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Research Article

Phytochemical Analysis of Seaweed *Halymenia dilatata* with its Antioxidant Activity and against the Mosquito Vectors *Culex quinquefasciatus*

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Abstract

Background and Objective: Common human diseases like malaria, dengue, chikungunya, yellow fever, Zika and West Nile are spread by mosquitoes. One of the most important methods for controlling mosquito populations is the application of biological pesticides. This study explores the antioxidant activity of bioactive compounds obtained from *Halymenia dilatata* and the mosquito-larvicidal capability of marine macroalgae, providing a natural and environmentally acceptable substitute for synthetic chemical insecticides.

Materials and Methods: Phytochemical analysis was conducted on various extracts of the marine macroalgae. The antioxidant potential was evaluated using DPPH, ABTS and hydroxyl radical scavenging assays, while the larvicidal capability was tested against *Culex quinquefasciatus* larvae. Experiments were performed in triplicate and data were analyzed using two-way ANOVA with the SNK *post hoc* test in SPSS, while LC₅₀ and LC₉₀ values with 95% confidence limits were estimated using probit regression. **Results:** Phytochemical analysis revealed that the ethanolic extracts contained 13 compounds, exceeding the number found in other extracts. The DPPH ethanolic extract demonstrated the highest antioxidant activity (74.71 ± 0.49%), followed by the ABTS chloroform extract (68.55 ± 0.95%) and the hydroxyl radical scavenging methanol extract (71.42 ± 1.0%). The IC₅₀ values for DPPH, ABTS and hydroxyl radical scavenging activities were 41.06 ± 1.0 µg/mL, 65.81 ± 1.1 µg/mL and 69.04 ± 1.0 µg/mL, (p < 0.05), respectively. These findings suggest that the compound exhibits strong antioxidant properties, as lower IC₅₀ values correspond to greater antioxidant activity. Chloroform extracts exhibited significant effects on *Cx. quinquefasciatus* larvae. **Conclusion:** Bioactive compounds isolated from these extracts may serve as alternatives for antioxidant and larvicidal properties, which could mitigate the environmental impacts commonly associated with synthetic insecticides. Additionally, seaweed represents a promising resource for mosquito control due to its demonstrated larvicidal properties.

Key words: Seaweed, antioxidant, DPPH, ABTS, Hydroxyl radical, larvicidal property

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mosquito-borne diseases comprise an important worldwide public health challenge, account for 17 % of all infectious diseases and cause approximately 700,000 deaths annually¹. Mosquitoes function as biological vectors, transferring a diverse array of pathogens (viruses, bacteria and parasites) to humans and vertebrate hosts. The intricate interplay between these vectors, pathogens and hosts generates complex transmission dynamics that are highly responsive to ecological and socioeconomic shifts^{2,3}.

Environmental toxicity, bioaccumulation and the concerning increase in pesticide resistance among mosquito populations are some of the issues that traditional vector control techniques, which rely mostly on synthetic insecticides⁴. As a result, there is a pressing need to investigate sustainable, ecologically friendly and efficient alternatives. A promising approach is to search for marine insecticides within floral biodiversity. A method that could lead to the discovery of novel and more sustainable pest control methods⁵. Unlike traditional insecticides that rely on one active ingredient, algae-based formulations utilize diverse seaweed blends containing multiple bioactive compounds. This multi component nature makes them a more effective and sustainable solution for controlling mosquito populations⁶.

Seaweeds are a rich natural source of valuable bioactive compounds such as polysaccharides, polyphenols and essential fatty acids. These compounds hold therapeutic promise for humans (e.g., as antioxidants and anti-inflammatory agents) and are utilized in aquaculture to improve fish health and provide disease resistance⁷. Numerous natural antioxidants, antimicrobials, anticancer agents, larvicidal property and other health-enhancing substances have been isolated from algae⁸. Consequently, seaweed and seaweed-derived compounds are potential sources of natural antioxidants due to their high bioactive compound content. These antioxidants have strong anti-inflammatory, anti-cancer and anti-microbial properties. Making them suitable for various applications such as functional foods, nutraceuticals and pharmaceuticals⁹.

Seaweeds (macroalgae) produce diverse phytochemicals, such as terpenes, phlorotannins and halogenated compounds, as secondary metabolites. These compounds act as powerful chemical defenses to deter herbivorous insects¹⁰. Phytochemicals occurring naturally secondary metabolites in seaweed represent highly effective, eco-friendly and biodegradable alternatives to synthetic insecticides¹¹. There are several advantages to using phytochemicals, including their rapid biodegradation, their low cost compared to

synthetic insecticides, their minimal harm to non-target organisms and their multiple target sites that drastically reduce the likelihood of resistance development¹².

This study, evaluate the phytochemical compounds *in vitro* antioxidant activity and larvicidal efficacy of extracts from *Halymenia dilatata* sourced from the Manapad coastline, Thoothukudi, against the mosquito *Culex quinquefasciatus*. Thus, this study aimsto determine the vector control and biological the efficacy of *Halymenia dilatata* on *Culex quinquefasciatus*.

MATERIALS AND METHODS

Seaweed materials: Specimens of the red seaweed *Halymenia dilatata* were collected from the coastal region of Manapad (8.3957°N, 78.05886°E) in Thoothukudi District, Tamil Nadu, India, from January, 2024 to May, 2024. Immediately following collection algal material was manually separated from epiphytic organisms. The seaweed samples were thoroughly cleaned to remove undesirable parts, rinsed with sterile distilled water and identified with support from the Central Marine Fisheries Research Institute (CMFRI) at the Mandapam Regional Centre (Government of India). Samples were then air-dried at $26 \pm 1^\circ\text{C}$ in the shade, finely powdered and stored in an airtight container.

Preparation of seaweed extracts: The Soxhlet extraction of the seaweed samples was carried out sequentially with different solvents, namely aqueous, ethanol, methanol and chloroform, by the samples in the respective solvents (10 g sample, 300 mL solvent) with a Soxhlet apparatus at a temperature of $65 \pm 2^\circ\text{C}$. The sample was extracted for a total of 72 cycles (12 to 18 hrs at a rate of 4-6 cycles per hour). After extraction, the samples were evaporated in a hot-air oven at 50°C and then stored in a sterile container in a refrigerator until they were ready for further use.

Preliminary qualitative phytochemical analysis: The research focused on identifying sixteen distinct phytochemicals, which included steroids, tannins, terpenoids, flavonoids, saponins, alkaloids, reducing sugars, cardiac glycosides, coumarins, phlobatannins, anthraquinones, quinones, glycosides, phenols, anthocyanins and betacyanins, as referenced by Harborne¹³ and Sadasivam and Manikam¹⁴.

In vitro antioxidant assays

DPPH radical scavenging activity assay: DPPH radical scavenging activity was adopted by Singh *et al.*¹⁵ with slight modifications. A DPPH is a violet-colored stable free radical

solution that decolorizes into a colorless solution with the addition of a substrate that can donate a hydrogen atom. Briefly, a total of 1 mL of 0.135 mM DPPH prepared in methanol was mixed with 1.0 mL of seaweed extract ranging from 20 to 100 µg/mL (1 mg/mL concentration). The reaction mixture was vortexed thoroughly and left in the dark at room temperature (23-25 °C) for 30 min. Changes in the absorbance of the samples were measured at 517 nm using a UV-visible double-beam spectrophotometer 2205 (Systronics; Ahmedabad, Gujarat, India). All the tests were performed in triplicate. The inhibition percentage was calculated using the following formula:

$$\text{Radical scavenging activity (\%)} = \left[\frac{(\text{Abs}_{\text{con}} - \text{Abs}_{\text{sample}})}{(\text{Abs}_{\text{con}})} \right] \times 100$$

where, Abs_{con} is the absorbance of control, $\text{Abs}_{\text{sample}}$ is the absorbance of the test sample/standard.

ABTS radical scavenging assay: The ABTS scavenging activity of the extracts was performed using the method described by Re *et al.*¹⁶. The antioxidant agents present in the extract reduce the blue-green chromogen of ABTS^{+} to ABTS and decolorizes it. The working solution was prepared by mixing stock solutions of 7 mM ABTS and 2.4 mM Potassium persulfate in equal amounts and allowing them to react for 12 hrs (overnight) at room temperature in the dark. The solution was then diluted by mixing 1 mL ABTS^{+} solution with 60 mL methanol to obtain an absorbance of 0.706 ± 0.01 units at 734 nm using a spectrophotometer. A fresh ABTS solution was prepared for each assay. A total of 1 mL of freshly prepared ABTS solution was added to 1 mL of the seaweed extracts, ranging from 20 to 100 µg/mL (1 mg/mL concentration), the reaction mixture was vortexed for 10 sec and the absorbance was measured at 734 nm after 6 min using a UV-visible double-beam spectrophotometer 2205 (Systronics; Ahmedabad, Gujarat, India). All the tests were performed in triplicate. The inhibition percentage was calculated using the following formula and compared with that of the standard BHT.

$$\text{Radical scavenging activity (\%)} = \left[\frac{(\text{Abs}_{\text{con}} - \text{Abs}_{\text{sample}})}{(\text{Abs}_{\text{con}})} \right] \times 100$$

where, Abs_{con} is the absorbance of control, $\text{Abs}_{\text{sample}}$ is the absorbance of test sample/standard.

Hydroxyl radical scavenging assay: Hydroxyl radical scavenging activity was determined according to a slightly modified method of the 2-deoxy-ribose oxidation method¹⁷.

Hydroxyl radical was generated by the Fenton reaction in the presence of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. A reaction mixture containing 0.2 mL each of 10 mM $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 10 mM EDTA and 10 mM 20-deoxyribose was mixed with 0.2 mL of the extract solution, ranging from 20 to 100 µg/mL (1 mg/mL concentration) and 0.1 M phosphate buffer (pH 7.4) was added to the reaction mixture until the total volume reached 1.8 mL. Then 0.2 mL of 10 mM H_2O_2 was finally added to the reaction mixture and incubated at 37 °C for 4 hrs. After the incubation, 1 mL of 2.8% TCA (Trichloroacetic acid) and 1.0% TBA (Thiobarbituric acid) were added. Then, the mixture was placed in a boiling water bath for 10 min. Absorbance was measured at 532 nm using a UV-visible double-beam spectrophotometer 2205 (Systronics; Ahmedabad, Gujarat, India). All the tests were performed in triplicate. The inhibition percentage was calculated using the following formula and compared with that of the standard BHT.

$$\text{Radical scavenging activity (\%)} = \left[\frac{(\text{Abs}_{\text{con}} - \text{Abs}_{\text{sample}})}{(\text{Abs}_{\text{con}})} \right] \times 100$$

where, Abs_{con} is the absorbance of control, $\text{Abs}_{\text{sample}}$ is the absorbance of test sample/ standard.

Collection of mosquito samples and rearing: The mosquito eggs of *Culex quinquefasciatus* were collected from Tiruchendur (8.500038°N, 78.117141°E), Thoothukudi District, Tamil Nadu, India. Collected egg samples were brought to the testing laboratory and transferred to the enamel trays, each containing 300 mL of water and allowed to hatch. The mosquito larvae were fed a diet consisting of a 3:1 ratio of dog biscuit to yeast powder, suspended in 500 mL of deionized water. The larval colonies were maintained in the laboratory at a temperature of 25 °C with a relative humidity of $75 \pm 5\%$ and 14 hrs of photoperiod. The emerging fourth instar stage of the mosquito larvae was used for the experiment.

Larvicidal activity: The assays were conducted according to the guidelines provided by the World Health Organization¹⁸ for mosquito *in vivo* testing. We utilized 20 late 3rd instar and early 4th instar larvae per replicate that were collected in disposable plastic cups filled with dechlorinated water. Then, various concentrations of metabolites were prepared using 100 mL of water. The seaweed metabolite was dissolved in 10% DMSO for preparation at a 1 mg/mL concentration (stock solution 10 mL) and experiments were performed using different concentrations (0.5, 1.0, 1.5, 2.0 and 2.5 µg/mL) of metabolites from seaweed extract. The control of each experiment (treated with 10 % DMSO and distilled water) was

tested three times. The mortality (using Abbott's formula) and survival rate were determined after 24 hrs and 48 hrs of exposure¹⁹. All the test containers were kept at room temperature without any disturbances. The LC₅₀ and LC₉₀ values of exposed larvae were calculated using probit analysis²⁰.

$$\text{Mortality (\%)} = \frac{\text{Treated mortality (\%)} - \text{Control mortality (\%)}}{100 - \text{Control mortality (\%)}} \times 100$$

Statistical analysis: Experiments were performed in triplicate and the results were presented as Mean $\pm$ Standard Deviation. The data were analyzed by applying the Student-Newman-Keuls (SNK) *post hoc* test for a two-way ANOVA in SPSS (Statistical Package for Social Science). To determine the LC₅₀ and LC₉₀ (lethal concentration) values for the average larval mortality data, a probit regression model was fitted to the observed relationship between the substance's logarithmic concentration and the percentage of larval mortality. The 95% confidence limits of these values were then estimated²⁰.

RESULTS

Preliminary qualitative phytochemical analysis: The investigate involved an initial phytochemical analysis of 16 distinct compounds in the seaweed species *Halymenia dilatata* using four solvents (aqueous, ethanol, methanol and chloroform). The findings from (Table 1) demonstrate that the highest number of phytochemical compounds was found in the methanolic extract of *Halymenia dilatata*, revealing a total of thirteen phytochemical compounds (steroids, tannins, terpenoids, flavonoids, alkaloids, reducing sugar, cardiac

glycosides, coumarins, anthraquinones, quinones, glycosides, phenols and betacyanin) while the aqueous extract contained the lowest count of eight compounds.

In vitro antioxidant activity

DPPH radical scavenging activity: The DPPH radical scavenging ability of aqueous, ethanol, methanol and chloroform extracts from the seaweed *Halymenia dilatata* was evaluated at various concentrations, with the results illustrated in Fig. 1 ($p < 0.05$). The ethanolic extract demonstrated strong DPPH activity at 74.71 ± 0.49 %, followed by the chloroform extract at 70.18 ± 1.0 %, the methanolic extract at 68.04 ± 0.5 % and the aqueous extract at 62.54 ± 1.0 % when tested at concentrations ranging from 20-100 $\mu\text{g/mL}$, while the reference antioxidant Butylated Hydroxytoluene (BHT) displayed an activity of 81.67 ± 0.66 %. The ethanolic extracts showed the lowest IC₅₀ values at 41.06 ± 1.0 . Among all the samples examined, the ethanolic extract exhibited the highest DPPH radical scavenging activity.

ABTS radical scavenging activity: The ABTS radical scavenging activities of the aqueous, ethanol, methanol and chloroform extracts from the seaweed *Halymenia dilatata* were assessed at concentrations ranging from 20 to 100 $\mu\text{g/mL}$ and compared to the standard antioxidant Butylated Hydroxytoluene (BHT) (Fig. 2). The ABTS radical scavenging activity of all extracts increased with higher concentrations ($p < 0.05$). Particularly, the chloroform extracts exhibited a significantly greater ABTS free radical scavenging activity (68.55 ± 0.95 %) compared to the ethanolic extracts (67.43 ± 0.72 %), followed by the methanolic and aqueous extracts, while BHT demonstrated a free radical scavenging activity of (90.0 ± 1.0 %). The chloroform and methanolic

Table 1: Preliminary qualitative phytochemical analysis of solvents and their fractions of different extracts of *Halymenia dilatata*

Phytochemical compounds	Solvents			
	Aqueous	Ethanol	Methanol	Chloroform
Steroids	-	+	+	+
Tannins	+	+	+	-
Terpenoids	-	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	-	+
Alkaloids	+	+	+	-
Reducing sugar	-	-	+	-
Cardiac glycosides	+	+	+	+
Coumarins	-	+	+	+
Phlobatannins	-	-	-	-
Anthraquinones	-	-	+	+
Quinones	+	+	+	+
Glycosides	-	+	+	-
Phenols	+	+	+	+
Anthocyanin	-	-	-	-
Betacyanin	+	+	+	+

+: Indicates presence of phytochemicals and -: Indicates the absence of phytochemicals

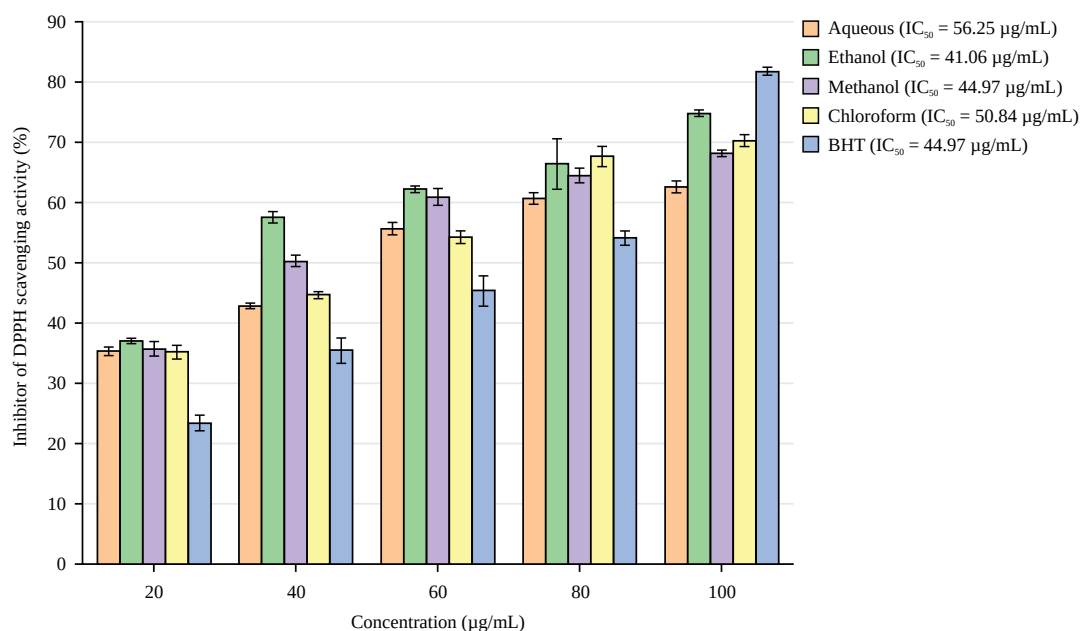


Fig. 1: Inhibitory effect of *Halymenia dilatata* seaweed extracts on 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity compared to Butylated Hydroxytoluene (BHT)
Each value is expressed as the (Mean $\pm$ Standard Deviation) (N = 3)

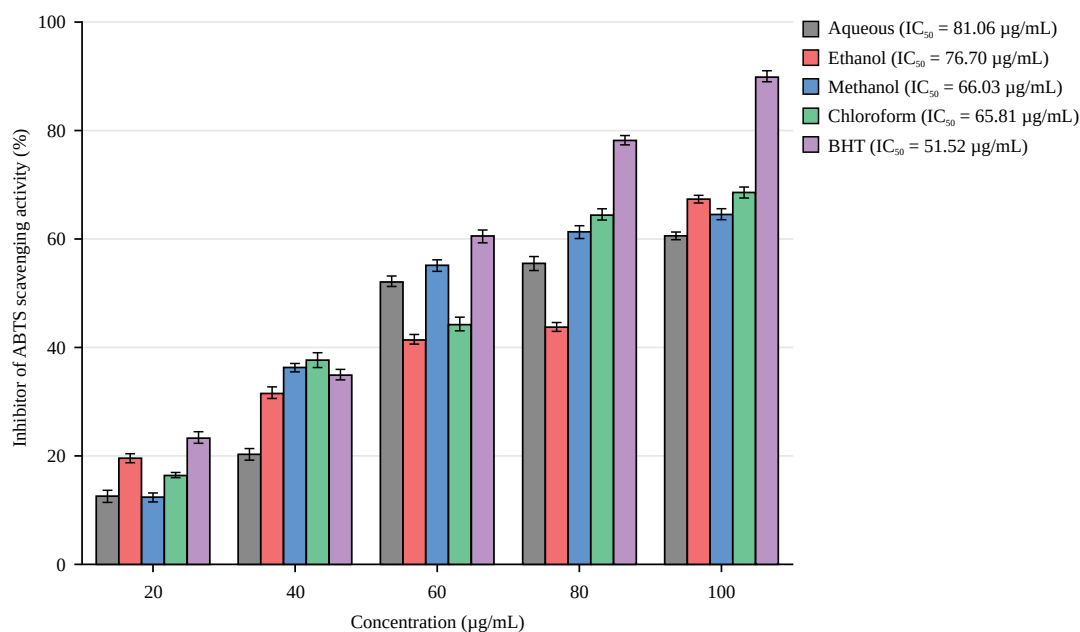


Fig. 2: Inhibitory effect of *Halymenia dilatata* seaweed extracts on 2, 2-azinobis-3-ethylbenzothiazoline-6-sulfonic (ABTS radical scavenging activity compared to Butylated Hydroxytoluene (BHT)
Each value is expressed as the (Mean $\pm$ Standard Deviation) (N = 3)

extracts recorded the lowest IC₅₀ values (65.81 ± 1.1 and 66.03 ± 1.0), while the IC₅₀ values for the ethanol and aqueous extracts were the highest.

Hydroxyl radical scavenging activity: The hydroxyl radical scavenging activity of *Halymenia dilatata* was assessed using various extracts aqueous, ethanol, methanol and

chloroform at concentrations ranging from 20 to 100 µg/mL and the results were compared to those of BHT (Fig. 3). The methanolic extract demonstrated a significantly greater ($P < 0.05$) H₂O₂ scavenging activity of $71.42 \pm 1.0\%$ at the 100 µg/mL concentration, along with other concentrations of 20, 40, 60 and 80 µg/mL. The standard antioxidant BHT exhibited an H₂O₂ scavenging

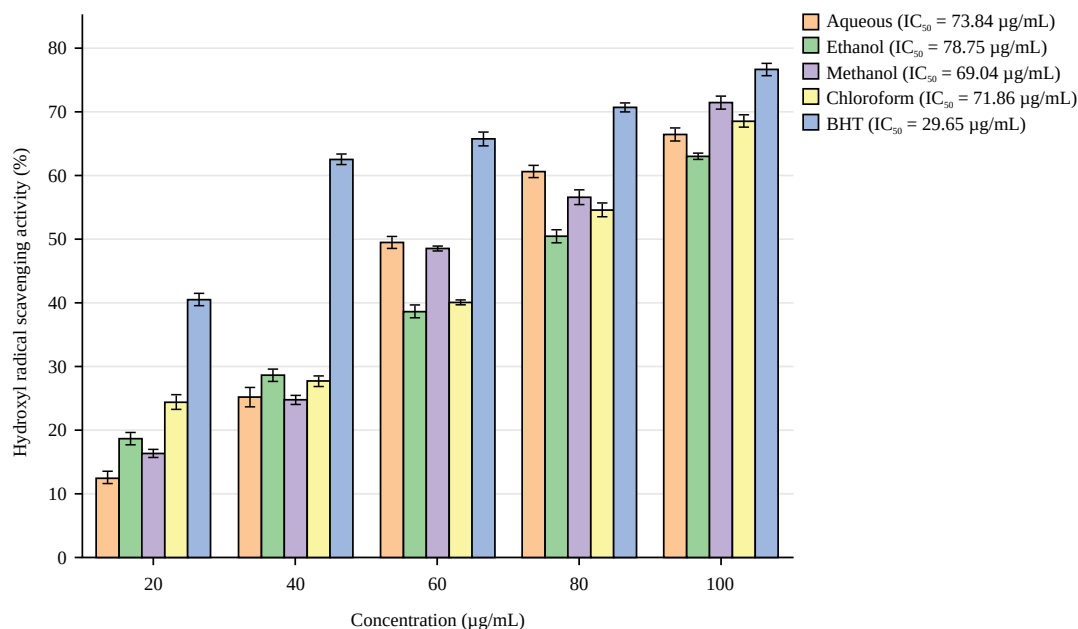


Fig. 3: Scavenging activity of *Halymenia dilatata* seaweed extracts on hydroxyl radical scavenging activity compared to Butylated Hydroxytoluene (BHT)

Each value is expressed as the (Mean $\pm$ Standard Deviation) (N = 3)

Table 2: Observed and expected mortality of *Culex* larvae exposed to *Halymenia dilatata* with aqueous, ethanol, methanol and chloroform extracts. Expected mortality is based on probit regression analysis

Extracts	Duration	LC ₅₀ (µg/mL)	LC ₉₀ (µg/mL)	95% Confidence limit LC ₅₀ (LC ₉₀)		Regression equation	X ² (df = 3)
				Lower (µg/mL)	Upper (µg/mL)		
Aqueous	24 hrs	2.963	5.694	2.235 (3.271)	9033.8 (7.634)	y = 4.517+2.131x	0.757
	48 hrs	2.540	4.384	2.064 (2.964)	9.165 (198.6)	y = 5.409+2.190x	1.064
Ethanol	24 hrs	2.706	6.854	1.955 (3.534)	23.119 (3546.4)	y = 3.175+1.375	0.432
	48 hrs	1.854	4.279	1.411 (2.781)	2.930 (24.139)	y = 3.528+0.946x	0.699
Methanol	24 hrs	2.538	4.878	2.002 (3.078)	10.371 (338.35)	y = 4.521+1.828x	1.149
	48 hrs	2.349	4.127	1.920 (2.867)	4.898 (44.265)	y = 5.235+1.942x	0.641
Chloroform	24 hrs	0.969	2.713	0.566 (1.834)	1.328 (8.849)	y = 2.866+0.039x	3.008
	48 hrs	0.446	1.220	0.065 (0.822)	0.688 (3.393)	y = 2.928+1.028x	0.943

95% CL: 95% Confidence limit, LC₅₀: 50% Lethal concentration and LC₉₀: 90% Lethal concentration

activity of $76.60 \pm 1.0\%$. The methanolic extracts recorded the lowest IC₅₀ values (69.04 ± 1), suggesting a high level of antioxidant activity.

Larvicidal activity: The larvicidal activity assays were conducted to evaluate the efficacy of seaweed derived extracts against selected vector species. LC₅₀ and LC₉₀ values were determined at 24 and 48 hrs. The results are presented in Fig. 4a-b. After 24 hrs of exposure to *Halymenia dilatata* chloroform extract, significantly higher mortality was observed ($p < 0.05$), with mortality rates ranging from 30% to 100% against *Cx. quinquefasciatus* and LC₅₀ and LC₉₀ values of 0.969 and 2.713 µg/mL, respectively. After 48 hrs of treatment at a concentration of 2.5 µg/mL, *Cx. quinquefasciatus* exhibited increased mortality rates of 60% to 100%, with LC₅₀ and LC₉₀ values of 0.446 and 1.220 µg/mL, respectively (Table 2).

DISCUSSION

Due to a global trend away from hazardous synthetic chemical insecticides and toward environmentally acceptable alternatives, the search for natural pesticide substitutes, especially for mosquito control, has attracted a lot of attention. Plants and marine organisms are important sources of these naturally occurring bioactive chemicals, which can be used for a variety of ecological purposes²¹. Specifically secondary metabolites of natural origin show great promise as alternative agents to manage the threat of vector-borne diseases by disrupting mosquitoes physiological and behavioural systems²².

Among these marine sources, seaweed have demonstrated high potential across various fields including agriculture, food, medicine and vector control²³. Utilizing

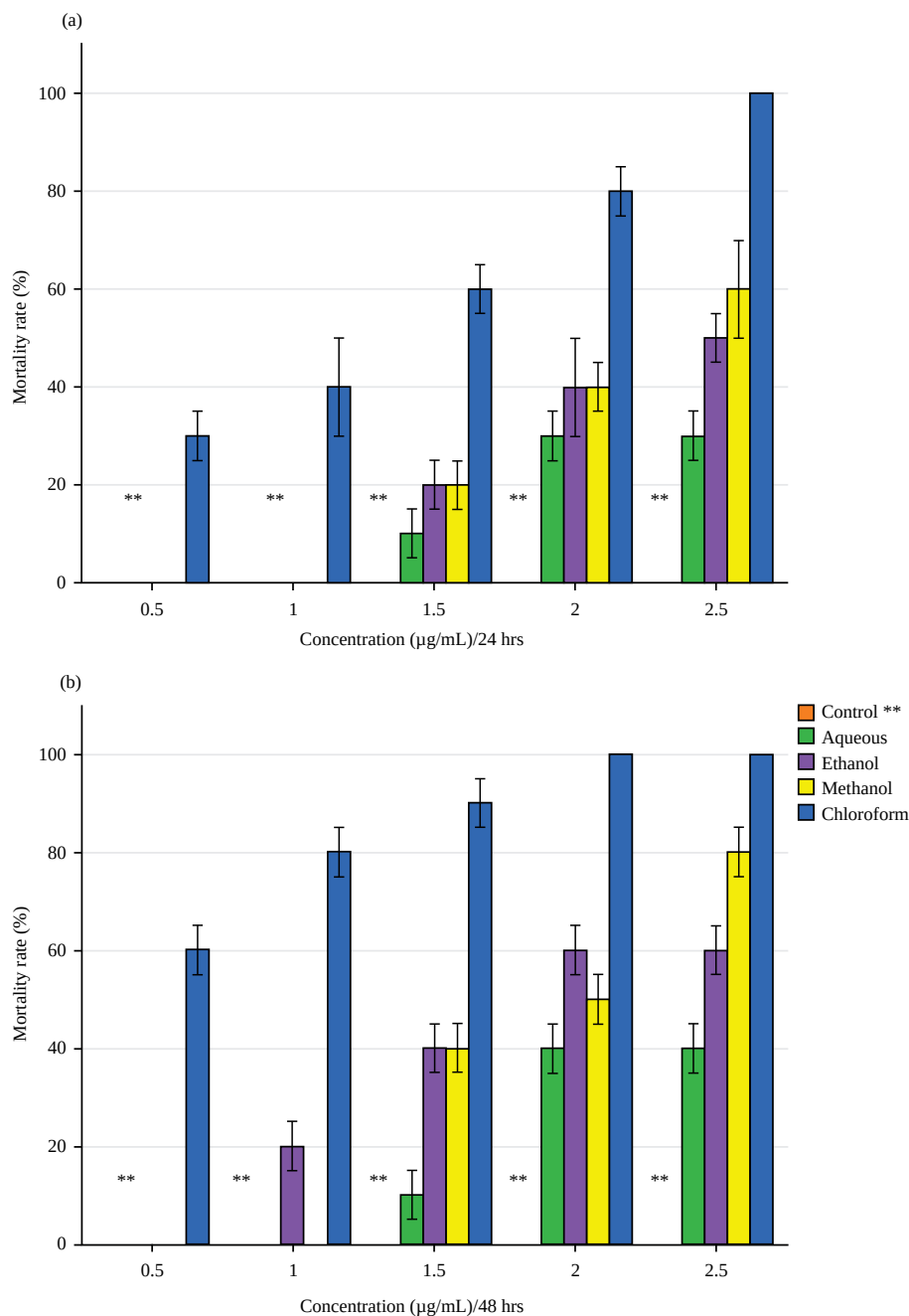


Fig.4(a-b): Percent larvicidal activity of potential of *Halymenia dilatata* (a) 24 hrs, (b) 48 hrs, seaweed extracts against *Cx. quinquefasciatus*. The positive control consisted of 10% DMSO, while the negative control was distilled water, both resulting in a 0% mortality rate

Each value is expressed as the (Mean $\pm$ Standard Deviation) (N = 3) and **: 0 (%) Mortality rate

seaweed-derived compounds could be crucial in reducing the transmission of devastating diseases like malaria, yellow fever, dengue fever and filariasis. However, effectively controlling these vectors remains highly challenging due to a combination of drug-resistant parasites, rising insecticide resistance and a lack of reliable vaccines, anti-cancer, or antimicrobial drugs²⁴.

The investigate involved an initial phytochemical analysis of 16 distinct compounds in the seaweed species *Halymenia dilatata* using four solvents (aqueous, ethanol, methanol and chloroform). The demonstrate that the highest number of phytochemical compounds was found in the methanolic extract of revealing a total of thirteen phytochemical compounds (steroids, tannins, terpenoids,

flavonoids, alkaloids, reducing sugar, cardiac glycosides, coumarins, anthraquinones, quinones, glycosides, phenols and betacyanin) while the aqueous extract contained the lowest count of eight compounds. The wide variety of phytochemicals found in marine seaweeds indicates possible bioactive profiles, that exhibit antioxidant, anti-inflammatory, parasite and insecticide properties^{6,7}. Specifically, Seaweed-derived tannins, saponins, flavonoids, polyphenols, steroids are valuable bioactive compounds, possessing insecticidal, antimicrobial, antiparasitic and cardiogenic properties²⁵. Because these secondary metabolites have numerous therapeutic benefits, they are used extensively in the drug and pharmaceutical industry⁷.

Seaweed has emerged as a premier natural source for reactive oxygen species (ROS) inhibitors, prized for its unique profile of marine-derived antioxidants. These macroalgae adapt to intense sunlight and harsh marine environments by producing specialized, robust secondary metabolites that effectively neutralize oxidative stress^{26,27}. In the present study, high DPPH radical scavenging activity was recorded in the ethanolic extract of *H. dilatata* (74.71%), a value that were lower than the activity of the BHT. The *H. dilatata* exhibited a high DPPH radical scavenging activity with a low IC₅₀ 41.06 ± 1.0 µg/mL, indicating that these seaweed extracts possess substantial antioxidant properties, comparable to a commonly used synthetic antioxidant. These finding align with several recent studies examining ethanolic extract for Annamalai⁸ reported high DPPH scavenging activities of 77% in the ethanol extract of *Codium fragile* and 81.48% in *Turbinaria conoides*. Similarly, Cho *et al.*²⁷ observed that ethanolic extracts enhanced the levels of antioxidant and scavenging activity in *Enteromorpha compressa* and *Capsosiphon fulvescens*, identifying them as highly efficient DPPH scavengers. Tanna *et al.*²⁸ reported that the methanol extract of *Caulerpa racemosa* var. *macrophyssa* showed 60% DPPH scavenging activity. When evaluating three edible green algae species, Ganesan *et al.*²⁹ found that the methanolic extract of *Ulva compressa* IC₅₀ 1.89 mg/mL exhibited the highest antioxidant activity, while Farasat *et al.*³⁰ recorded a strong DPPH radical scavenging activity for *Ulva clathrata* with a lower IC₅₀ 0.715 ± mg/mL.

In evaluating the antioxidant potential via the ABTS assay, the chloroform and ethanolic extracts of *H. dilatata* exhibited radical scavenging activities of 68.55% and 67.43% respectively. For comparison, the synthetic standard BHT demonstrated a scavenging efficacy of (90.0 %). The chloroform extract significantly quenched ABTS radical with a low IC₅₀ 65.81 ± 1.1 µg/mL a strong performance that correlate

well with the presence of flavonoids, phenolic compounds and tannins in the species for comparison, the synthetic standard BHT scavenging efficacy of 90.0%. Other marine algae have shown similarly highest ABTS scavenging activity. The ethyl acetate (EtOAc) fraction of the seaweed *Turbinaria ornata* exhibited significant ABTS radical scavenging activity with an IC₅₀ value of 3.16 µg/mL, indicating a strong antioxidant potential³¹. Pangestuti *et al.*³² reported that subcritical water extraction of *Ulva lactuca* displayed a higher ABTS activity than *Caulerpa racemosa*. Furthermore, Maheswari and Salamun³³, the highest ABTS radical scavenging activity (96.95 ± 0.41%) was observed in *C. verticillata* than standard ascorbic acid (90.99 ± 0.30%).

The study suggests that the methanolic extracts of *H. dilatata* have novel hydroxyl radical scavenging activities, reaching 71.42 %. Which is comparable to the activity of BHT recorded at 76.60 %. This high antioxidant capacity in red seaweeds is largely attributed to the presence of polyphenolic compounds like phlorotannins, which act as electron traps responsible for multifunctional antioxidant properties, including the scavenging of hydroxyl radicals, peroxy radicals, or superoxides^{34,35}. Similar enhanced hydroxyl (HO[•]) radical scavenging activities in methanolic extracts have been reported for other red seaweeds, such as *J. rubens*³⁶. Furthermore, earlier findings by Ganesan *et al.*³⁷ showed up to 90% hydroxyl radical scavenging activity in the dichloromethane and butanol fractions of the red seaweeds *A. spicifera* and *G. edulis*.

Free radicals are frequently associated with cellular damage in mosquitoes, a process that increases their vulnerability to various insecticides. In seaweed, endogenous antioxidants naturally protect the algae's own tissues from this type of oxidative stress; however, the concentrated presence of these antioxidant constituents particularly flavonoids and phenolic compounds can concurrently indicate substantial larvicidal capability when introduced to mosquito vectors^{37,38}.

The larvicidal activity of various seaweed extracts against *Cx. quinquefasciatus* mosquitoes were assessed; with effectiveness varying based on the seaweed species, the extraction solvent (aqueous, ethanol, methanol and chloroform), the concentration and the length of exposure. Overall chloroform extract of *H. dilatata* demonstrated the highest efficacy, establishing significant larval mortality rate of 30-100% at 24 hrs and 60-100% at 48 hrs across tested concentrations (0.5, 1, 1.5, 2, 2.5 µg/mL). Consequently, *H. dilatata* chloroform extracts exhibited the lowest lethal concentration values, with an LC₅₀ of 0.446 µg/mL and an LC₉₀ of 1.220 µg/mL. These secondary metabolites within the

chloroform and ethanol extracts of *C. fragile* and *T. conoides* seaweeds likely drive this larvicidal activity by disrupting the physiological development *Cx. quinquefasciatus* larvae⁶, as well as and *Ae. aegypti* vectors²³.

The toxicity of seaweed extracts against mosquito vectors is primarily caused by the phytochemicals' ability to react with cellular components and potentially produce damaging byproducts, disrupting normal growth and function and ultimately leading to mortality, rather than acting as an antioxidant³⁹. Each solvent's potency in these activities is determined by the particular phytochemical components it extracts, such as alkaloids in chloroform or phenols and flavonoids in ethanol. For instance, the mosquito larvicidal activity of ethanol extracts from *D. dichotoma*, *E. intestinalis* and *A. spicifera* against *Aedes aegypti* is driven by these extracted phytochemicals, including flavonoids, phenols, terpenoids and saponins⁴⁰. Other extracts have also demonstrated insecticidal effects against various vectors: crude ethanol and aqueous extracts of *T. decurrens* and crude *Gracilaria corticata* extract have all proven effective against *Ae. aegypti*, *Cx. quinquefasciatus* and *An. Stephens*⁴¹.

CONCLUSION

The findings demonstrate that *Halymenia dilatata* contains diverse bioactive compounds with notable antioxidant and larvicidal activities. Among the tested extracts, the ethanolic extract showed the highest DPPH scavenging activity, while chloroform extracts exhibited significant larvicidal effects against *Culex quinquefasciatus* larvae. The observed antioxidant and larvicidal activities highlight *H. dilatata* as a promising natural source of bioactive compounds for potential antioxidant applications and environmentally sustainable mosquito control. Further studies are recommended to isolate and characterize the active compounds and to evaluate their efficacy, safety and effects on non-target organisms under laboratory and field conditions.

SIGNIFICANCE STATEMENT

This study highlights the potential of the marine macroalgae *Halymenia dilatata* as a natural source of antioxidant and mosquito-larvicidal compounds. Its demonstrated activity against *Culex quinquefasciatus* larvae suggests that seaweed-derived bioactive compounds could contribute to the development of alternative mosquito-control agents and help reduce reliance on conventional

synthetic insecticides. Further validation of their safety, active constituents and field effectiveness may support their future application in sustainable vector-management programs.

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