https://doi.org/10.48047/AFJBS.6.15.2024.3343-3362

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

POTENTIAL OF *ULVA SPECIES* AS A SAFE BIOPESTICIDE FOR SUSTAINABLE AGRICULTURE AND ENVIRONMENTAL DEVELOPMENT

Mahalakshmi [1], Kamakshi S [1], Dr. K. Arulmeha Ponradha[2] and Dr. R. Siva[2]

1. Research Scholar,
Department of Plant Biology and Plant Biotechnology,
Shrimathi Devkunvar Nanalal Bhatt Vaishnav College for Women,
Chromepet, Chennai, 600044, Tamil Nadu, India
2. Associate Professor,
Department of Plant Biology and Plant Biotechnology,
Shrimathi Devkunvar Nanalal Bhatt Vaishnav College for Women,
Chromepet, Chennai, 600044, Tamil Nadu, India

Volume 6, Issue 15, Sep 2024

Received: 15 July 2024

Accepted: 25 Aug 2024

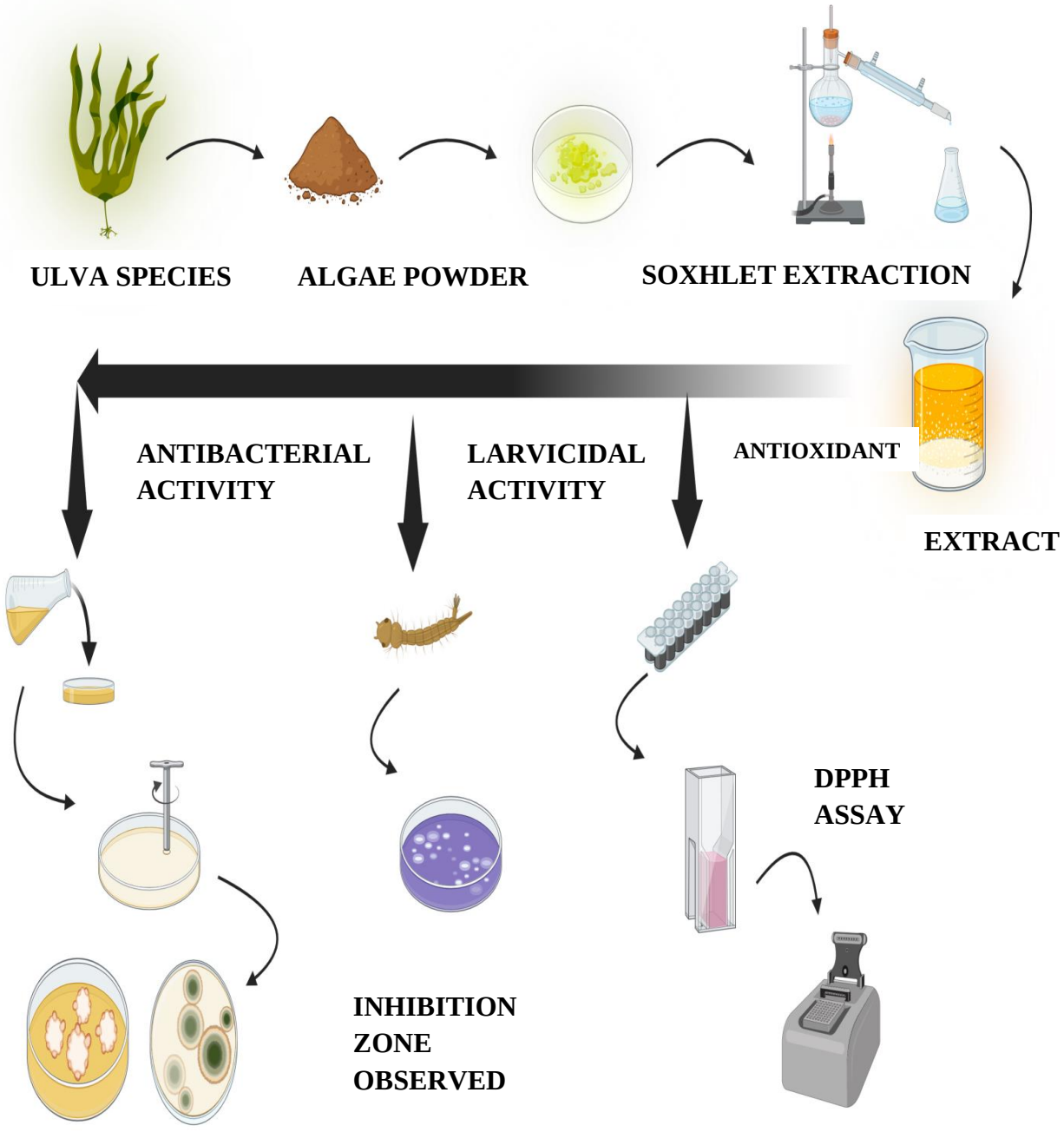
Published: 05 Sep 2024

doi:10.48047/AFJBS.6.15.2024.3343-3362

Abstract
The essential nutrients for a well-balanced feed supplement, such as polysaccharides, fatty acids, amino acids, antioxidants, vitamins, and minerals are often abundant in marine macroalgae. The recent study examined and compared the phytochemical, antibacterial, and antioxidant properties of *Ulva lactuca* and *Ulva fasciata* Lin (Chlorophyceae). Different extracts (acetone, benzene, methanol, and aqueous) at various doses (100, 200, 300, 400, and 500 ppm) were used to study the larvicidal effects of *Ulva lactuca* Lin (Chlorophyceae) against *Anopheles stephensi*. The study used early 4th instar larvae of *Anopheles stephensi* and followed the WHO procedure to determine the treated larvae's fatal concentrations (LC50 and LC90). The observed mortality was recorded 24 to 48 hours after treatment. The acetone extracts of Ulva lactuca and Ulva fasciata contained phenolic components, tannins, and terpenoids. *The results* showed that each extract had different levels of larvicidal efficacy against *Anopheles stephensi*. *Ulva lactuca* methanol extract exhibited exceptional larvicidal action against *Anopheles stephensi*. *Ulva lactuca's* aqueous extract had the lowest larval mortality compared to acetone, benzene, methanol, and ethanol extracts. This study found *Ulva lactuca*'s methanolic extract is effective against *Anopheles stephensi* larvae, making it an eco-friendly mosquito control option.

Key words: *Ulva lactuca*, *Ulva fasciata*, *Anopheles stephensis*, Quinone, seaweed, Larvicidal activity

GRAPHICAL REPRESENTATION OF ULVA SPECIES AS A SAFE BIOPESTICIDES FOR SUSTAINABLE AGRICULTURE AND ENVIRONMENTAL DEVELOPMENT



INTRODUCTION

Mosquitoes belong to the order Diptera and the family Culicidae. There are over 3,500 species of mosquitoes worldwide, each with its unique characteristics and behaviours. Female mosquitoes are the primary vectors of disease, as they require blood for egg development. They have specialized mouthparts designed for piercing the skin and extracting blood. Male

mosquitoes, on the other hand, feed solely on plant nectar. Mosquitoes breed in stagnant water, laying their eggs in various water bodies, ranging from ponds and marshes to artificial containers like discarded tires and flowerpots. The larvae, commonly known as wrigglers, develop in water, feeding on organic matter until they emerge as adults. Their breeding sites are diverse and ubiquitous, making mosquito control a challenging endeavor.

Mosquito-Borne Diseases: Mosquitoes are notorious for transmitting a wide array of pathogens, including viruses, parasites, and bacteria. Among the most significant mosquito-borne diseases is malaria, caused by the Plasmodium parasite and transmitted by Anopheles mosquitoes. Malaria remains a leading cause of morbidity and mortality in many tropical and subtropical regions, particularly in sub-Saharan Africa. Natural products constitute a source of compounds with insecticidal properties (Newman, D. J., & Cragg, G. M. 2020). Numerous studies have reported the activity of edible plant extracts and essential oils against Ae. aegypti, highlighting readily available matrices Other notable diseases spread by mosquitoes include dengue fever, caused by the dengue virus and transmitted primarily by Aedes mosquitoes. Dengue is endemic in over 100 countries, with millions of cases reported annually. Zika virus, transmitted by Aedes mosquitoes, gained global attention due to its association with birth defects, including microcephaly, in newborns.

Additionally, mosquitoes transmit diseases such as chikungunya, yellow fever, West Nile virus, and Japanese encephalitis, posing significant public health threats worldwide. The geographic distribution of these diseases is expanding due to factors like urbanization, climate change, and globalization, underscoring the importance of vigilance and proactive measures to mitigate their spread. However, synthetic insecticides are commonly used to control various vector-borne diseases,but they have several disadvantages, including an impact on non-targeted species, negative effects on the environment, and the development of resistance in vector species because of target site alteration. The concerns about insecticide resistance and environmental impacts have stimulated renewed interest in larval control involving temporary or permanent removal of anopheline larval habitats, as well as larvicide with biological agents or plant-based products that are eco-friendly in nature. Plant extracts can be used as larvicides and repellents which will have minimal environmental impact. These plant-derived compounds provide broad-spectrum resistance to many mosquito species, are less expensive, environmentally safer, biodegradable, freely available, and non-toxic to non-targeted creatures. Many studies aiming to identify representative genera of marine algae that produce bioactive substances have been conducted. In the last three decades, the rate of discovery of biologically

active metabolites produced by macroalgae has increased (Cabrita *et al.*,2010).

Ulva species, also known as sea lettuce, have gained attention for their diverse range of biological activities, leading to extensive research focusing on their potential applications in various fields, including medicine, agriculture, and environmental sustainability (Siva R and Kowsalya G). The following sections will explore the specific biological activities of *Ulva lactuca* and *Ulva fasciata* species and their potential implications for human health, environmental sustainability, and innovative uses in pest control (Kulkarni *et al.*,2021). They also contain a rich array of bioactive compounds, including phenolic compounds, flavonoids, and polysaccharides, which contribute to their diverse biological activities (Santos *et al.*,2015). Understanding the phytochemical composition of *Ulva lactuca* and *Ulva fasciata,* the present study is essential for unlocking their full potential in various applications, from pharmaceuticals to agricultural products.

In addition to their phytochemical diversity, *Ulva* species exhibit remarkable antioxidant capabilities (Gomathi and Anna Sheba 2018; Praba and Sumaya 2022; Uddin *et al.*,2020). The presence of antioxidants such as vitamins C and E, carotenoids, and phycobiliproteins in *Ulva* species contributes to their ability to neutralize reactive oxygen species and protect cells from oxidative damage. Furthermore, exploring the underlying mechanisms of antioxidant activity in *Ulva* species can provide valuable insights for developing natural antioxidant products with diverse health and wellness applications (Zaatout *et al.,* 2019).

The antimicrobial properties of *Ulva* species have been widely studied in recent years, with promising results indicating their potential as natural sources of antimicrobial agents. Various extracts of Ulva species have shown activity against a wide range of microorganisms, including bacteria, fungi, and viruses. The phytochemical composition of Ulva extracts, such as polysaccharides, polyphenols, and terpenoids, is thought to be responsible for their antimicrobial effects (El-Mesallamy *et al.*,2021). These compounds have demonstrated inhibitory effects on the growth and proliferation of pathogenic microorganisms, making Ulva species a valuable resource for the development of novel antimicrobial agents. Further research is needed to elucidate the specific mechanisms underlying the antimicrobial activity of Ulva species and to optimize their potential applications in the field of medicine and biotechnology. The antimicrobial properties of Ulva species have sparked interest in exploring their potential as natural alternatives to conventional antibiotics. These macroalgae have demonstrated inhibitory effects against a broad spectrum of bacteria, highlighting their potential in combating

infectious diseases. Investigating the mode of action of Ulva's antibacterial components can offer a deeper understanding of their mechanisms and pave the way for the development of novel antimicrobial agents.

Ulva species have been found to possess significant antioxidant properties, making them a promising source of natural antioxidants. Various studies have reported that Ulva species contain compounds such as phenolic compounds, flavonoids, and carotenoids that exhibit potent antioxidant activity (Arsianti 2023). These antioxidant compounds help to neutralize harmful free radicals in the body, thereby reducing oxidative stress and preventing damage to cells and tissues. Moreover, Ulva species have been shown to have a high total antioxidant capacity, making them effective in scavenging reactive oxygen species and protecting against chronic diseases associated with oxidative stress (Farasat *et al.,* 2014). Overall, the antioxidant properties of Ulva species underscore their potential as valuable sources of bioactive compounds for therapeutic and nutraceutical applications.

Ulva species have shown promising potential as a natural source for mosquito larvicidal activity. Studies have demonstrated that extracts from various Ulva species exhibit significant larvicidal effects against mosquito larvae, making them a promising candidate for environmentally friendly mosquito control strategies. The phytochemical composition of Ulva species, including polyphenols, flavonoids, and other bioactive compounds, contributes to their larvicidal activity. Additionally, the antioxidant properties of Ulva species play a role in their ability to disrupt the development and survival of mosquito larvae.

In this study, we compared the phytochemical, antioxidant, antimicrobial, and mosquito larvicidal activity of Ulva lactuca and Ulva fasciata species. The goal is to develop new antimicrobial agents and mosquito control strategies by fully understanding their beneficial properties and potential uses.

MATERIALS AND METHODS

Collection of algae

Ulva lactuca and *Ulva fasciata* were collected from a variety of locales in Mandapam (Lat. 09° 17.417'N; Long. 079° 08.558'E) within the Rameshwaram district of the Gulf of Mannar Marine Biosphere Reserve in Tamil Nadu. The collections were made in November and

December 2023. Mr. Rajendra Kumar of the R.K. Algae Center in Rameshwaram, Tamil Nadu, India, identified the algae.

Preparation of extracts

Algal species were manually collected from submerged rocks and shells during low tide. They were carefully cleaned with seawater to remove sand particles, animal droppings, contaminants, and epiphytes. The collected samples were packed in fresh polythene bags, and morphologically diverse algal thalli were kept in an ice box filled with slush ice and brought to the laboratory. Dry blotting was done with the samples on sterile tissue paper. The seaweed was allowed to dry at room temperature for a week, and then each sample was finely powdered. The Whatman filter paper was used to encase 500g of finely ground algal powder. Powdered materials were extracted in a Soxhlet apparatus for 72 hours using a variety of organic solvents with varying degrees of polarity, including hexane, chloroform, ethyl acetate, acetone, methanol, and distilled water. The extracts were concentrated at temperatures lower than 40 °C in a rotary vacuum evaporator (Heidolph, Germany). For the further assay, the crude extracts were stored at 4 °C.

Phytochemical screening

Qualitative phytochemical analysis was carried out on hexane, chloroform, ethyl acetate, acetone, Petroleum ether, aqueous extract, and methanol extracts using *Ulva lactuca* and *Ulva fasciata*. Among the phytochemicals Flavonoids, alkaloids, phenolic compounds, cardiac glycosides, terpenoids, and tannins were investigated.

Detection of flavonoids:

Ferric chloride test:

A milliliter of ferric chloride solution was added to one milliliter of the extract. The appearance of brown colour indicates the presence of flavonoids .

Detection of alkaloids:

Wagner's test:

Wagner's test was made by dissolving 2 gms of potassium iodide and 1.29 gms of iodine in 5 mm of deionized water. Distilled water is used to make the solution up to 100 mm. When Wagner's reaction is introduced to 1 mm of extract, a dark precipitate appears. The presence of

alkaloids is shown by the production of brown precipitate.

Detection of tannins:

Ferric chloride test:

One ml of the extract and, a few ml of 5% ferric chloride were added. The development of dark bluish-black indicates the presence of tannins.

Detection of terpenoids:

Libermann-burchard's test:

2ml of extract and a few drops of acetic anhydride were added, boiled, and cooled. Then one ml of concentrated sulphuric acid was added on the sides of the test tube. The formation of a brown ring at the junction of two layers and the formation of deep red colour indicates the presence of terpenoids.

Detection of glycosides:

Keller-killani test:

Two ml of extract was dissolved in 2 ml of glacial acetic acid containing one drop of ferric chloride solution. The mixture was then poured into a test tube containing one ml of conc.H2So4. a brown ring at the inner phase indicates the presence of deoxyribose sugar.

Detection of saponins:

Frothing test:

One ml of extract was added with 4 ml of distilled water and the mixture was shaken vigorously. The formation of persistent foam which lasts for at least a few minutes indicates the presence of saponins.

Test for carbohydrate:

Fehlings test:

Molisch test:

Take 2ml of extract in a dry test tube, 2ml of distilled water in another test tube as control add 2-3 drops of molisch reagent to the solution and gently pipette one ml of conc. H_2SO_4 along

the sides of the test tube.so two distinct layers are formed. Purple colour indicates the presence of carbohydrate

Benedict's test:

Mix one milliliter of the sample with two milliliters of the Benedict reagent. Place the mixture in a hot water bath and heat it for three minutes. colour shift from blue to brick red when precipitation falls.

Detection of protein:

Ninhydrin test:

Take one ml of extract in a dry test tube and add a few drops of ninhydrin reagent, place the test tube in a water bath for five minutes, and allow cooling at room temperature. appearance of purple colour.

Test for Anthraquinone:

Take 0.1 gm of the drug and add 5 ml of 5% solution of ferric chloride and 5 ml dilute hydrochloric acid and heat on a boiling water bath for 5 minutes, cool the solution, and shake gently with an organic solvent like benzene. Separate the organic solvent layer and add an equal volume of dilute ammonia. A pinkish-red color is formed in the ammoniacal layer. This test is of C. glycoside.

DPPH scavenging activity of *Ulva lactuca* and Ulva *fasciata*

Antioxidant activity was evaluated by the DPPH method according to Darsih *et al.*, (2019) with modification. Several concentrations of *Ulva lactuca* and *Ulva fasciata* extracts were dissolved in methanol and then reacted with DPPH 1.01 mM at room temperature. The reaction was incubated in the dark condition for 30 min. Absorbance was measured using a UV-Vis spectrophotometer at 517 nm. The scavenging activity was calculated by the equation as follows:

$$\text{DPPH radical scavenging activity (\%)} = \left\{ \frac{A_{\text{sample}} - A_{\text{control}}}{A_{\text{control}}} \right\}$$

Antibacterial assay of *Ulva lactuca* and *Ulva fasciata*

The agar well diffusion method was used to examine the antibacterial activity of *Ulva lactuca* and *Ulva fasciata*. The extracts of methanol, acetone, and aqueous were examined against the pathogenic bacterium, *Escherichia coli*, *Bacillus subtilis*, *Streptococcus mutans*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa*. Pure colonies of *Escherichia coli*, *Bacillus subtilis*, *Streptococcus mutans*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa* were cultured in nutritive broth media to prepare the inoculums for the antibacterial experiment. After 24 hours, the Mueller Hinton Agar (MHA) medium was sterilized by autoclaving and then uniformly poured into Petri plates. Using a sterile glass spreader, 100µL of the pathogenic microbe culture broth (CFU 106 cells/mL) was carefully distributed across different sterilized Petri plates to form a homogenous layer of the bacterial suspension. A solution of gentamicin was prepared by mixing 4µ g/mL of gentamicin with sterile distilled water was placed in the well as a reference. Petri dishes were sealed and stored in an incubator at 37 °C. After 24 hours, the Petri plate wells had distinct inhibition zones around them, and their diameter (measured in millimeters) was recorded.

MIC determination

The methanolic, acetone, and aqueous extracts of *Ulva lactuca* and *Ulva fasciata* were tested for their minimum inhibitory concentration (MIC) alongside the standard antibiotic gentamicin (4 µg/ml) using a micro-dilution assay. Different concentrations of the algal extract and antibiotic were prepared with 5% DMSO. Concentrations of Ulva extract (1.25, 2.5, and 5 mg/mL) were combined with the antibiotic (4 µg/ml) in Mueller Hinton broth (MHB) tubes, which were then inoculated with 100 mL of pathogenic bacterium (1.5 × 108 CFU/mL). The control tubes contained MHB and the examined bacterium, and were incubated at 37 °C for 24 hours. The MIC values were determined as the lowest concentration of antibacterial substances that visibly inhibited the growth of *Klebsiella pneumoniae* RCMB 003 (1) ATCC 13,883.

Mosquito larvicidal assay

For the mosquito larvicidal assay, Anopheles stephensis larvae were collected from a stagnant water yard in Chennai. The larvae were kept at a temperature of 27 ± 2°C and a relative humidity of 75-85%. They were fed a mixture of dog biscuits and yeast at a ratio of 3:1. The larvicidal activity was evaluated following standard protocols. Aqueous, benzene, acetone, and methanol extracts were prepared at concentrations of 100, 200, 300, 400, and 500 ppm. Each larvicidal assay consisted of 20 early fourth instar stage larvae placed in a 100 mL beaker, with

three replicates for each concentration. Throughout this trial, the larvae were not given any nourishment. Their mortality rate was measured after 24 and 48 hours of being exposed. Additionally, the mortality of the larvae was observed when they were exposed to water, as well as when they were exposed to benzene, acetone, and methanol separately.

Statistical analysis

The results are expressed as the mean ±SD. All statistical analyses were performed using SPSS version 16.0 statistical software (SPSS Inc., Chicago, IL, USA). The average larval mortality data were subjected to probit analysis calculating LC_{50}, LC_{90}, and other statistics, and 95% coincidence limits and chi-square values were calculated.

RESULTS AND DISCUSSION

Phytochemi cal analysis

Phytochemical analysis was performed on the extracts of hexane, chloroform, ethyl acetate, acetone, and methanol. According to Table 1, the results showed the presence of terpenoids, tannins, cardiac glycosides, alkaloids, flavonoids, and anthraquinone. Compared to the other extracts, the methanolic extracts of *Ulva lactuca* had a stronger content of phytochemicals such as terpenoids, tannins, cardiac glycosides, alkaloids, flavonoids, and anthraquinone (Arsianti *et al.*,2016). Among the phytochemicals, cardiac glycosides were present in all the extracts except Hexane (Joseph 2019). Anthraquinone, Alkaloids, Flavonoids, and reducing sugar were present in all the extracts of *U. lactuca* and *U. fasciata*. Terpenoids are present in Aqueous extract, acetone, and ethanol. (Putra *et al.,* 2024)

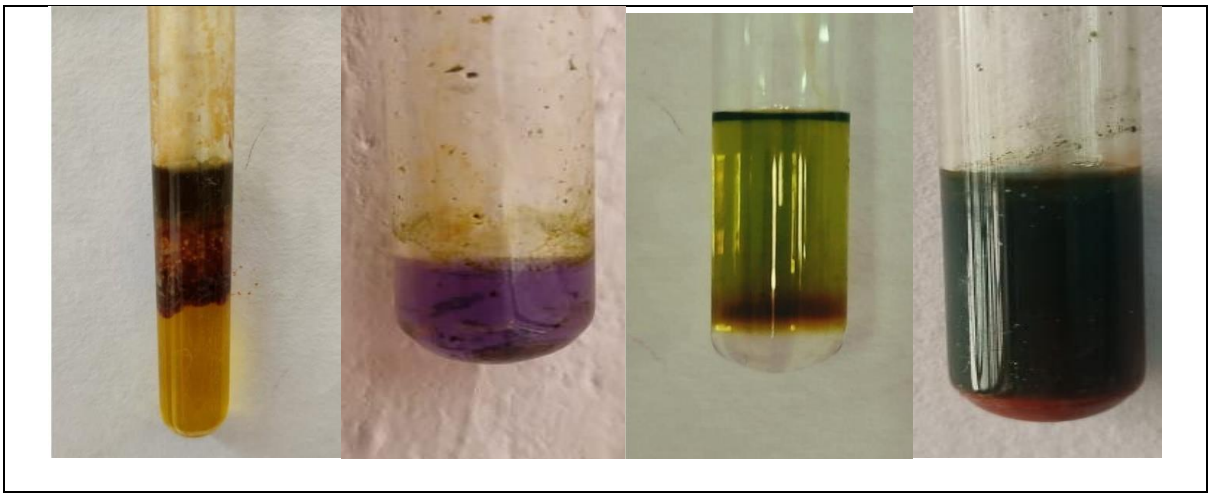


Figure 1, (A) Presence of Anthroquinone; (B) Detection Of Glysocide; (C) Detection Of Carbohydrates; (D) Presence Of Carbohydrate.

TABLE 1, QUALITATIVE PHYTOCHEMICAL ANALYSIS WITH ULVA LACTUCA

TESTS	DISTILLED WATER	ACETONE	ETHANOL	ETHYL ACETATE	PETROLEUM ETHER	BENZENE	CHLOROFORM	HEXANE
MOLISCH	+	+	-	+	+	-	+	-
FEHLING	+	+	-	+	-	-	+	-
BENEDICT	+	+	-	+	-	-	+	-
NINHYDRIN	+	+	+	-	+	-	-	-
SAPONIN	+	+	+	-	+	+	+	+
TERPENOIDS	-	-	-	-	-	-	+	+
ALKALOIDS	-	-	-	-	-	-	+	-
GLYCOSIDE	-	+	-	-	+	-	+	--
ANTHRAQUINONE	-	+	+	-	+	+	+	+
TANNINS	+	+	-	-	-	-	-	-
FLAVONOIDS	-	+	+	-	-	-	+	-

TABLE 2, QUALITATIVE PHYTOCHEMICAL ANALYSIS WITH ULVA FASCIATA

TESTS	HEXANE	DISTILLED WATER	ACETONE	ETHANOL	ETHYL ACETATE	BENZENE	CHLOROFORM

FLAVONOIDS	+	+	+	+	+	+	+
ALKALOIDS	+	+	+	+	+	+	+
TANNINS	-	-	-	-	-	-	-
TERPENOIDS	-	+	+	+	-	-	-
GLYCOSIDES	-	+	+	+	+	+	+
SAPONINS	-	+	+	+	+	+	-
FEHLING	+	+	+	+	+	+	+
BENEDICT	-	-	-	-	-	-	-
NINHYDRIN	-	+	+	+	+	-	+
ANTHRAQUINONE	+	+	+	+	+	+	+

DPPH radical scavenging activity

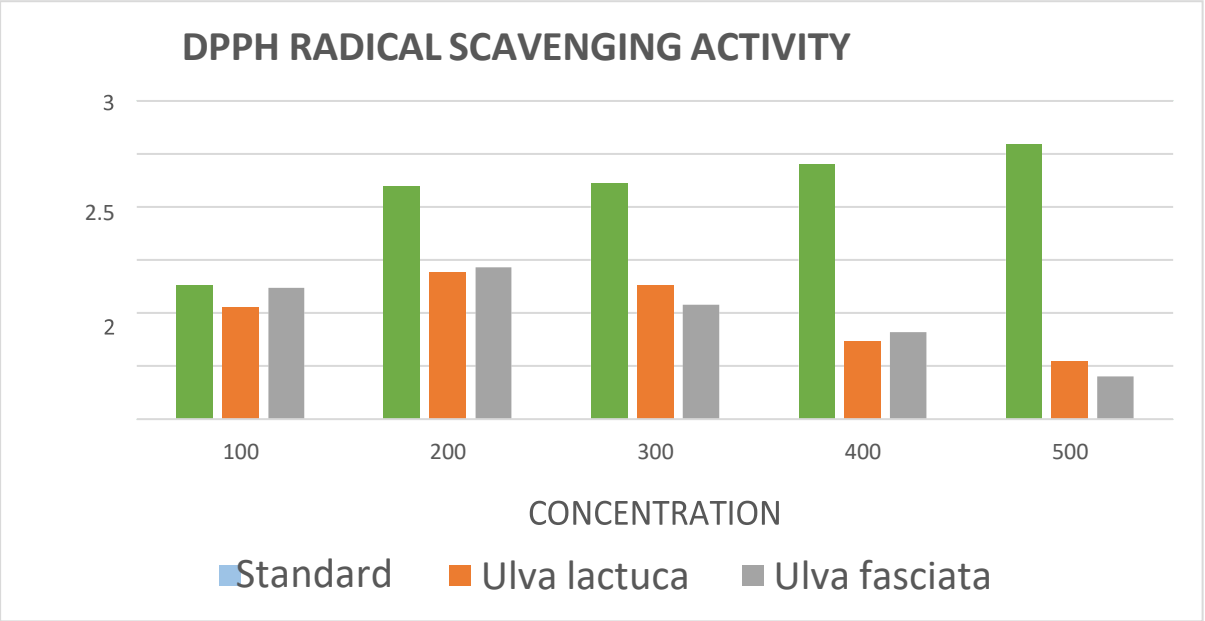
The radical scavenging method was used to evaluate the antioxidant activity of *U. lactuca and U. fasciata.* The most widely used technique to assess an extract's potential to scavenge radicals from natural sources is the DPPH method. *Ulva fasciata* extract had lower antioxidant activity than *Ulva lactuca* extract, according to the DPPH scavenging activity study. *Ulva lactuca* extract demonstrated 32.67±4.23% DPPH radical scavenging activity at the maximum concentration of 0.8 mg/mL, while *Ulva fasciata* extract demonstrated 22.34±9.71% radical scavenging activity (Figure 2). At 0.025 mg/mL, ascorbic acid, used as a positive control, had a radical scavenging activity of 76.67±1.20%. According to (Farasat *et al.*,2014), *Ulva rigida* exhibited 92.5% scavenging activity at 1 μg/mL in the DPPH assay. Additionally, it was shown that *U. rigida* showed a defense against hydrogen peroxide, preventing the death of yeast cells and zebrafish embryos. Because of their high protein content and polyphenolic components, the marine macroalgae *Ulva* sp. and *Gracilaria* sp. demonstrated strong antioxidant activity, according to another study.

Table 3, Antioxidant activity of Ulva lactuca and Ulva fasciata

S.no	Concentration	Standard (ascorbic acid)	Ulva lactuca	Ulva fasciata	% RSA (u.l)	% RSA (u.f)

1	100	1.264	1.053	1.689	16.693	0.34
2	200	2.201	1.386	1.432	37.028	0.349
3	300	2.216	1.267	1.076	42.824	0.514
4	400	2.40	0.738	0.821	69.25	0.657
5	500	2.596	0.549	0.401	78.852	0.845

Figure 2, Graphical representation of the antioxidant activity of *Ulva lactuca* and *Ulva fasciata*



Antibacterial activity of *Ulva lactuca* and *Ulva fasciata*

The antibacterial activity of the sample has been investigated in this study using the agar well diffusion method. A parameter used to evaluate the antibacterial activity of an agar plate is the diameter of the inhibition zone. The antibacterial activity of *Ulva lactuca* and *Ulva fasciata* extracts was tested against *Escherichia coli*, *Bacillus subtilis*, Streptococcus *mutans*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa*. The solvent without the sample served as the negative control and Gentamicin as the positive control. Table 3-6 displays the outcomes. The Aqueous extract of *Ulva lactuca* and *Ulva fasciata* did not exhibit bacterial growth inhibitory action at a dosage of 100 µl. Acetone extract of *Ulva lactuca* shows maximum inhibition against *P. aeruginosa, S. mutans, and E. coli* growth at a dose of 40 µl, with an inhibition zone measuring 4 and 5 mm respectively. *Ulva lactuca* showed stronger antibacterial activity against P. *aeruginosa* in comparison to *Ulva fasciata* acetone extract. While acetone and aqueous, the solvent utilized, showed no inhibition against the bacteria, *ulva lactuca* extract at a dosage of 80 µl suppressed bacterial growth with an inhibition zone of 5 mm. It is thought that several active compounds in the *U. lactuca* extract have a variety of antibacterial and anti-inflammatory qualities that can overcome the antibiotic resistance of Methicillin-resistant Staphylococcus aureus and hasten the growth of new tissue during the healing process of wounds (Ardita *et al.*, 2021).

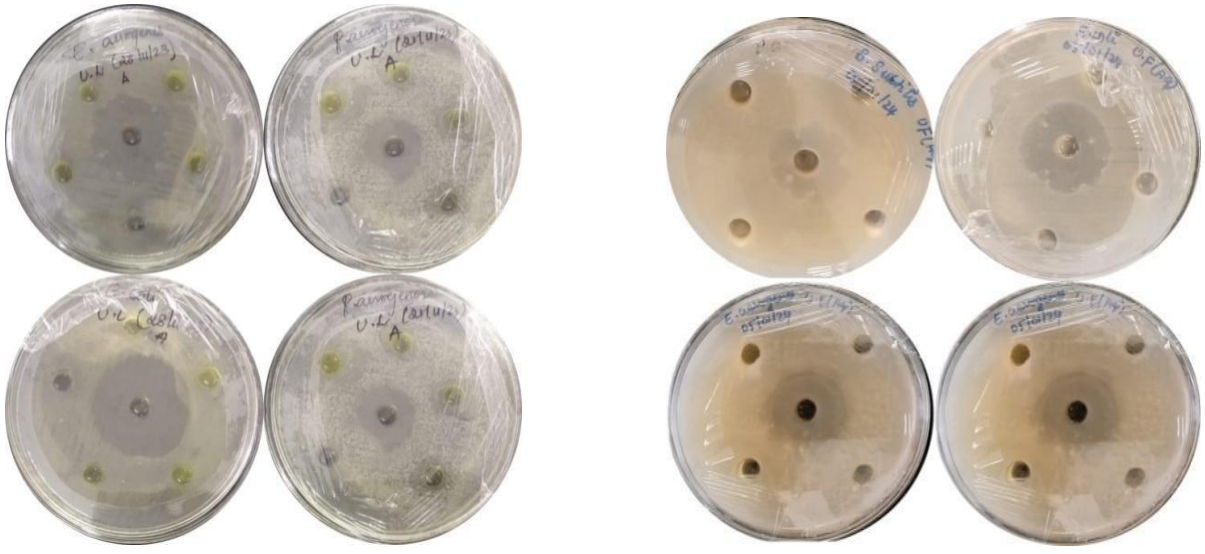


Figure 3, A) Antimicrobial Activity of *Ulva Lactuca* With Acetone; **B)** AntimicrobialActivity of *Ulva fasciata* With aqueous extract;

ANTIBACTERIAL ACTIVITY OF *ULVA LACTUCA*

TABLE 4, ACTIVITY OF *ULVA LACTUCA* WITH ACETONE EXTRACT

S.no	**Name of bacteria**	**20µl**	**40µl**	**60µl**	**80µl**	**Negative control (solvent)**	**Positive control (antibiotic)**
1	*P. aeruginosa*	-	0.3cm	0.3cm	0.5cm	-	1.3cm
2	*S.mutans*	-	0.2cm	0.2cm	0.4cm	0.2cm	1cm
3	*E.coli*	-	-	0.2cm	0.4cm	-	1cm
4	*B.subtilis*	-	-	-	-	-	1.2cm
5	*E..aerogenes*	-	-	-	-	-	1cm

TABLE 5, ACTIVITY OF *ULVA LACTUCA* WITH AQUEOUS EXTRACT

S.no	Name of bacteria	20µl	40µl	60µl	80µl	Negative control(solvent)	Positive control(antibiotic)
1	*P. aeruginosa*	-	-	-	-	-	1cm
2	*S.mutans*	-	-	-	-	-	1.6cm
3	*E.coli*	-	-	-	-	-	1cm
4	*B..substilis*	-	-	-	-	-	1.2cm
5	*E..aerogenes*	-	-	-	-	-	1cm

TABLE 6, ACTIVITY OF *ULVA FASCIATA* WITH ACETONE EXTRACT

S.no	Name of the bacteria	20µl	40µl	60µl	80µl	Negative control(solvent)	Positive control (antibiotic)
1	*P.aeruginosa*	-	-	-	0.5cm	-	1cm
2	*S.mutans*	-	-	0.2cm	0.4cm	-	1cm
3	*E.coli*	-	-	0.2cm	0.3cm	-	1.2cm
4	*B..substilis*	-	-	0.1cm	0.3cm	-	1cm

5	*E..aerogenes*	-	-	0.2cm	0.5cm	-	1cm

TABLE 7, ACTIVITY OF *ULVA FASCIATA* WITH AQUEOUS EXTRACT

S.no	Name of bacteria	20µl	40µl	60µl	80µl	Negative control(solvent)	Positive control (antibiotic)
1	*P. aeruginosa*	-	-	-	-	-	1cm
2	*S.mutans*	-	-	-	-	-	1.3cm
3	*E.coli*	-	-	-	-	-	1.2cm
4	*B..substilis*	-	-	-	-	-	1cm
5	*E..aerogenes*	-	-	-	-	-	1.cm

Mosquito larvicidal activity

The larvicidal activity of different solvents *viz.*, aqueous, acetone, benzene, and methanol extracts of *U. lactuca* and *U. fasciata* against *Anopheles Stephens* and the results are presented in (Tables2-3) which shows that in the case of 4^{th} instar of *Anopheles stephensis*, 500 ppm concentration exhibits mortality at 24 and 48 hours. The larvicidal mortality rate increases with time of exposure (24 h>48h). Tables 2-3 reveal significant differences in larvicidal mortality of log probit analysis at 95% confidence level where LC_{50} and LC_{90} values. The highest activity was observed in the methanolic extract of *Ulva lactuca* against the larva of *Anopheles stephensis.*

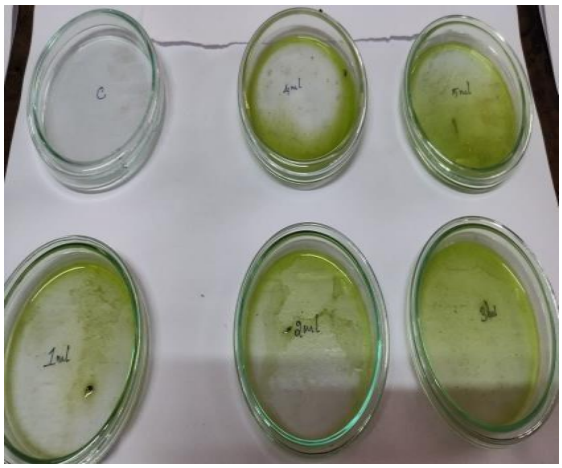


Table 8, Larvicidal activity of *Ulva fasciata* with four different extracts

Seaweed extracts	LC50 (mg/L)	95% CL (LB-UB)	LC90 (mg/L)	95% CL (LB-UB)	Regression Line	Chi square	
AQUEOUS EXTRACT	**1756.01955**	**687.86830-4482.84167**	**37278.74079**	**14602.83512-95166.76067**	**Y= 1.866+0.966 X**	0.247	0.970
ACETONE EXTRACT	**47.1018**	**25.23506 - 87.91671**	**9.39872**	**5.03541-17.54293**	**Y=8.202-1.952 X**	0.681	0.878
BENZENE EXTRACT	**61.65189**	**37.05259 - 102.58272**	**17.15388**	**10.30943-28.54239**	**Y=9.129-2.307 X**	0.140	0.987
METHANOL EXTRACT	**54.01759**	**32.86460 - 88.78550**	**25.86894**	**15.73881-42.51925**	**Y=11.944 -4.008X**	0.668	0.881

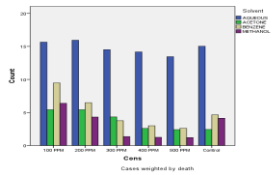


Figure 4, Larvicidal activity of Ulva lactuca extracts

CONCLUSION

In the present study, preliminary phytochemical analysis concluded that due to the presence of extra phytochemicals in *Ulva lactuca* has efficient larvicidal activity when compared to *Ulva fasciata*. In the DPPH scavenging assay, both the seaweed extracts showed high antioxidant activity. The *Ulva lactuca* samples have more effective antioxidant activity when compared to the Ulva fasciata and the percentage of scavenging was found to be about 83.95% for *Ulva lactuca* and 63.77% for *Ulva fasciata*. The results of antimicrobial activity by the well diffusion assay also clearly expressed that *Ulva lactuca* has a high concentration of active principles when compared to *Ulva fasciata.* The larvicidal activity of hexane, aqueous, chloroform, acetone, and methanol extracts of *U. lactuca* against *Anopheles stephensis* was tested. The methanolic crude extract of U. lactuca shows potential for mosquito control. This extract can be used as biocides or insecticide formulations, but further study is needed to understand its effects on non-target organisms and to evaluate its performance in the field. In that way, the results of the present study offer a viable way for further investigations to find out the active molecule needed to elucidate this activity against a wide range of all stages of mosquito species.

References:

1. Ardita, Nadya Fianny, Lenny Mithasari, Daris Untoro, and Siti Isrina Oktavia Salasia. 2021. “Potential Antimicrobial Properties of the Ulva Lactuca Extract against Methicillin-Resistant Staphylococcus Aureus-Infected Wounds: A Review.” *Veterinary World* 14 (5): 1116–23.

2. Arsianti, Ade. 2023. “ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF

GREEN SEAWEED ULVA LACTUCA TOWARDS MCF-7 BREAST CANCER CELLS." *Jurnal Multidisiplin Indonesia* 2(6). doi: 10.58344/jmi.v2i6.264.

3. Arsianti, Ade Arsianti, Fadilah Fadilah, Yazid Fatmawaty, Lies Kurniatis Wibisono, Suid Kusmardi, Norma Nur Azizah, Rista Putrianingsih, Tutik Murniasih, Abdullah Rasyid, and Ratih Pangestuti. 2016. "Phytochemical Composition and Anticancer Activity of Seaweeds Ulva Lactuca and Eucheuma Cottonii against Breast MCF-7 and Colon HCT-116 Cells." *Asian Journal of Pharmaceutical and Clinical Research* 9(6). doi: 10.22159/ajpcr.2016.v9i6.13798.

4. Babu, M., DP Raja, AA Arockiaraj, and J. Vinnarasi. 2014. "Chemical Constituents and Their Biological Activity of Ulva Lactuca Linn." *Int.J.Pharm Drug Anal* 2(7).

5. El-Mesallamy, Amani M. D., Talaat N. Amer, Sally Z. Mohamed, Yossef M. Ali, and Sahar A. M. Hussein. 2021. "Phytochemical Constituents of Ulva Lactuca and Supplementation to Improve the Nile Tilapia (Oreochromis niloticus) Haemato-Biochemical Status." *Egyptian Journal of Chemistry* 64(5). doi: 10.21608/EJCHEM.2021.60453.3296.

6. Farasat, Massoumeh, Ramazan-Ali Khavari-Nejad, Seyed Mohammad, Bagher Nabavi, and Foroogh Namjooyan. 2014a. "Antioxidant Activity, Total Phenolics and Flavonoid Contents of Some Edible Green Seaweeds from Northern Coasts of the Persian Gulf." Vol. 13.

7. Farasat, Massoumeh, Ramazan-Ali Khavari-Nejad, Seyed Mohammad, Bagher Nabavi, and Foroogh Namjooyan. 2014b. "Antioxidant Activity, Total Phenolics and Flavonoid Contents of Some Edible Green Seaweeds from Northern Coasts of the Persian Gulf." Vol. 13.

8. Gomathi, K., and L. Anna Sheba. 2018. "Phytochemical Screening and Heavy Metal Analysis of Ulva Reticulata." *Asian Journal of Pharmaceutical and Clinical Research* 11(4). doi: 10.22159/ajpcr.2018.v11i4.23012.

9. Kamaraj, C., Bagavan, A., Elango, G., Zahir, A. A., Rajakumar, G., Marimuthu, S., ... & Rahuman, A. A. (2011). "Larvicidal activity of medicinal plant extracts against Anopheles stephensis subpictus & Culex tritaeniorhynchus." *Indian Journal of Medical Research*, 134(1), 101-106.

10. Kulkarni, Seema A., Sabari B. B. Krishnan, Bavya Chandrasekhar, Kaushani Banerjee, Honglae Sohn, and Thirumurthy Madhavan. 2021. "Characterization of Phytochemicals in Ulva intestinalis L. and Their Action Against SARS-CoV-2 Spike Glycoprotein Receptor-Binding Domain." *Frontiers in Chemistry* 9. doi: 10.3389/fchem.2021.735768.

11. M.T. Cabrita, C. Vale, and A.P. Rauter. "Halogenated compounds from marine algae," *Marine Drugs*, vol. 8, no. 8, pp. 2301–2317, 2010.

12. Manilal, A., N. Thajuddin, J. Selvin, A. Idhayadhulla, R. S. Kumar, and S. Sujith. "In vitro mosquito larvicidal activity of marine algae against the human vectors, Culex quinquefasciatus (say) and Aedes aegypti (Linnaeus) (diptera: Culicidae)," *International Journal of Zoological Research*, vol. 7, no. 3, pp. 272–278, 2011.

13. Newman, D. J., & Cragg, G. M. (2020). "Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019." *Journal of Natural Products*, 83(3), 770-803.

14. P.A. Miyasato, T. Kawano, J.C. Freitas, R.G.S. Berlinck, E. Nakano, and L. F. Tallarico. "Molluscicidal activity of some marine substances against the snail Biomphalaria glabrata (Mollusca, Planorbidae)," *Parasitology Research*, vol. 110, pp. 1873–1879, 2012.

15. Praba, N., and Dr. S. Sumaya. 2022. "Study On Phytochemical and Antioxidant Properties of Padina Gymnospora and Ulva Lactuca." *International Journal of Life Science and Pharma Research*. doi: 10.22376/ijpbs/lpr.2022.12.6.l155-160.

16. Putra, Nicky Rahmana, Sofa Fajriah, Lailatul Qomariyah, Ariyanti S. Dewi, Dwila Nur Rizkiyah, Irianto Irianto, Devi Rusmin, Melati Melati, Ni Wayan Trisnawati, Ireng Darwati, and Nyoman Ngurah Arya. 2024. "Exploring the Potential of Ulva Lactuca: Emerging Extraction Methods, Bioactive Compounds, and Health Applications – A Perspective Review." *South African Journal of Chemical Engineering* 47: 233–45.

17. Rajan, K., Archana, A., & Anusuya, S. (2021). "Larvicidal potential of certain plant extracts of the family Lamiaceae against Anopheles stephensis subpictus (Diptera: Culicidae)."

18. Santos, Sonia A. O., Carla Vilela, Carmen S. R. Freire, Maria H. Abreu, Silvia M. Rocha, and Armando J. D. Silvestre. 2015. "Chlorophyta and Rhodophyta Macroalgae: A Source of Health Promoting Phytochemicals." *Food Chemistry* 183. doi 10.1016/j.foodchem.2015.03.006.

19. T., Rajasekar, Mary Shamya A., and Jerrine Joseph. 2019. "SCREENING OF PHYTOCHEMICAL, ANTIOXIDANT ACTIVITY AND ANTI-BACTERIAL ACTIVITY OF MARINE SEAWEEDS." *International Journal of Pharmacy and Pharmaceutical Sciences* 11(1). doi: 10.22159/ijpps.2019v11i1.29119.

20. Uddin, S. Aftab, S. Akter, S. Hossen, and MA Rahman. 2020. "Antioxidant, Antibacterial and Cytotoxic Activity of Caulerpa racemosa (Forsskål) J. Agardh and Ulva (Enteromorpha) Intestinalis L." *Bangladesh Journal of Scientific and Industrial Research* 55(4). doi: 10.3329/bjsir.v55i4.50959.

21. Zaatout, Hala, Doaa Ghareeb, Alshimaa Abd-Elgwad, and Amany Ismael. 2019. "Phytochemical, Antioxidant, and Anti-Inflammatory Screening of the Egyptian Ulva Lactuca Methanolic Extract." *Records of Pharmaceutical and Biomedical Sciences* 0(0). doi: 10.21608/rpbs.2019.12251.