the testing period was run between 0-6 h. Three to four days old blood-starved 100 adult females of *Culex quinquefasciatus* mosquito was randomly selected and placed in an experimental cage (30 x 30 x 30 cm) and left to acclimatize for 1h. The arm tested person was cleaned with ethanol. After air drying the arm of the test person, only 25 cm<sup>2</sup> dorsal side of the skin on each arm was exposed and the remaining area being covered with rubber gloves. The ethanolic seaweed extract of *Halophila ovalis* with different concentration (100 µL, 150 µL, 200 µL, 250 µL) was applied. The control and treated arms were introduced simultaneously into the cage. The first bite by *Culex quinquefasciatus* was noted from 5 minutes for every 1h upto 6 h. Subsequently, the test arm was introduced into the cage for the same period of time and the numbers of mosquitoes that landed and attempted to feed were recorded. The experiment was conducted for three times. It was observed that there was no skin irritation by the extracts of *Halophila ovalis*. The percentage protection was calculated by using the following formula;

No. of bites received by control - No. of bites received by treated

$$\text{Percentage Protection} = \frac{\text{No. of bites received by control} - \text{No. of bites received by treated}}{\text{No. of bites received by control}} \times 100$$

## Adulticidal activity

The repellent activity was conducted with slightly modified as per the method of Govindarajan et al. [13] and was determined by the percentage mortality rate in relation to dose method WHO [18,19]. The toxicity of the ethanolic extract derived from seaweed against four to five days old female adults of *Aedes aegypti* and *Culex quinquefasciatus* was examined. Glass tubes of 20 ml capacity were used as exposure chambers during the fumigation test. The ethanolic seaweed extract was applied on Whatman no. 1 filter paper (12×15 cm<sup>2</sup>) placed inside the glass tubes at concentrations of (100 µL, 150 µL, 200 µL) of extracts. The extract papers were rolled and placed in exposure tubes, the hole on the top of the tube sucrose fed and blood starved 20 mosquito adults was allowed inside and plugged with cotton. They kept for acclimatize for 1hr and fumigated adults were observed and tabulated. A pre dried Whatman no. 1 filter paper consists ethanol added was served as control. At the end of exposure the mosquitoes were placed in holding

glass tube. Cotton pads soaked in 10% sugar solution with vitamin B complex were placed during the holding period of 24 h. The number of dead mosquitoes and mortality percentages were determined after 24 hrs of treatment. Triplicates of each treatment and control were set up.

## GC-MS analysis

GC-MS technique was used to examine the constituents of extracts from *H. Ovalis* was carried out in IITM, Tamil Nadu. It was performed using Agilent and Jeol GC mateII (Mass spectrometry) by HP-5 column capillary equipped with a high temperature column (DB-5 mm 30 × 0.25 mm × 0.25 µm) was used and works with 70 eV. The injector and detector temperature can set at 250°C. A 1µL sample volume was injected into the column and employed using split less mode. High pure Helium is carrier gas was programmed to maintain a constant flow rate of 1 ml/min. The column oven temperature was initially kept at 80°C for 2 min, then programmed at 200°C/min, which was held at 20 min. Identification of organic compound was matching their recorded spectra with the data bank mass spectra of NIST library provided by the instrument.

## Statistical analysis of data

The average mortality data were subjected to profit analysis to calculate LC<sub>50</sub> and 95% fiducial limits of upper confidence limit (UCL) and lower confidence limit (LCL), regression equation, Chi-square and analysis variation values were calculated using the Stat plus 2009 software. Results with p≤0.05 were considered to be statistically significant.

## Results and Discussion

The in a progress study have tested with marine seaweed extract of *Halophila ovalis* (Figure 1) against the vector borne disease causing mosquito like *Culex quinquefasciatus*. It reveals that the extract of *Halophila ovalis* showed various ranges of repellent and adulticidal activities. *Halophila ovalis* showed the maximum percentage of showed protection percentage of *Culex quinquefasciatus* mosquito protection (95) and protection time (4.4 hrs) was observed at the 250 µL of *Halophila ovalis* extract. The results were presented in Table 1. However, bites were observed between 10.00-16.00 hrs with the remaining concentration of extract treated arms. The result of the adulticidal activity from *Halophila ovalis* against *Culex quinquefasciatus* are presented in Table 2. Among the five concentrations of seaweed extract tested, the highest adulticidal activity was observed in 200 µL against *Culex quinquefasciatus* with the LC<sub>50</sub>=51.2 ± 0.9 and LCL-UCL values of, 50.2-56.8 respectively, and the regression value R<sup>2</sup> = 0.959 and analysis of variation was significant at p≤0.05 level. The relative percentage of identifying compounds from the ethanolic extract of *Halophila ovalis* were depicted in Table 3 were Hexadecanoic acid, followed by Glucobrassicin, Ethanol, 2-(9-octadecenyloxy)-,(Z), Ethanone, 1,1'-(1,4-dihydro-2,4,6-trimethyl-3,5-pyridinediyl)bis., Tridecanoic acid, methyl ester, 1,2,4-Trioxolane-2-octanoic acid, 5-octyl-, Methyl ester, Pentetic Acid and 9-Hexadecenoic acid.

The studies on mosquito larvicidal activities with seaweed extracts are too restricted. The seaweed extract of *Halophila ovalis* showed significant repellent and adulticidal activities. The results were comparable with early studies of Ali et al., reported the seaweed extract, *C. racemosa* showed toxicity against 4th instar larvae of *Aedes aegypti*, *Culex quinquefasciatus*, *Anopheles stephensi* with equivalent (LC<sub>50</sub>=0.0556 ± 0.0103 µg/mL, 0.0675 ± 0.1360 µg/mL and 0.0661 ± 0.0076 µg/mL) respectively [12]. Ali et al., [10] reported that column chromatographic fractions of *R. mucronata* bark extracts (E1) showed

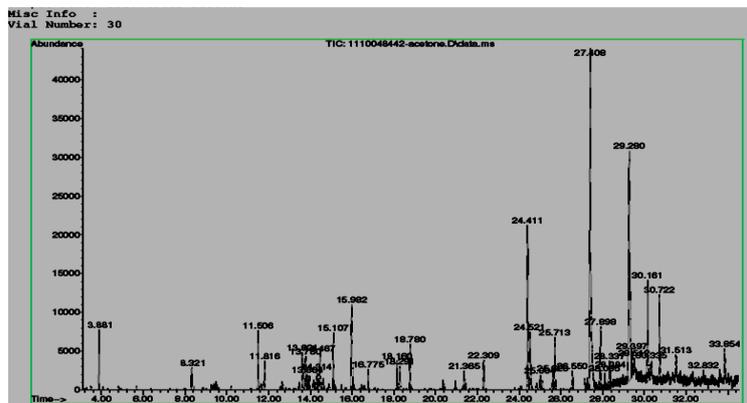


Figure 1: GCMS analysis of seagrass extract of *H. ovalis*.

Concentration (%)	Treatments Period (hrs)												% of protection	Protection Time (hrs)
	10-11		11-12		12-13		13-14		14-15		15-16			
	T	C	T	C	T	C	T	C	T	C	T	C		
100	0	0	0	0	3	5	4	7	3	9	3	10	59.3	1.2
150	0	0	0	1	2	5	2	7	3	9	4	10	65.6	1.5
200	0	0	0	1	1	4	2	6	2	9	3	11	74.1	2.1
250	0	0	0	3	0	5	1	8	2	10	3	11	95	4.4

T-Treatment, C-Control

Table 1: Repellent activity of ethanolic seagrass extract of *H. ovalis* against *C. Quinquefasciatus*.

No. of Mosquito	Concentration (µL)	LC <sub>50</sub> (mg/ml)		R <sup>2</sup>	P≤0.05
		Mean ± SE	LCL - UCL		
25	100	32.6 ± 0.58	29.6-34.9	1.048	2.87
25	150	45.1 ± 0.25	43.1-46.1	1.125	1.26
25	200	51.2 ± 0.9	50.2-56.8	0.959 *	0.032*

\*Significant at P<0.05 level, LCL: Lower confidence level, UCL: Upper confidence level, R<sup>2</sup> – Regression equation of significant level of P≤0.05

Table 2: Adulticidal activity from seagrass extract of *H. ovalis* against *C. quinquefasciatus*.

Retention time	Compound	Molecular weight	Peak Area
11.67	Hexadecanoic acid	256.42	15272544
12.63	Glucobrassicin	447.46	5986464
15.33	Ethanol, 2-(9-octadecenyloxy)-(Z)	312.53	7423168
15.45	Ethanone, 1,1'-(1,4-dihydro-2,4,6-trimethyl-3,5-pyridinediyl)bis-	165.18	7269504
15.07	Tridecanoic acid, methyl ester	228.37	7721632
56.23	1,2,4-Trioxolane-2-octanoic acid, 5-octyl-, methyl ester	344.48	6614032
73.33	Pentetic Acid	393.35	5986464
56.23	9-Hexadecenoic acid	254.40	8790688

Table 3: Identification of compound from seagrass extract of *H. ovalis* using GCMS.

maximum larvicidal activity (LC<sub>50</sub> = 0.0496 ± 0.0085 µg/ml and LC<sub>90</sub> = 0.1264 ± 0.052 µg/ml), acetone extract (LC<sub>50</sub> = 0.0564 ± 0.0069 µg/ml and LC<sub>90</sub> = 0.1187 ± 0.05 µg/ml), ethanolic fraction (E4) of *R. mucronata* stilt root extracts showed maximum larvicidal activity (LC<sub>50</sub> = 0.0484 ± 0.0078 µg/ml and LC<sub>90</sub> = 0.1191 ± 0.025 µg/ml) and acetone fraction (A3) (LC<sub>50</sub> = 0.0419 ± 0.0059 µg/ml and LC<sub>90</sub> = 0.0955 ± 0.069 µg/ml). Kovendan et al. [11] evaluated LC<sub>50</sub> values of hexane, chloroform, ethyl acetate, acetone and methanol extract of *O. thymiflorus* third instar larvae of *An. stephensi* were LC<sub>50</sub> = 201.39, 178.76, 158.06, 139.22 and 118.74 ppm; *Cx. quinquefasciatus* were LC<sub>50</sub> = 228.13, 209.72, 183.35, 163.55 and 149.96 ppm and *Ae. aegypti* were LC<sub>50</sub> = 215.65, 197.91, 17505, 154.80 and 137.26

ppm respectively. Several authors have investigated that ethanolic extract show mosquito larvicidal activity [10,12]. Similarly, the present study was made an attempt and against *C. quinquefasciatus* were observed at maximum activity of minimum concentration at 250 µL.

Today, worldwide consumption of synthetic repellents has increased to prevent losses in store foodstuff materials. Unlike the larvicides, mosquito repellents only reduce the bites and cannot be considered as a control measure. Investigated Chloroform: methanol of mature leaf extract of *S. mahagoni* exhibits 100% repellency up to 2 h 15 min as no mosquito bites up to that time periods in the treated hands [20]. Ali et al., [10] reported repellency of *R. mucronata* done

in stilt root and bark extracts (A3) showed maximum protection (97.5%) with 9.1 h protection time at 4 mg concentration and ethanolic fraction of the stilt root (E4) extract showed maximum (100%) with 10 h protection time at 4 mg concentration. Plant based insecticides are nontoxic, easily available and show target specific activities. Kamaraj et al., [21] observed maximum repellent activity was observed at 500 ppm in methanol extracts of *N. nucifera*, ethyl acetate and methanol extract of *P. nigrum* and methanol extract of *T. ammi* protection time from 30 to 150 min. Chemical repellents are not secure for public consume due to their apparent toxicity. Many researchers proved phytochemical constituents such as n-Hexadecanoic acid, Furfural, Glucobrassicin acts as insecticidal agents. Marimuthu Govindarajan *et al* evaluated the methanol extract of *E. alba* and *A. paniculata* was produced maximum repellency against *An. Stephensi* [22]. Skin repellent test by Pushpanathan et al, at 1.0, 2.5 and 5.0 mg/cm<sup>2</sup> concentration of *C. citratus* gave 100% protection up to 3.00, 4.00 and 5.00 hours respectively. Likewise, the repellency percentage of 95% against *C. quinquefasciatus* at 250 µL of concentration extract of *Halophila ovalis* was determined in the present study.

The plant produces a great array of secondary metabolites as a result of metabolic activities. These compounds either alone or in combination are responsible for the specific therapeutic action administered as a medicament or a health supplement. Kovendan et al., [6] reported the adult mortality was found in ethanol extract of *C. sinensis* with the LC<sub>50</sub> and LC<sub>90</sub> values of 272.19 and 457.14 ppm, *A. stephensi*; 289.62 and 494.88 ppm, *Ae. aegypti*; and 320.38 and 524.57 ppm, respectively. Comparatively, the adulticidal activity was observed in 200 µL concentration against *Cx. quinquefasciatus* with LC<sub>50</sub> (51.2 ± 0.9) µL/ml. Many novel control agents from botanicals previously have proven to be a significant potential mosquito killer as they are relatively safer, cost effective, less toxic and easily degradable.

The present study have shown repellent, and adulticidal activity might be due to the presence of phytochemicals constituents such as Hexadecanoic acid, Glucobrassicin, Ethanol, 2-(9-octadecenyloxy)-(Z), Tridecanoic acid, 1,2,4-Trioxolane-2-octanoic acid 5-octyl- methyl ester, Ethanone, 1,1'-(1,4-dihydro-2,4,6-trimethyl-3,5-pyridinediyl) bis-, Pentetic Acid and 9-Hexadecenoic acid which may cause inhibition of poly (ADP-ribose) polymerase enzyme which is involved in the DNA repair in adult mosquito, alterations in the siphon and toxicity of prothoracic glands in instar larvae. Hexadecanoic acid, Tridecanoic acid and Glucobrassicin are natural insecticides and nematocides. Similarly *Sargassum polycystum* showed a lethal effect in mosquitocidal activity on *Ae. aegypti* and *Cx. quinquefasciatus*. Earlier reports showed the essential oil compounds Eucalyptol, Caryophyllene, Germacrene-D and α-humulene showed significant adulticidal activity whereas Cyclopentane, Hydrazinecarboxamide, Benzamide, Pentadecanoic acid, Cyclopentanone, Hexanedioic acid, 2-Hydroxy-1-(Hydroxymethyl) ethyl ester and mono (2-ethylhexyl) ester showed larvicidal and repellent activity [10]. Comparing earlier authors evaluation our results revealed that the experimental *Halophila ovalis* extract was effective to mosquito vectors.

## Conclusion

The structural elucidation of the seagrass *Halophila ovalis* extract showed the presence of most effective, unique chemical classes. The results as well as the significance of this preliminary investigation highlight the importance of *Halophila ovalis* as a novel source for natural insecticidal products. The structural elucidation of the *Halophila ovalis* extract throws light into the development of potential pesticide, insecticide and repellent cream at a large scale level for wider use for humankind. Further studies on identification of active compounds,

toxicity and field trials are needed to recommend the active fraction of these plant extracts for development of eco-friendly chemicals of insect vectors.

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