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# Bioactive Component Analysis and Antioxidant Activity of Various Solvent Extracts of Green Algae *Ulva lactuca*

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## Abstract

In recent years, the quest for bioactive molecules to create novel medicines and healthful meals has focused on marine resources. Particularly significant and economically beneficial as a source of food, fodder, soil conditioners, and medications are seaweeds. Thus, this investigation aims to assess bioactive components and antioxidant property of various extracts of green algae *Ulva lactuca,* collected from coastal region of Rasthacad, Kanyakumari district, Tamil Nadu, India. Soxhlet extracts of *Ulva lactuca* were prepared by using different slovents like aqueous, methanol and chloroform. All the extracts were analysed by GC-MS and 15 varieties of bioactive metabolites were chemically characterized. Methanolic and aqueous extracts of *Ulva lactuca* showed maximum scavenging property in all the tested assays (DPPH, FRAP and $H_2O_2$) in relation to control, Ascorbic acid. *Ulva lactuca*'s methanolic extract had the greatest levels of antioxidant and free radical scavenging activity compared to the extracts of aqueous and chloroform. The *Ulva lactuca* contains significant bioactive chemicals, according to GC-MS, which may have anti-cancerous potential.

***Key words*:** Antioxidant, DPPH, GC-MS, Sea weeds/Marine algae, Soxhlet extraction

In the field of life science, seaweeds are frequently employed as a source of chemicals with various structural forms, biological activity, and potential as novel antioxidant sources [1]. Hence it was studied as possible biocide and pharmacological agents [2]. Seaweeds' phytochemical constitution varies depending on their species, habitats, age, and environmental factors. There are number of reports from all over the world regarding the medicinal significance of seaweeds belonging to Rhodophyceae (red algae), Phaeophyceae (brown algae) and Chlorophyceae (green algae) [3-6].

With more than 7000 species that can thrive in a range of habitats, green algae are the most varied category of algae. The green macro marine algae are found in all beaches. It may thrive in regions where rivers meet the sea and can endure low salinity. Some of the common green seaweeds are *U. lactuca* (Sea grapes) causes "green tides" in the sea, *Caulerpa racemosa* and *Ulva intestinalis* etc., found abundantly along Indian coast line [7]. The green algae *U. lactuca*, which has a fruity flavour and a variety of chemical qualities, has a significant amount of potential as a commercial product. It is also rich in dietry fiber [2]. *Ulva lactuca* has strong antioxidant potential, and the phytochemicals they contain can be utilised as a natural antioxidant source and agents that scavenge free radicals. Many marine algae were said to have antioxidant qualities [8]. Inhibiting and scavenging free radicals is a critical function of antioxidants, which protects against infection and degenerative disorders [9-11]. The suppression of chain initiation, reductive capacity, binding of catalysts for transition metal ions, and radical scavenging are only a few of the processes and mechanisms associated with antioxidant activities [12]. The synthetic antioxidants butylate hydroxyl anisol (BHA) and butylated hydroxyl toluene (BHT) is frequently employed in foods that contain lipids [13]. Natural anti-oxidants like a phenols, tocopherol, and beta-carotene, which are discovered in *U. lactuca* and utilised to prevent lipid peroxidation in the food sector. In addition, they can protect the body from free radicals and delay the onset of many chronic illnesses by preventing radical - damage linked to ageing [14].

There is presently rising interest in natural antioxidants because of the toxicity and safety concerns with synthetic antioxidants [15]. Biomolecules including nucleic acids, proteins, lipids, and other biological components can become damaged by ROS (reactive oxygen species). This is associated with various chronic diseases [16]. Antioxidant substances are frequently utilised to reduce oxidative damage as a result of these factors. Consequently, it is crucial to create and use efficient natural antioxidant molecules. Hence it is being planned to find out the chemical profiles and antioxidant potential of aqueous, chloroform and methanol extracts of the

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Green algal species *U. lactuca* using GC-MS followed by DPPH radical scavenging activity, Ferric reducing antioxidant assay and $H_2O_2$ radical scavenging activity.

## MATERIALS AND METHODS

*Seaweed collection and identification*

Sample of seaweed was obtained from the coastal area of Rastacad, Kanyakumari district, Tamil Nadu, India and identified as *Ulva lactuca* by experts. Collected algae were cleaned with sea water to eliminate epiphytes, and necrotic parts. Using sterile water, the cleaned algae were washed to get rid of any related dirt. The recently cleaned algae was ground into a fine powder and dried in the shade.

*Seaweed extracts preparation*

Ground, fine powder of *U. lactuca* was extracted with various solvents like aqueous, chloroform, and methanol. It is used to extract the phytochemical constituents present in the selected seaweed, and GC-MS was used to detect the Bioactive Components.

*GC-MS (Gas Chromatography-Mass Spectrometry) analysis*

A Shimadzu GCMS-QP2010 gas chromatograph mass spectrometer was utilized to analyze the material, coupled with a Rtx-5 fused silica capillary column, a Turbo Mass quadrupole mass spectrometer, and (30 X 0.25 mm, with 1 Cm film thickness). The oven's heat was set to rise from 100°C to 320°C at a rate of 100°C per minute, with a 10-minutes pause. The carrier gas, helium, was employed at a rate of 1.0 mL/minute. The injector temperature was 250°C, the split ratio was 1:10, and the injection volume was 1 L neat. The mass spectra were obtained at 70eV with a mass scan range of 40-700 amu, while maintaining the interface and the MS ion source, respectively, at 320°C and 200°C (atomic mass unit). The GCMS solution software was used to handle the data.

*Estimating antioxidant activity*

*DPPH radical scavenging assay*

According to the accepted approach, the 2,2′-diphenyl-1-picrylhydrazyl (DPPH) test was used to assess the fractions' *in vitro* free radical scavenging activity [17]. 24 mg of DPPH was combined with 100 ml of ethanol to create the stock solution, which was then kept at 20°C until needed. A 3 ml aliquot of the working solution, which was made by diluting the DPPH solution with ethanol were added to 1 ml of test solution at concentrations between 100 and 300 µg/ml. After thoroughly shaking, the reaction solution was allowed to sit at room temperature for 15 minutes in the dark. Next, the absorbance at 517 nm was measured. The control was created without any samples, and the amount of DPPH radicals that were scavenged was used to assess the scavenging activity.

*Hydrogen peroxide-scavenging assay*

In phosphate buffer, a hydrogen peroxide solution (2 mmol/l) was made (pH 7.4). Extract was added to a hydrogen peroxide solution at varied doses (0.6 ml). For background subtraction, a distinct blank sample was utilized for every concentration. The absorbance of hydrogen peroxide at 230 nm after 10 minutes was measured using the UV visible spectrometer Shimadzu, UV-1800, Japan, in comparison to a blank solution made up of phosphate buffer without hydrogen peroxide [18].

*Ferric reducing antioxidant potential (FRAP) assay*

To evaluate the ability of plant extracts to reduce ferric iron, a modified version of the FRAP test was utilized. This method depends on the transformation of an indigestible ferric complex ($Fe^{3+}$ -tripyridyltriazine) into a blue ferrous complex ($Fe^{2+}$ tripyridyltriazine) at low pH, which is brought on by the action of antioxidants that give electrons. The decrease in absorbance at 593 nm is used to gauge the reduction. Every day, 10 volumes of 300 mM acetate buffer, pH 3.6, were combined with 1 volume of 10 mM TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) in 40 mM hydrochloric acid, and 1 volume of 20 mM ferric chloride to create the functioning FRAP reagent. $FeSO_4 + 7H_2O$ were used in various concentrations to create a standard curve. On the day of the preparation, all solutions were applied. 300 µL of deionized water and 100 µL of sample solutions were each added to 3 mL of freshly made FRAP solution. A water bath was used to heat the reaction mixture for 30 minutes at 37 °C. The absorbance of the samples was then measured at 593 nm. Acetate buffer was also used to take a sample blank reading [19]. The FRAP value was determined using the difference between sample absorbance and blank absorbance. Using the reaction signal provided by a $Fe^{2+}$ solution, the reducing capacity of the plant extracts under test was determined in this experiment. Values for FRAP were given as mmol $Fe^{2+}$/g of material. Every measurement was made three times.

*Antibacterial activity*

Chandigarh's Microbial Type Culture Collection and Gene Bank (MTCC) provided the *Klebsiella pneumoniae*, *Bacillus subtilis*, *E. coli*, *Lactobacillus* sp, *Staphylococcus aureus*, *Enterococcus* sp, and *Pseudomonas aeruginosa* strains. Nutrient Agar was used to sustain the bacterial strains (NA).

Pure cultures were transferred from the plate to nutrient agar plates and sub-cultured for 24 hours at 37°C. The fresh culture was aseptically added to 2 ml of a sterile 0.145 mol/L saline tube to create a 1.5108 cfu/ml bacterial suspension. Next, the cell density was modified to meet 0.5 McFarland turbidity criteria. Antimicrobial test employed standardised inoculum.

The medium was made by dissolving 38 g of Mueller-Hinton Agar Medium (Hi Media) in 1 L of distilled water. The dissolved media was autoclaved for 15 minutes at 121°C and 15 Lbs of pressure (pH 7.3). After cooling and being thoroughly mixed, the autoclaved medium was added to Petri plates (25 ml each plate). After swabbing the plates with a bacterial pathogen such as *S. aureus*, *Lactobacillus* sp., *Enterococcus* sp., *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *B. subtilis*. Afterwards, Mueller-Hinton Agar media with the Sample loaded Disc on top was applied, the plates were incubated for 24 hours at 37°C. Following incubation, the inhibition zones around the disc were examined and measured in millimetres using a transparent ruler. The disc included; the zone of inhibition's size was expressed in millimetres. It was believed that the absence of zone inhibition meant there was no activity [20-21]. Activities are categorised as resistant when the zone of inhibition is much less than 7 mm, intermediate (8–10 mm), and sensitive (more than 11 mm).

## RESULTS AND DISCUSSION

*Screening of phytochemical constituents*

There were phytochemicals present in aqueous extract *of U. lactuca*, including Flavonoid, Tannin, Terpenoids, Glycoside, Fat and Fixed oils. The Methanol extract of *Ulva lactuca* showed the presence of phytochemicals like Flavonoid, Tannin, Saponin, Terpenoids, Glycoside, and Fat and Fixed oils. The Chloroform extract of *U. lactuca* revealed the presence of phyto compounds like Terpenoids, Steroids,

Glycoside, Fat and Fixed oils and the results are presented in Tables 1-3. Among the phytochemicals, Terpenoids, Glycoside, Fat and Fixed oils were present in all the extracts. Carbohydrate, Alkaloid and Phenol were absent in all the extracts of *U. lactuca*. *U. lactuca* extracts in aqueous and methanol both contained tannin and flavonoids. Both aqueous and chloroform extracts of *U. lactuca* contained steroids. Saponins were present only in the Methanolic extract of *U. lactuca*. The possibility of using flavonoids as natural antioxidants and antimicrobials makes their existence in the aqueous and methanolic extracts attractive. There have been several studies indicating the existence of flavonoids in marine algae, and some of them have had their biological function examined. Saponins' antifungal, antiviral, and antibacterial properties are well known [22].

Table 1 Screening of phytochemicals of aqueous, chloroform and methanol extracts of *Ulva lactuca*

| Phytochemical qualitative tests | Aqueous | Methanol | Chloroform |
|---|---|---|---|
| Alkaloid | - | - | - |
| Flavonoid | + | + | - |
| Tannin | + | + | - |
| Phenol | - | - | - |
| Saponin | - | + | - |
| Terpenoids | + | + | + |
| Carbohydrate | - | - | - |
| Glycoside | + | + | + |
| Fat and fixed oils | + | + | + |
| Steroids | + | - | + |

+ Presence., - Absence

*GCMS analysis*

The relative concentrations of different compound eluting as a function of retention time were displayed on the gas chromatogram. The peak heights reveal the relative concentrations of the several plant-present components. The composition and structure of the compounds are determined by the mass spectrometer's analysis of the chemicals eluted at various periods. The big component breaks down into smaller compounds, causing peaks to form at various m/z ratios [23-26]. In addition to their retention duration, molecular weight, molecular formula, percentage peak area, and configuration, the detected compounds' chemical profiles are provided in (Table 1-3).

The studies of the biocompounds in aqueous, chloroform and methanol extracts of *U. lactuca* by GC-MS analysis revealed the presence of 15 compounds, like 1. Naphthelene, 2. Azulene, 3.1 H-Indole, 5- methyl-2- phenyl-, 4. 1,4-Benzenediol, 2,5 – bis (1,1- dim…5. 2-(Acetoxymetnyl)- 3-methoxycarb…6. Phenol, 2,4, bis (1,1- dimethylethyl), 7. Phenol, 3,5, bis (1,1- dimethylethyl), 8. 1,2- Benzisothiazol-3-amine tbdms, 9. Cyclotrisiloxane, hexamethyl-, 10.1 H-Indole,1-methyl-2-phenyl-, 11.5-methyl-2-phenylindilizine, 12. Benzo(h)quinolone, 2,4-dimethyl-,13. Acetamide, N-(4-(trimethylsilyl)…, 14.Silane,trimethyl (5-Amethyl-2-(1-..,), 15. 1,1,1,3,5,5 Heptamethyltrisilo, and the GC-MS Spectrum confirmed the existence of 15 main peaks of various compounds with retention times of 3.600, 3.600, 15.031, 15.126, 15.163, 6.918, 6.918, 16.733, 16.733, 17.149, 17.149, 17.149, 17.253, 17.253, 17.253 minute respectively (Fig 1-3). Abbassy *et al.* [27] confirm that Methanolic extract of *U. lactuca* possess 42 peaks and the five main peaks confirmed the presence of components like 1,2-benzene dicarbolicacid, bis (2-ehthylhexyl) ester, benzene,1,2,4-trimethyl, palmitic acid, 8-octadecanoic acid, 1-ethyl -2-methyl and methyl ester and benzene. However, a significant Antioxidant activity was not observed. The GC-MS screening of several *U. lactuca* extracts used in the current investigation revealed a variety of different chemicals. There were 15 primary peaks detected for *U. lactuca* in total. There were 31 major peaks in total for the aqueous *U. lactuca* extracts that were obtained early in the morning from the Red Sea shore of the Haql Tabuk area, Saudi Arabia [28]. Five main peaks were observed in the aqueous extracts of *U. lactuca* collected from Abu Qir Bay near Boughaz ElMaadya [29]. In this study main peak observed for aqueous extracts of *U. lactuca* collected from Rasthakad, beach was one and was identified as Naphthalane. There were 14 major peaks in total for the methanolic *U. lactuca* extracts taken from Al Selsela. Nevertheless, the methanolic *Ulva lactuca* extract from Abu Qir Bay showed 13 peaks [30]. In this study main peak observed for methanolic extracts of *U. lactuca* collected from Rasthakad, beach was Four and was identified as 1. Azulene, 2.1 H-indole, 5- methyl-2-phenyl-, 3. 1,4- Benzenediol, 2,5 – bis(1,1- di. The component was compared with previously isolated compounds. *U. lactuca* chloroform extracts from the Okha coast in Western India contained two primary peaks in total (Dibutylepthalate 18.61 1.45 278.34 1,2 benzendicarboxylic acid) [31].

In this study main peak observed for Chloroform extracts of *U. lactuca* collected from Rasthakad, beach was Ten and was identified as 1. Phenol, 2,2, bis (1,1- dimethylethyl), 2. Phenol, 3,5, bis (1,1- dimethylethyl)-, 3. 1,2- Benzisothiazol-3- amine tbdms, 4. Cyclotrisiloxane, hexamethyl-, 5.1 H- Indole,1-methyl-2-phenyl-,6.5-methyl-2-phenylindilizine, 7. Benzo(h)quinolone,2,4-dimethyl-8.Acetamide,N-(4-(trimethylsilyl)…, 9.Silane,trimethyl (5-Amethyl-2-(1-..,), 10. 1,1,1,3,5,5 Heptamethyltrisilo. Components isolated were differing from the previously studied components. This variation may be because of the variation in environmental conditions, place and time of sample collection. According to the prior study, the majority of the chemicals found in *U. lactuca* have therapeutic qualities [32]. To our knowledge, though, this is the initial report of research about the GC-MS analysis of *U. lactuca* from Rasthakad.

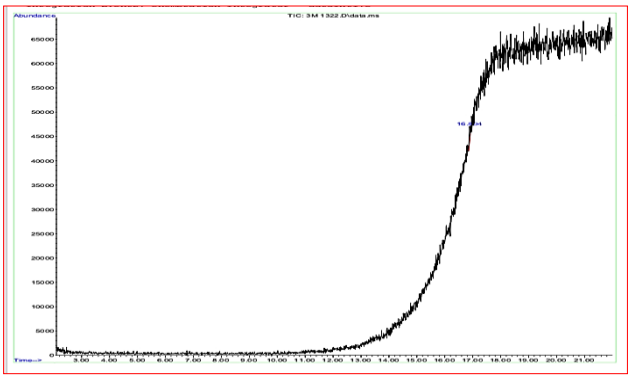


Fig 1 GC-MS analysis of aqueous extract of *Ulva lactuca*

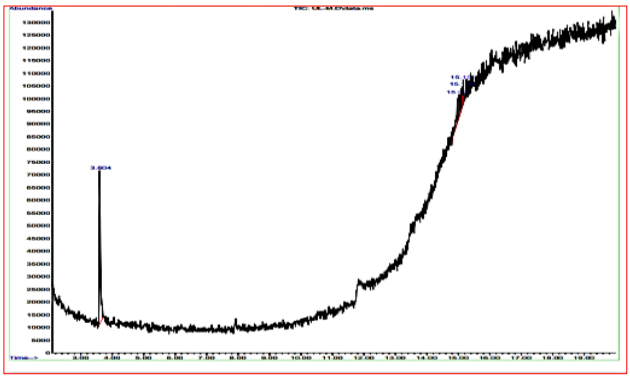


Fig 2 GC-MS analysis of methanol extract of *Ulva lactuca*

Table 2 GCMS analysis of aqueous extract of *Ulva lactuca*

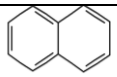
| S. No | Name | Retention time | Molecular formula | Molecular weight | Peak area (%) | Structure |
|---|---|---|---|---|---|---|
| 1 | Naphthalene | 3.600 min | $C_{10}H_8$ | 128.17g/mol | 100.00 |  |

Table 3 GCMS analysis of methanol extract of *Ulva lactuca*

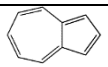
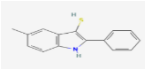
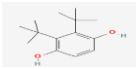
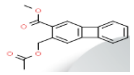
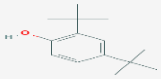
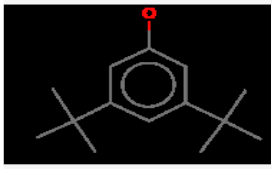
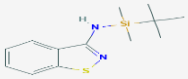
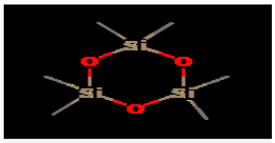
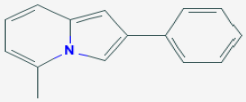
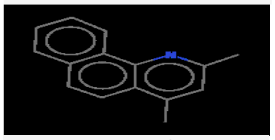
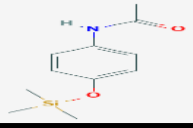
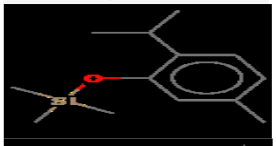
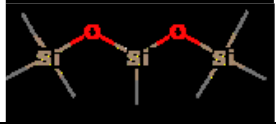
| S. No. | Name | Retention time (min) | Molecular formula | Molecular weight | Peak area (%) | Structure |
|---|---|---|---|---|---|---|
| 1 | Azulene | 3.600 | $C_{10}H_8$ | 222.3987 g/mol | 3.604 |  |
| 2 | 1 H- Indole, 5-methyl-2-phenyl- | 15.031 | $C_{15}H_{13}N$ | 207.27g/mol | 15.031 |  |
| 3 | 1,4-Benzenediol, 2,5-bis (1,1- dim… | 15.126 | $C_{14}H_{22}O_2$ | 222.32 g/mol | 15.130 |  |
| 4 | 2- (Acetoxymethyl)-3- (metthoxycarb… | 15.163 | C17H14O4 | 289.29 g/mol | 15.159 |  |

Table 4 GCMS analysis of chloroform extract of *Ulva lactuca*

| S. No. | Name | Retention time (min) | Molecular formula | Molecular weight | Peak area (%) | Structure |
|---|---|---|---|---|---|---|
| 1 | Phenol, 2,4-bis(1,1-dimethylethyl)- | 6.918 | $C_{14}H_{22}O$ | 206.32 g/mol | 35.01 |  |
| 2. | Phenol, 3,5-bis(1,1-dimethylethyl)- | 6.918 | $C_{14}H_{22}O$ | 206.3239 g/mol | 35.01 |  |
| 3. | 1,2-Benzisothiazol-3-amine tbdms | 16.733 | $C_{13}H_20N_2SSi$ | 264.46 g/mol | 20.85 |  |
| 4. | Cyclotrisiloxane, hexamethyl- | 16.733 | C6H18O3Si3 | 222.4618 g/mol | 20.85 |  |
| 5. | 1H-Indole, 1-methyl-2-phenyl- | 17.149 | $C_{15}H_{13}N$ | 207.27 g/mol | 37.83 |  |
| 6. | 5-Methyl-2-phenylindolizine | 17.149 | $C_{15}H_{13}N$ | 207.27 g/mol | 37.83 |  |
| 7. | Benzo[h]quinoline, 2,4-dimethyl- | 17.149 | $C_{15}H_{13}N$ | 207.2704 g/mol | 37.83 |  |
| 8. | Acetamide, N-[4-(trimethylsilyl)... | 17.253 | $C_{11}H_{17}NO_2Si$ | 223.34 g/mol | 6.31 |  |
| 9. | Silane, trimethyl[5-methyl-2-(1-... | 17.253 | $C_{13}H_{22}OSi$ | 222.3987 g/mol | 6.31 |  |
| 10. | 1,1,1,3,5,5,5-Heptamethyltrisilo | 17.253 | $C_7H_{22}O_2Si3$ | 222.5049 g/mol | 6.31 |  |

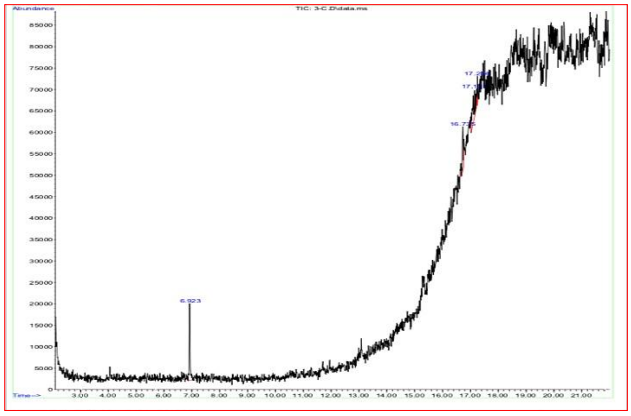


Fig 3 GC-MS analysis of chloroform extract of *Ulva lactuca*

*Anti-oxidant assay*

*Radical scavenging test for DPPH*

*U. lactuca* extracts in aqueous, methanol, and chloroform were shown to have free radical-scavenging action in (Table 4, Fig 4). It showed that three extracts' radical-scavenging abilities improved with concentration, from 100 µg/ml to 300 µg/ml. It showed that the methanolic *U. lactuca* extract had extremely potent antioxidant activity. This extract exhibits the highest Antioxidant activity with a low $IC_{50}$ (56.396). This is significantly low to standard $IC_{50}$ value of Ascorbic acid (456.67). *U. lactuca* aqueous and chloroform extracts were shown to have about two times the antioxidant capacity of the methanolic extract. Environmental conditions significantly impact phytochemical synthesis, which in turn affects antioxidant activity [33]. However, this is the first research to identify the antioxidant characteristics of *U. lactuca* discovered in the Rastacad coastal area. Earlier studies have also shown the potential antioxidant activity of *U. lactuca* [34].

Table 4 DPPH scavenging activity of extracts of *Ulva lactuca*

| Concentration | Aqueous | Methanol | Chloroform | Standard (Ascorbic acid) |
|---|---|---|---|---|
| 100 µg/ml | 32.343 ± 0.012 | 21.806 ± 0.052 | 34.362 ± 0.018 | 28.734 ± 0.007 |
| 200 µg/ml | 34.327 ± 0.05 | 38.210 ± 0.010 | 52.835 ± 0.017 | 34.627 ± 0.002 |
| 300 µg/ml | 36.365 ± 0.020 | 43.422 ± 0.055 | 67.814 ± 0.002 | 40.636 ± 0.040 |
| $IC_{50}$Value | 978.32 | 56.396 | 190.014 | 456.67 |

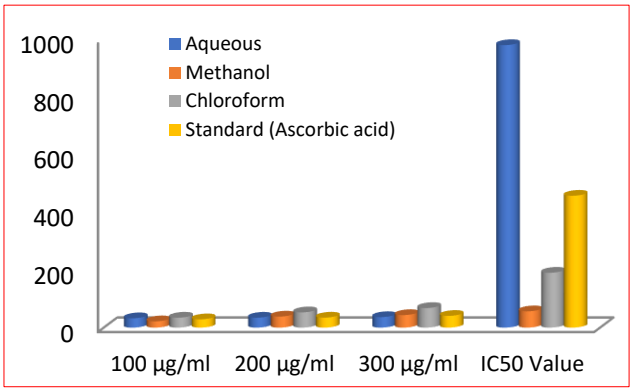



Fig 4 DPPH scavenging activity of extracts of *Ulva lactuca*

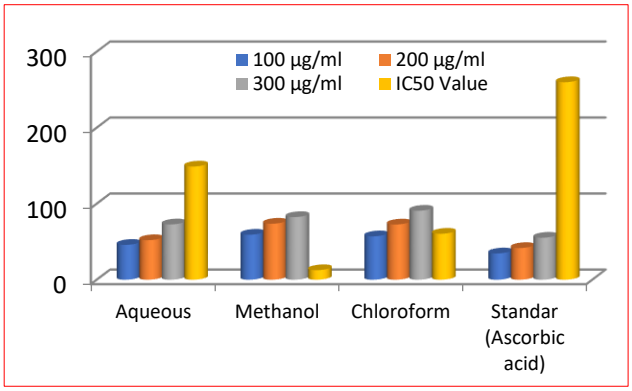



Fig 5 FRAP antioxidant potential of extracts of *Ulva lactuca*

*FRAP radical scavenging assay*

Ferric reducing antioxidant potential activity of Aqueous, Methanol and, Chloroform extracts of *U. lactuca* were given in (Table 5, Fig 5). It showed that three extracts' radical-scavenging abilities improved with concentration, from 100 µg/ml to 300 µg/ml. It showed that, a very strong antioxidant activity was noted in the aqueous extract of *U. lactuca*. This extract exhibits the maximum antioxidant activity with a very low $IC_{50}$ (51.884). This is significantly low to standard $IC_{50}$ value of Ascorbic acid (456.67). *U. lactuca*'s aqueous extract was said to have roughly two times the antioxidant activity of its methanolic and chloroform extracts.

Table 5 FRAP antioxidant potential of extracts of *Ulva lactuca*

| Concentration (µg/ml) | Aqueous | Methanol | Chloroform | Standard (Ascorbic acid) |
|---|---|---|---|---|
| 100 | 20.653 ± 0.017 | 44.921 ± 0.013 | 33.396 ± 0.008 | 7.151 ± 0.021 |
| 200 | 33.154 ± 0.002 | 57.857 ± 0.007 | 39.081 ± 0.016 | 13.099 ± 0.021 |
| 300 | 44.00 ± 0.012 | 69.955 ± 0.003 | 57.093 ± 0.003 | 20.482 ± 0.008 |
| $IC_{50}$Value | 51.884 | 139.461 | 257.476 | 746.436 |

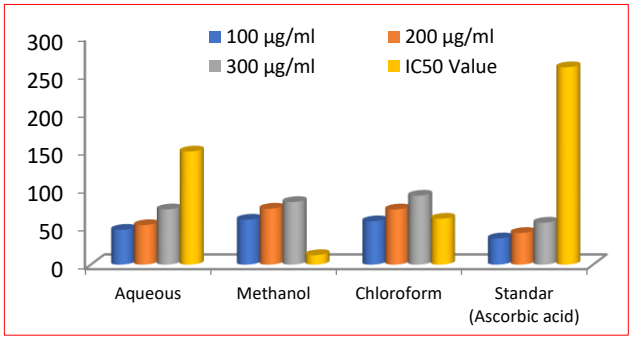



Fig 6 $H_2O_2$ radical scavenging activity of extracts of *Ulva lactuca*

*$H_2O_2$ radical scavenging assay*

Hydrogen peroxide radical scavenging antioxidant action of aqueous, methanol and chloroform extracts of *Ulva lactuca* was given in (Table 6). It showed that three extracts' radical-scavenging abilities improved with concentration, from 100 µg/ml to 300 µg/ml. It demonstrated that the methanolic *Ulva lactuca* extract had extremely potent antioxidant activity. The extract exhibits the highest Antioxidant activity with a very low $IC_{50}$ (12.552). This is significantly low to standard $IC_{50}$ value of ascorbic acid (259.849). *Ulva lactuca's* methanolic extract was shown to have roughly two times the antioxidant activity of its aqueous and chloroform counterparts. The results

of antioxidant activities of various assays (DPPH, FRAP, and $H_2O_2$) showed that, *Ulva lactuca* has higher radical scavenging property (Fig 6).

*Antibacterial activity*

The bactericidal efficacy of crude methanolic and aqueous extracts of *U. lactuca* taken from the Libyan coast was also investigated by Alghazeer *et al.* [35]. Eight pathogenic bacteria such as Gram positive (*B. subtilis*, *S. aureus*, *S. epidermidis*, and Bacillus spp.) and negative (*E. coli*, *P. aeruginosa*, *Klebsiella* spp., and *Salmonella typhi*) bacteria, were examined for the crude extract's antibacterial properties. However, in other instances, methanolic extract was shown to be more effective against the pathogenic bacteria. There are now greater indications that it is possible to create natural compounds with antibacterial properties that can aid in the creation of highly desirable medicines. *U. lactuca* extract underwent yet another in vitro examination against a variety of Gram positive and Gram-negative bacteria as well as a fungus [16]. Ethyl-ether was used to extract bioactive components, and wide spectrum activity was seen. Particularly against methicillin-resistant *S. aureus*, a greater activity was seen.

Table 6 $H_2O_2$ radical scavenging activity of extracts of *Ulva lactuca*

| Concentration | Aqueous | Methanol | Chloroform | Standard (Ascorbic acid) |
|---|---|---|---|---|
| 100 µg/ml | 45.840 ± 0.008 | 59.190 ± 2.764 | 57.076 ± 0.027 | 34.576 ± 0.167 |
| 200 µg/ml | 52.030 ± 0.222 | 73.679 ± 0.089 | 72.655 ± 0.043 | 41.677 ± 3.099 |
| 300 µg/ml | 72.730 ± 0.255 | 82.405 ± 0.013 | 90.740 ± 0.059 | 55.217 ± 0.136 |
| $IC_{50}$Value | 148.928 | 12.552 | 60.442 | 259.849 |

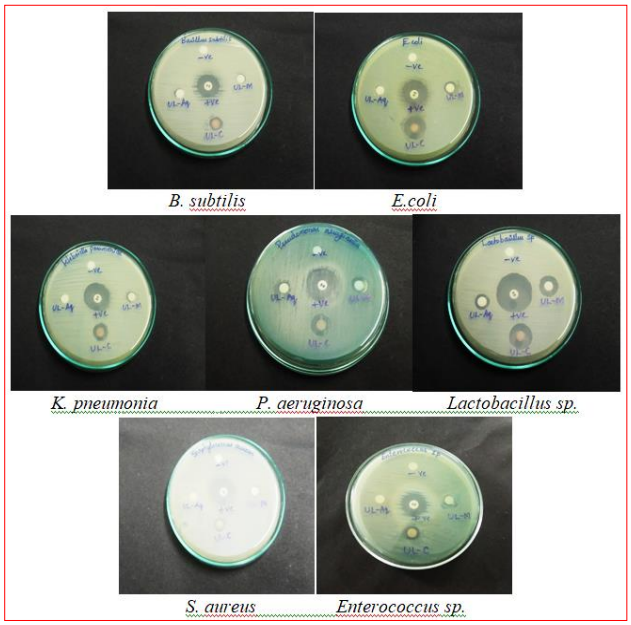



Fig 7 Antibacterial activity of various extracts of *Ulva lactuca*

In these investigations, the antibacterial activity of aqueous extract of *U. lactuca* showed maximum zone of inhibition against the Human Pathogenic bacteria *Lactobacillus* sp (10 mm) followed by *P. aeruginosa* (8 mm), *K. pneumonia* (7 mm), *E. coli* (7 mm), *S. aureus* (7 mm), *B. subtilis* (7 mm), *Enterococcus* sp (NZ). From this analysis, the antibacterial assay of chloroform extract of *U. lactuca* showed highest zone of inhibition against the Human Pathogenic bacteria *E. coli* (16 mm), followed by *Lactobacillus* sp (15 mm), *K. pneumonia* (13 mm), *P. aeruginosa* (14 mm), *S. aureus* (12 mm), *B. subtilis* (10 mm), Enterococcus sp (10 mm). In this investigation, the antibacterial activity of methanol extract of *U. lactuca* exhibited highest zone of inhibition against the bacterial strains such as *E. coli* (12 mm), followed by *P. aeruginosa* (12 mm), *Lactobacillus* sp (12 mm), *S. aureus* (10 mm), *K. pneumonia* (9 mm), *Enterococcus* sp (8 mm) and *B. subtilis* (8 mm).

Aqueous, methanol and chloroform extracts of *U. lactuca* was tested for their antibacterial activity against seven strains of bacterial strains by disc diffusion method (Table 7, Fig 7). *U. lactuca* showed high antibacterial activity with chloroform extract against *E. coli* (16 mm), Lactobacillus sp (15 mm) *P. aeruginosa* (14 mm), *K. pneumonia* (13 mm), *S. aureus* (12 mm), *B. subtilis* (10mm), Enterococcus sp (10 mm), with methanol extract of *Ulva lactuca* against *E. coli* (12 mm), followed by *Lactobacillus* sp (12 mm) *P. aeruginosa* (12 mm), *S. aureus* (10 mm), and with aqueous extract of *U. lactuca* against *Lactobacillus* sp (10 mm) (Fig 7). Meanwhile, aqueous extract of *Ulva lactuca* had no effect on *Enterococcus* sp. Otherwise, moderate to low activity was observed for aqueous and methanol extract of *Ulva lactuca* against the human pathogenic bacterial strains.

Table 7 Antibacterial activities of three different extracts of *Ulva lactuca* bacteria represented by inhibition zone diameter (mm)

| Organisms | Aqueous (mm) | Chloroform (mm) | Methanol (mm) | +ve (mm) control | -ve control (mm) |
|---|---|---|---|---|---|
| *Klebsiella pneumoniae* | 7 | 13 | 9 | 22 | NZ |
| *Staphylococcus aureus* | 7 | 12 | 10 | 21 | NZ |
| *E. coli* | 7 | 16 | 12 | 19 | NZ |
| *Bacillus subtilis* | 7 | 10 | 8 | 18 | NZ |
| *Lactobacillus sp* | 10 | 15 | 12 | 25 | NZ |
| *Enterococcus sp* | NZ | 10 | 8 | 20 | NZ |
| *Pseudomonas aeruginosa* | 8 | 14 | 12 | 22 | NZ |

NZ = No Zone of Inhibition

## CONCLUSION

The information provided by the current discoveries on the seaweed *Ulva lactuca's* antioxidant capacity, nutritional value, and therapeutic action will be useful for the creation of seaweed-based foods and supplements for the food and pharmaceutical sectors. Seaweeds may be used to treat health issues brought on by protein, mineral, and carbohydrate shortages in food and medication. It is crucial to promote the use of seaweeds in commercial goods as a natural source of antioxidants since the bioactive chemicals isolated from seaweeds have several therapeutic benefits (antitumor, anticancer, antithrombin, etc.).

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