Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 27(5): 485 – 507 (2023)
www.ejabf.journals.ekb.eg

Evaluation of antioxidant and cytotoxic potential of some selected seaweeds: An *in vitro* study

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ARTICLE INFO

Article History:
Received: Sept. 6, 2023
Accepted: Sept. 28, 2023
Online: Oct. 1, 2023

Keywords:
Marine macroalgae,
Antioxidant,
Cytotoxicity,
Gas Chromatography/
Mass Spectroscopy.

ABSTRACT

In this study, four seaweed extracts, *Ulva fasciata*, *Ulva compressa* (Chlorophyta), *Amphiroa rigida* (Rhodophyta), and *Sargassum lacerifolium* (Phaeophyta) were evaluated for their antioxidant activity. The cytotoxic effects of the seaweed extracts on three different cell lines were also assessed. Using the DPPH (2, 2- diphenyl-1-picrylhydrazyl) scavenging assay, the ethanolic extract of *S. lacerifolium* had the maximum antioxidant activity (60.17±0.4 %) at 50 µg/ml. Similarly, *S. lacerifolium* ethanolic extract had the highest ABTS (2,2–azinobis (3-ethylbenzthiazoline-6-sulfonic acid) radical scavenging activity (61.4±0.14 %), at 60 µg/ml. While total antioxidant capacity of *S. lacerifolium* extract recorded(40.19±0.27mg ascorbic acid/gram dry weight (mgAA/gdw). MTT assay for cytotoxicity, *in vitro* was carried out for the highest antioxidant seaweed *S. lacerifolium* ethanolic extract, which showed weak cytotoxic activity against MCF7 cell line and moderate cytotoxic activity against HepG2 and Hela cell lines. According to gas chromatography/mass spectrometry (GC/MS) data, *S. lacerifolium* ethanolic extract displayed the presence of several distinct chemicals, including phytol (8.579%), heptadecane (3.080%), and 1,2,3,4-tetrahydro-1,4,6-trimethylnaphthalene (7.71%). The potential efficacy of the seaweed extract may be related to a synergistic interaction between their fatty acid, alkaloid, phytol, hydrocarbon, phenolic, and phthalate contents, according to GC/MS analyses. In conclusion, these seaweeds are a good source of natural products that contain antioxidant and cytotoxic compounds for the pharmaceutical industry.

INTRODUCTION

Oxidative damage caused by the release of reactive oxygen species (ROS) during metabolic activities in living organisms **(Santos-Sánchez *et al.*, 2019).** Damage caused by oxidation for biomolecules may be the main causes a majority of chronic diseases, like cancer, atherosclerosis, and ageing caused by reactive oxygen species **(Pirian *et al.*, 2017).** Antioxidants have potential to counter oxidative stress harmful effects **(Larson, 1995; Adwas *et al.*, 2019; Gheda *et al.*, 2023 a and b).** They are substances that delay or prevent oxidation by neutralizing or scavenging free radicals in human cells **(Abo-Shady**

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***et al*., 2023**). Various synthetic antioxidants are employed in commercial products. However, these artificial antioxidants may have negative side effects (**Wijesekara *et al*., 2011**). Antioxidants synthesized butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) were shown in animal models to be toxic and carcinogenic, and must be substituted with antioxidants from nature **(Safer & Al-Nughamish, 1999; Mbah *et al*., 2019)**. The quest to find natural antioxidants to replace these synthetic ones has become a major area of research in modern pharmacy **(Abo-Shady *et al*., 2023)**.

Seaweeds are large visible macroalgae that grow adhering to stones and over the seashore and can be seen in several different marine species. They are classified according to content of pigments, morphology, and anatomy into three types of algae, red algae (Rhodophyceae), brown algae (Phaeophyceae), and green algae (Chlorophyceae). There is a lot of interest in screening beneficial medications derived from marine algae because they contain biomolecules, such as vitamins, polysaccharides, peptides, pigments, lipids, minerals, and proteins. They perform a variety of biological processes, such as antimicrobial (**Gheda *et al*., 2013; Gheda *et al*., 2023a**), anti-inflammatory, antioxidant (**Ismail *et al.,* 2019; Ismail *et al.,* 2020; Gheda *et al*., 2021; Gheda *et al*., 2023b; Abo-Shady *et al*., 2023**) and anticancer activities (**Gheda *et al*., 2018**).

In vitro, seaweeds act as anticancer agents reduce growth of tumour cellsthrough apoptosis. This occurs as a result of cytotoxic cytokine stimulation of expression of genes **(Sznarkowska *et al*., 2017).** The anticancer properties of red algae *Jania rubens* versus colon and breast cancer cell lines is due to their contents of polysaccharides **(Gheda *et al.,* 2018).** *Gracilaria corticata*, a red marine alga, showed antitumour efficacyversus cell lines of leukaemia and breast cancer (**Zandi *et al.,* 2010; Namvar *et al.,* 2014).** Cytotoxicity test using MTT assay showed that *U. lactuca* extract (green seaweed) had strong activity against MCF-7 and Hela cell lines, while *U. fasciata* had strong activity against PC3 and HepG2 cell lines (**Saeed *et al.,* 2020).**

The goal of the present work is to examine the antioxidant effects of the different seaweed extracts, and the study also extends to estimate the cytotoxicity of the highest antioxidant seaweed extracts against three cell lines (mammary gland breast cancer (MCF7), hepatocellular carcinoma (HepG2), and epithelioid carcinoma of cervix (Hela) cell lines. The chemical bioactive compounds of the seaweed extract had the highest antioxidant and cytotoxic activity will be identified using gas chromatography/mass spectrometer (GC/MS).

MATERIALS AND METHODS

Collection of seaweed samples

Seaweed samples were collected in December 2019, from Abu Qir Bay area, Alexandria, Egypt . Only one sample was collected during July 2019 from the coast of Red Sea, Hurghada, Egypt. The collected seaweeds were brought to the laboratory in plastic bags

containing seawater to prevent evaporation. Seaweeds were cleaned from epiphytes and rock debris and given a quick freshwater rinse to remove epiphytes and rock debris and given a quick freshwater rinse to remove surface salts. Some of the collected samples were preserved in 5% formalin in seawater for taxonomical identification. The other portion of the harvested samples was air dried in the shade at room temperature (30°C) on absorbent paper. The dried samples were ground to fine powder in an electrical mill and stored at –20°C until use. The taxonomy of the seaweed samples was carried out according to **Aleem (1993) and Jha *et al.* (2009).** The names of the species were identified according to Guiry and Guiry (2022) by using the algae base website (http://www.Algaebase.Org).

Seaweed extracts preparation

The extraction was carried out by soaking known weight of each seaweed in ethanol solvent for *Sargassum lacerifolium* and methanol solvent for the other seaweeds (1:20 w/v) inside a flask, which was then sealed with cotton cover. The choice of solvents was based on the fact that it was the best solvent that gave an antifungal effect in another experiment (Bedair, 2023). The samples were then kept in shaking incubator (model VS-8480S) at 120 rpm and 30°C for three days. The extracts were filtered and placed the filtrate in an oven at 45°C to remove the solvent. The extracted substance was then soaked in a suitable solvent (ethanol in case of *Sargassum lacerifolium* and methanol in case of other seaweeds tested) to produce a solution with a concentration of 65 mg/ml. The solvent was then evaporated under low pressure to remove it. Keep the extract at – 20°C in a glass bottle with a tight coat for further experiments (**Kaushik and Chanhan, 2008**).

Antioxidant activity of seaweed extracts

DPPH radical scavenging activity

Electron donation potential of the seaweed extracts and their scavenging activities were evaluated by the purple- coloured solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) as stated by **Yen & Chen (1995)** with some modifications. Approximately, 2.0 ml of DPPH solution (0.03 g/l methanol) was mixed with 2.0 ml of the examined seaweed extract different concentrations (10:60 µg/ml), then vortexed the producing reaction mixture for 1 minute, and incubated for 1 hour at room temperature in the dark before detecting absorbance at 517 nm. The following is how the scavenging effect (%) was calculated:

DPPH radical scavenging activity % $= (A_{control} - A_{Sample} / A_{control}) \times 100$

A : Is the absorbance

$ABTS^+$ [2,2’-azinobis-(3-ethylbenzothiazoline-6-sulfonate)]

Test was carried out using the technique described by **Paixo *et al.* (2007).** To get 1, 5, and 10 ppm final concentrations, respectively, 3, 15 and 30 µl of algal extract were mixed with 3 ml of the $ABTS^+$ solution. Ascorbic acid was used as a positive control and $ABTS^+$ solution as a negative control. The absorbance was measured at 415 nm. The formula determined the percentage of inhibition as (% inhibition) as follows:

Scavenging of free radicals % = (Ac – As) /Ac ×100

Ac : Is the absorbance of control.

As : Is the absorbance of the sample.

Total antioxidant capacity (TAC)

Total antioxidant capacity was determined according the method of **Sun *et al.* (2011).** 3 ml of the reagent solution (28 mM sodium phosphate, 4 mM ammonium molybdate and 0.6 M sulfuric acid) was mixed with 0.3 ml of crude extracts. The mixture was kept at 95°C in the dark for 90 minutes. After cooling at room temperature, the absorbance at 695 nm was measured in comparison to (0.3 ml of solvent was used rather than the extract) as a blank. TAC was calculated as milligrams of ascorbic acid equivalents antioxidant capacity per gram of crude extract (mg ascorbic acid equivalents/g crude extract).

Cytotoxicity assay

Hepatocellular carcinoma (HepG-2), mammary gland breast cancer (MCF7), and epithelioid carcinoma of the cervix (Hela) cell lines were obtained from Holding Company for Biological Products and Vaccines (VACSERA), Cairo, Egypt.

MTT assay

To evaluate the inhibition of the extracted compounds on cell proliferation, MTT test was performed on the cell lines listed above **(Klajnert *et al.,* 2006).** Yellow tetrazolium bromide (MTT) is transformed into a purple formazan derivative in intact cells by the mitochondrial enzyme succinate dehydrogenase in this colorimetric assay. Cell lines were cultured in RPMI-1640 medium containing 10% foetal bovine serum. At 37°C in an incubator with 5% CO_2, antibiotics of 100 g/ml streptomycin and 100 units/ml penicillin were introduced. Then cultivated the cell lines in a 96-well plate at a density 1.0×10^4 cells per well and incubated for 48 hours at 37°C with 5% CO_2. After being exposed to various chemical concentrations, the cells were cultured for 24 hours. Approximately, 20 ml of 5 mg/ml MTT solution was added after the medicine had been taken for 24 hours and incubated for four hours. Dimethyl sulfoxide (DMSO, 100 ml) was added to each well in

order to dissolve the formed purple formazan. The test was measured at 570 nm absorbance by usage of a plate reader (EXL 800, USA).

As the calculation (A570 of treated samples/A570 of untreated sample) × 100,

The proportion of relates cell viability was estimated as (A570 of treated samples/A570 of untreated samples) × 100.

*IC_{50} (μg/ml): 1-10 (very strong), 11-20 (strong), 21-50 (moderate), 51-100 (weak) and above 100 (non-cytotoxic).

*DOX (μM): Doxorubicin as standard.

Determination of the active compounds of the highest antioxidant and cytotoxic seaweed extract.

UV spectra

Ultraviolet absorption of seaweed extract was determined using a UV spectrophotometrically (UV 2101/PC) operating in the range 200–900 nm wavelengths.

Fourier Transform infra-red spectra (FTIR)

Seaweed extract fraction was analysed by infra-red spectrophotometer in Central Laboratory of Tanta University using Perkin Elmer 1430 FTIR. A pellet was formed by combining 5 mg of the material in solid phase with 200 mg of FTIR grade KBr. The sample container was instantly filled with the pellet, and FTIR spectra between 400 and 4000 cm^{-1} were measured **(Boeriu *et al*., 2004).**

Gas chromatography-Mass spectrometry (GC-MS) analysis

Seaweed extract was analysed by usage of a GC-MS Perkin Elmer Clarus with model system 580/560 S at these circumstances: ramp 8°C/min to 280°C, hold 4 min, Inj = 280°C, oven: initial temperature 80°C for 6 min, volume = 1 μL, split = 20:1, solvent delay = 5.00 min, carrier gas: He, source temperature = 200°C, transfer temperature = 180°C, scan: 50 to 550 Da, column (Elite-5 MS , 30 m 0.25 mm ID 0.25 um df). In accordance with the customary protocol. using the NIST spectral database library programme to compare retention duration and fragmentation pattern with mass spectra, metabolites in extracts were identified. Peak area normalization was used to express each constituent's value of relative region (as overall percentage of total volatile composition) which was derived direct from total ion current (TIC) **(Sparkman *et al*., 2011).**

Statistical analysis

The results were all expressed as the mean ± standard deviation. In quantitative data, the one-way ANOVA test and Duncan´s test were used by SPSS. V. 19 **(Pipkin,1984).** The significance was calculated at various probability levels at P <0.001.

RESULTS

1. Identification of different seaweeds

As shown in **Fig. 1**, the collected seaweeds were identified as follows: *Ulva fasciata* and *Ulva compressa* (Chlorophyta), *Amphiroa rigida* (Rhodophyta), and *Sargassum lacerifolium* (Phaeophyta).

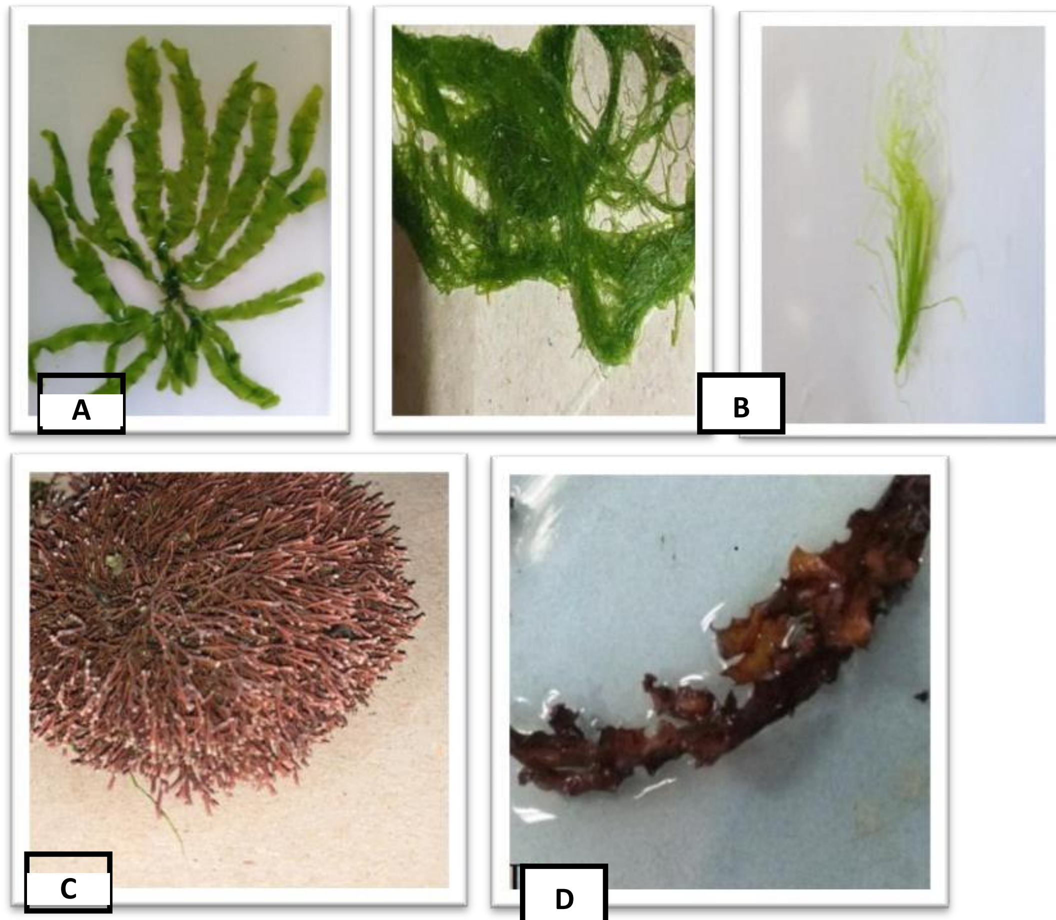
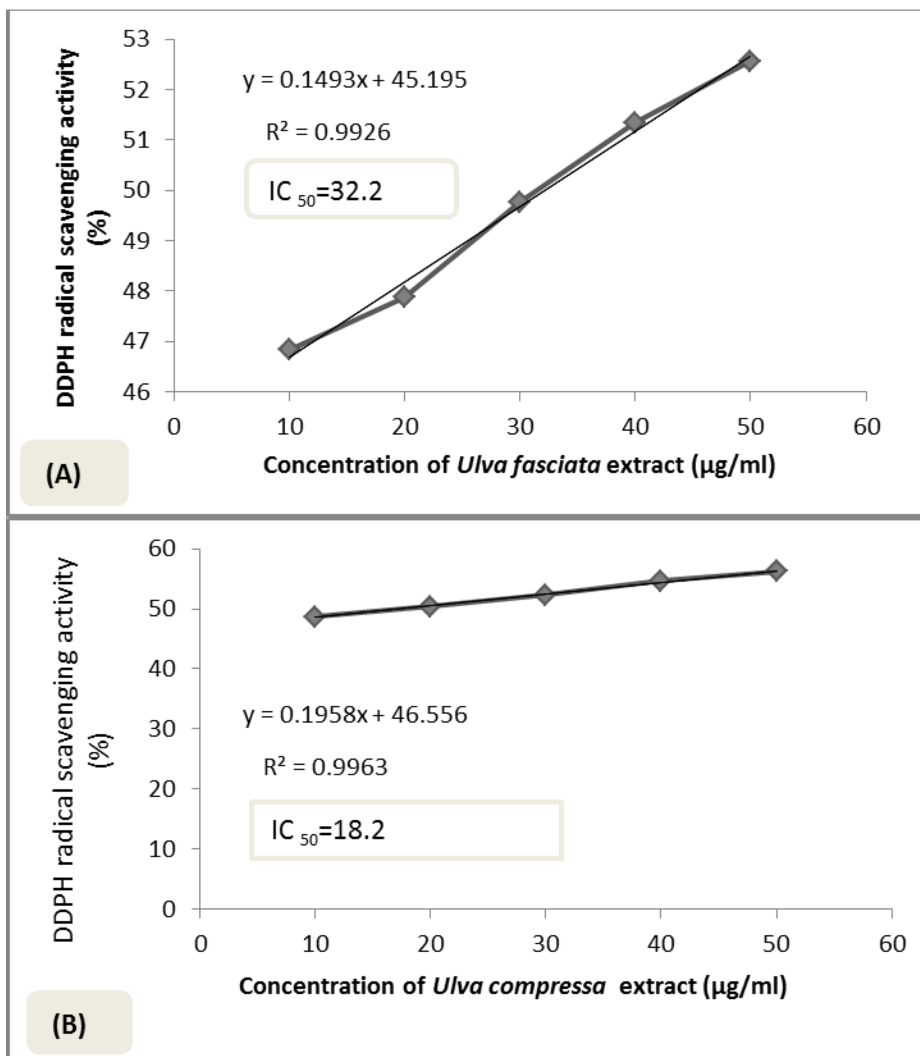


Fig 1: The collected seaweeds; A) *Ulva fasciata*; B) *Ulva compressa*; C) *Amphiroa rigida;* and D) *Sargassum lacerifolium*

2.Antioxidant activity of seaweeds extract

2.1. DDPH radical scavenging activity

Different concentrations of the crude algal extracts showed relatively equally DPPH antioxidant activity compared with the ascorbic acid as standard antioxidant, whose IC_{50} value was 18.4 µg/ml. Results in **Fig. 2** showed the strongest DPPH radical scavenging activity of *S. lacerifolium* ethanolic extract (60.17±0.4%) followed by *U. compressa, A. rigida*, and *U. fasciata* (56.25±0.27, 54.39±0.25, and 52.56±0.26%, respectively). The IC_{50} *S. lacerifolium* of detected as the lowest concentration expressed inhibition percentages greater than 50%, while the IC_{50} of *U. compressa* was 18.1 µg/ml.



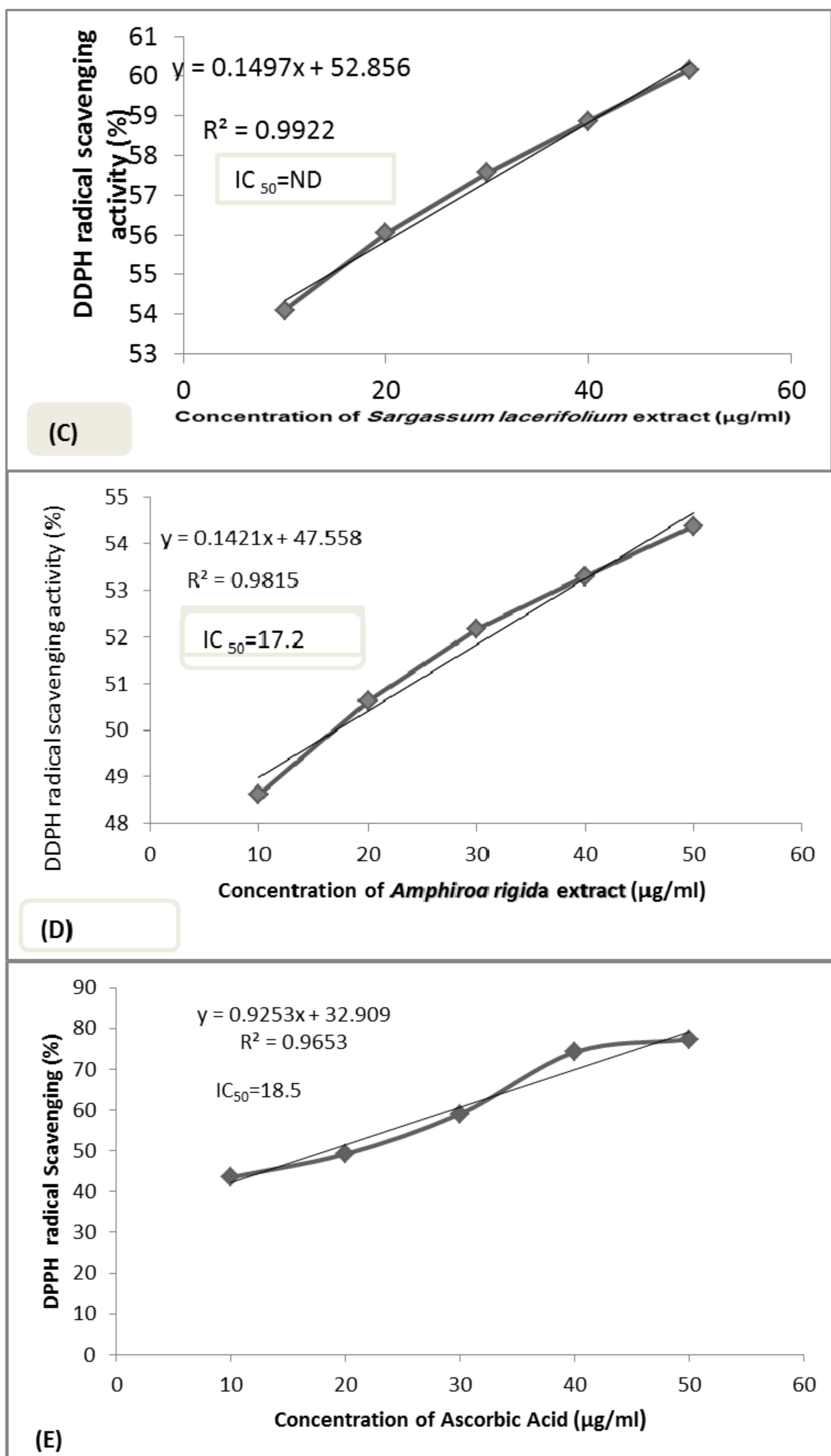
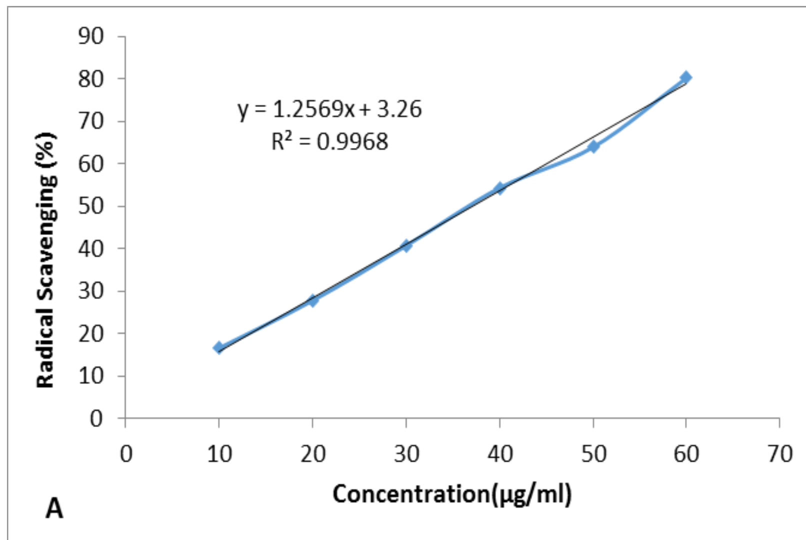
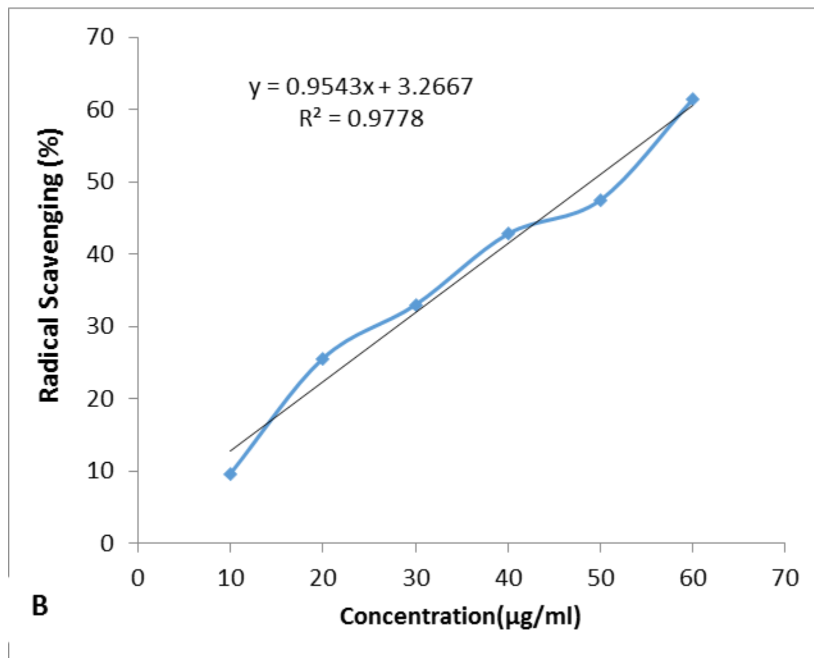


Fig 2: DDPH radical scavenging activity (%) and IC_{50} of different seaweeds; A) *Ulva fasciata*; B) *Ulva compressa*; C) *Sargassum lacerifolium,* D) *Amphiroa rigid*a; E) ascorbic acid.

2.2. Radical scavenging assay ($ABTS^{+}$)

Different concentrations of the crude algal extracts showed relatively equally ABTS antioxidant activity compared with the vitamin C as standard antioxidant **(Fig 3)**, whose IC_{50} value was 30.2 µg/ml. The highest ABTS radical scavenging activity was 61.4±0.14% for *S. lacerifolium* ethanolic extract at 60 µg/ml.





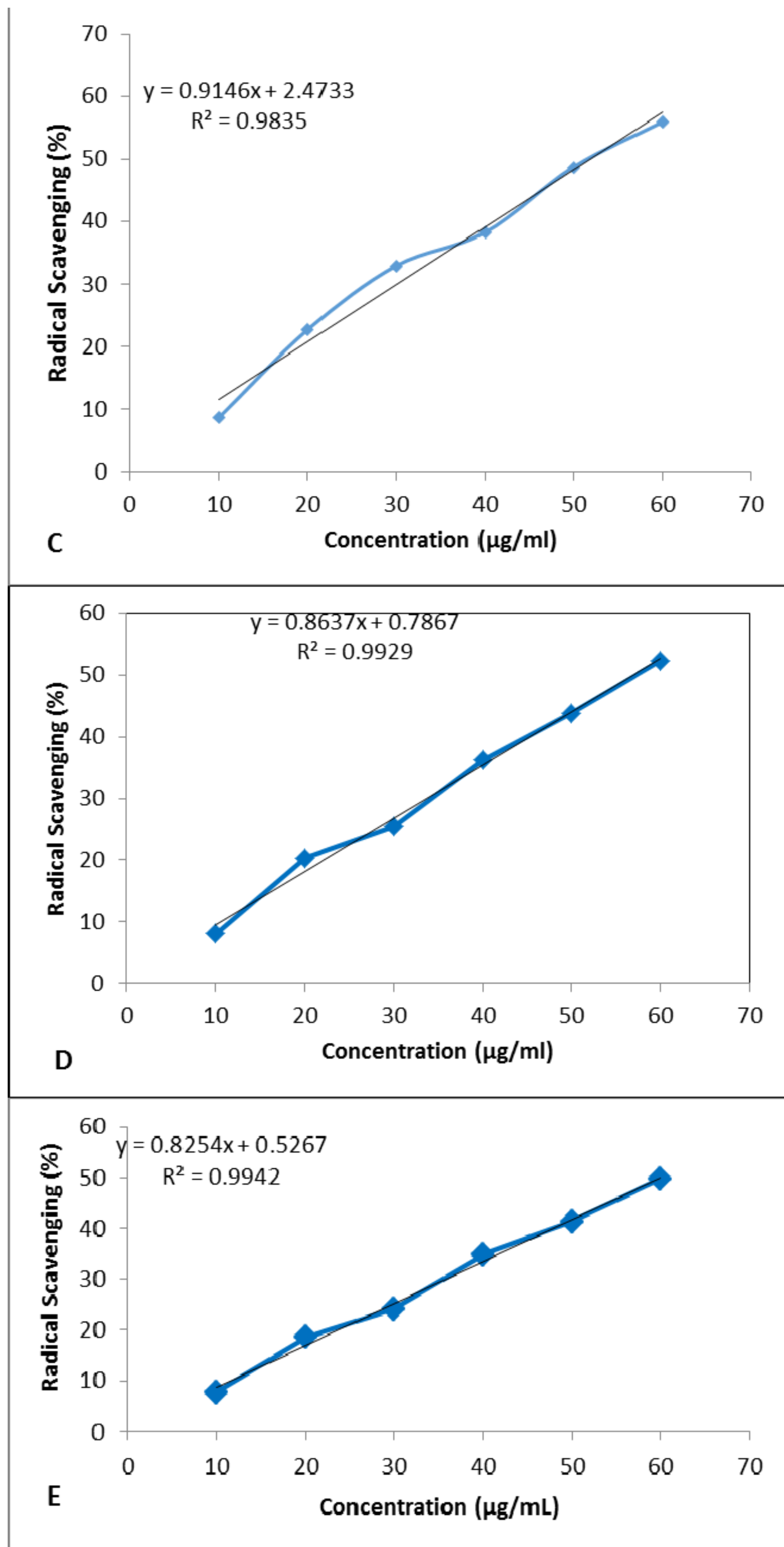


Fig. 3. Radical scavenging assay (ABTS$^+$) of different seaweeds; (A) ascorbic acid*;* B) *Sargassum lacerifolium*, (C) *Ulva fasciata*, (D) *Ulva compressa*; (E) *Amphiroa rigida.*

2.3. Total antioxidant capacity

The present data in **Table 1** demonstrated that *S. lacerifolium* showed the highest levels of antioxidant activity among the tested seaweeds (40.19±0.27mgAA/gDW) followed by *U. compressa* and *U. fasciata*, respectively (32.18±0.23 and 26.07±0.09 mgAA/gDW) while the lowest antioxidant activities was observed in *A. rigida* (25.34±0.16 mgAA/gDW).

Table 1 . Total antioxidant capacity (TAC) of different seaweeds

Tested seaweeds	Total antioxidant capacity (mg ascorbic acid/g dry weight)
Ulva fasciata	**26.07^{c}±0.09**
Ulva compressa	**32.18^{b}±0.23**
Sargassum lacerifolium	**40.19^{a}±0.27**
Amphiroa rigida	**25.34^{d}±0.16**
F Value	**5296.69^{*}**

Each value is mean of three replicates ±standard deviation. For each type of algae: Means within the same column of different letters are*highly Significant at ($p < 0.001$).

3. Cytotoxicity activity

Data recorded in **Table 2** indicated that crude extract of *S. lacerifolium* (the highest antioxidant activity among different seaweeds) possess cytotoxic activity against HePG2, Hela, and MCF7 cell lines. Cell growth inhibition increased by increasing the concentration of *S. lacerifolium* and reached maximum (67.5, 64.9, and 60.3%) in HePG2, Hela, and MCF7, respectively, at concentration 100 µg/ml. However, weak cytotoxic activity of *S. lacerifolium* was shown against the MCF7 cell line (IC_{50} 56.54±3.2 µg/ml). *S. lacerifolium* showed moderate cytotoxic activity against the HePG2 and Hela cell lines (IC_{50} 33.60 ± 2.3 and 47.71±2.8 µg/ml) respectively. The lower IC_{50} indicated the higher cell growth inhibition and cytotoxic activity (**Table 2**) and **(Fig. 4).**

Table. 2. Cytotoxicity (IC_{50}) of *Sargassum lacerifolium* ethanolic extract on different cell lines.

Compound	*In vitro* cytotoxicity IC_{50} (µg/mL)		
	HepG2	Hela	MCF7
DOX	4.50±0.2	5.57±0.4	4.17±0.2
Sargassum lacerifolium	33.60±2.3	47.71±2.8	56.45±3.2

• **IC_{50} (µg/ml)** : 1 – 10 (very strong), 11 – 20 (strong), 21 – 50 (moderate), 51 – 100 (weak), and above 100 (non-cytotoxic).

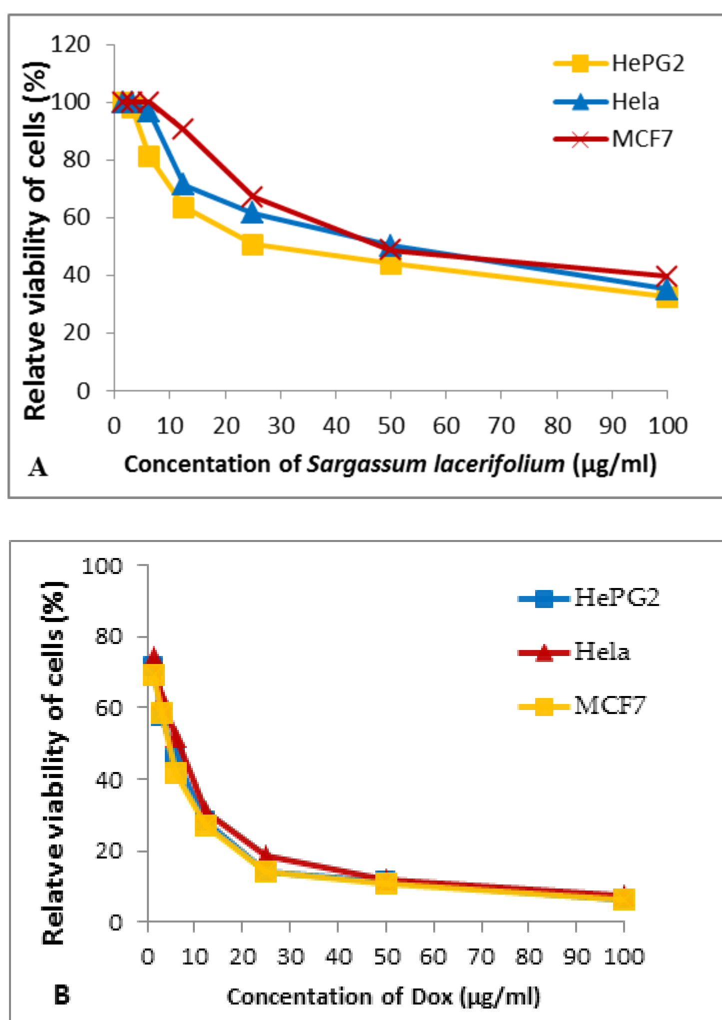


Fig. 4. Cytotoxicity assay (A) *Sargassum lacerifolium* ethanolic extract and (B) Doxorubicin (standard anticancer) on different cell lines by MTT method.

4. Chemical structure of the most effective seaweed extract that had antioxidant activity.

4.1. UV spectra

In contemporary practice, the ultraviolet spectrum of a particular compound is recorded in conjunction with other spectral data such as Fourier transform infrared as an attempt to deduce its molecular structure. Ultraviolet spectra, however, do not furnish prime information as such but tend to act as complimentary or even supplementary evidence to Fournier transform infrared. Before measuring the UV spectrum, the different pigments and impurities were removed by filtration using charcoal. The UV spectrum of *S. lacerifolium* extract were carried out in pure methanol. This spectrum shows absorption peak at 400 nm, and 664 nm indicating the presence of an aromatic compound (**Fig. 5**).

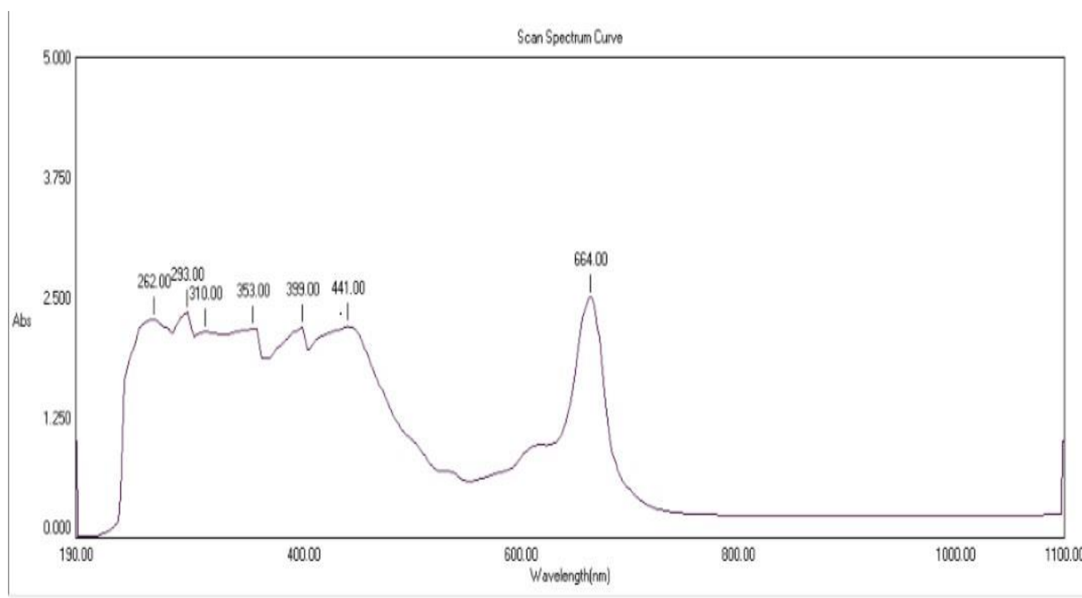


Fig. 5. UV spectrum of the crude ethanolic extract *Sargassum lacerifolium*.

4.2. Fourier transform infra-red spectra (FTIR) spectra analysis

The representative curve is shown in **Figure "6"**. In this region, the OH, CH aromatic and CH aliphatic stretching, vibration bands may appear. The FT-IR spectra reveal a weak band and a shoulder at 2855.94 cm^{-1} and 2924.66 cm^{-1} which can be indicate to the stretching vibrations of the CH aliphatic group and aromatic CH group. A broad band at 3398.50 cm^{-1} corresponding to the stretching vibration of the OH group. The presence of C = O group may be confirmed by the absorption at 1640.46 cm^{-1}. The absorption at 1079.71 and 1038.42 cm^{-1} region due to the stretching vibration of the C = C group.

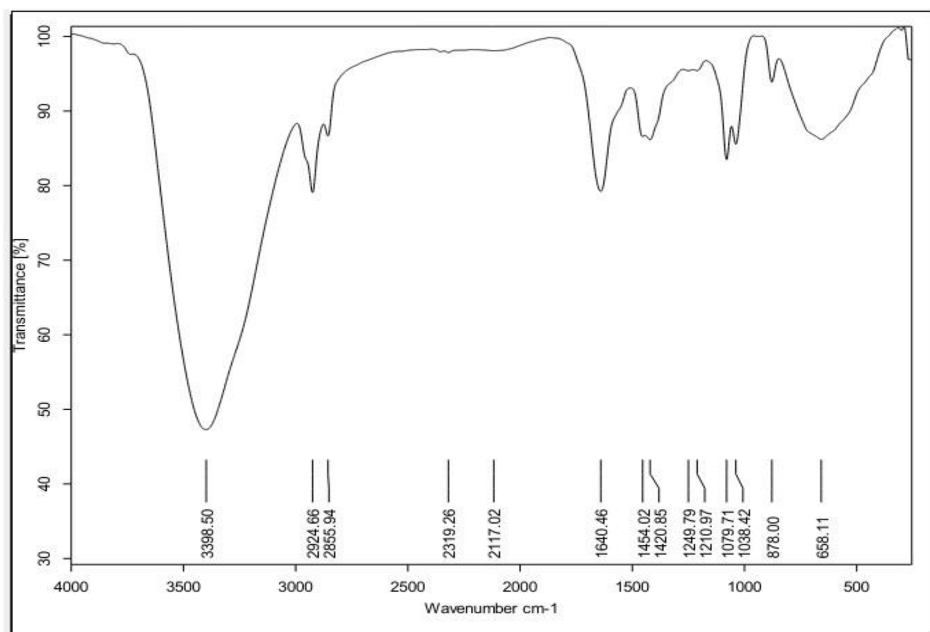


Fig 6. **FT-IR spectrum of the crude ethanolic extract of *Sargassum lacerifolium*.**

4.3. GC-MS analysis of seaweed ethanolic extract

Ethanolic extract of *Sargassum lacerifolium*, as indicated in **Table** 3 **and Fig**. 7 included various important biomolecules of antioxidant, anti-inflammatory, and antitumor potent activity. GC-MS analysis of *S. lacerifolium* ethanolic extract detected the following bioactive component: 3,7,11,15-tetramethyl-2-hexadecen-1-ol (36.822%), phytol (8.579 %), heptadecane (3.080%) naphthalene, 1,2,3,4-tetrahydro-1,4,6-trimethyl (2.81%), acetic acid 2,2'-[oxybis(2,1-ethanediyloxy)]bis- (2.38%), phenol,2,4-bis(1,1-dimethylethyl)- (2.302%), ethanone,1-(3 methylphenyl)- (2.272%), 2-nonenal,(E)- (2.249%), and dianhydromannitol (2.060%).

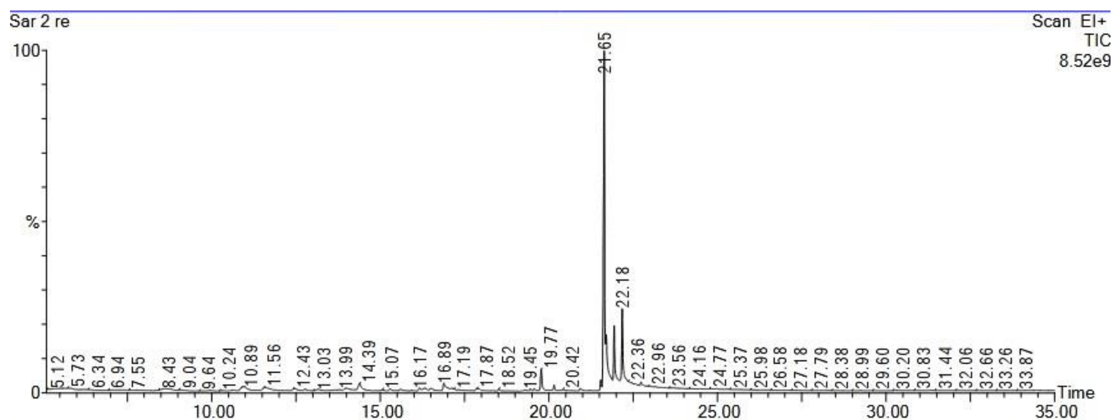


Fig. 7. GC-MS Chromatogram of *Sargassum lacerifolium* ethanolic extract .

Table 3. GC-MS analysis of *Sargassum lacerifolium* ethanolic extract.

	Compound name	Area %	MF	Biological activity*
1	Acetic acid, 2,2'-[oxybis(2,1ethanediyloxy)]bis-	2.381	$C_8H_{14}O_7$	Antioxidant, Anticancer, Anti-inflammatory
2	1-Deoxy-d-mannitol	0.429	$C_6H_{14}O_5$	Antimicrobial, Antioxidant, Anticancer
3	13,16-Octadecadiynoic acid, methyl ester	0.0665	$C_{19}H_{30}O_2$	Antimicrobial, Antioxidant. Anticancer
4	13,16-Octadecadiynoic acid, methyl ester	0.557	$C_{19}H_{30}O_2$	Mentioned before,
5	2-Nonenal, (E)-	2.249	$C_9H_{16}O$	Antioxidant, Anti-inflammatory, Anticancer
6	1,2-15,16-Diepoxyhexadecane	0.729	$C_{16}H_{30}O_2$	Anticancer, Antifungal, Antioxidant
7	Ethanone, 1-(3-methylphenyl)-	2.272	$C_9H_{10}O$	Antioxidant, Anticancer. Anti-inflammatory
8	Dianhydromannitol	2.060	$C_6H_{10}O_4$	Antioxidant, Anticancer, Anti-inflammatory
9	1-Cyclohexene-1-acetaldehyde, 2,6,6-trimethyl	0.806	$C_{11}H_{18}O$	
10	Cyclopropanemethanol,à,2-dimethyl-2-(4-methyl-3-pentenyl).	0.438	$C_{12}H_{22}O$	
11	1,2-15,16-Diepoxyhexadecane	0.475	$C_{16}H_{30}O_2$	Mentioned before
12	(Hydroxymethyl)ethylene acetate	0.367	$C_7H_{12}O_5$	Antioxidant, Anticancer
13	(Hydroxymethyl)ethylene acetate	1.470	$C_7H_{12}O_5$	Mentioned before,
14	Naphthalene,1,2,3,4-tetrahydro-1,4,6 trimethyl	2.840	$C_{13}H_{18}$	Antioxidant, Anti-inflammatory, Anticancer
15	Naphthalene,1,3-dimethyl-	0.450	$C_{12}H_{12}$	Antioxidant, Anticancer, Anti-inflammatory

16	2-Benzofuranmethanol,2,4,5,6,7,7a-hexahydro-4,4,7a-trimethyl	0.382	$C_{12}H_{20}O_2$	
17	5-Methyl-1-phenylhex-5-en-1-one	0.642	$C_{13}H_{16}O$	
18	trans-Z-à-Bisabolene epoxide	1.008	$C_{15}H_{24}O$	Antioxidant, Anti-inflammatory
19	3-Buten-2-one,4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	0.726	$C_{13}H_{20}O$	Antioxidant
20	Phenol, 2,4-bis(1,1-dimethylethyl)	2.302	$C_{17}H_{30}OSi$	Anticancer, Anti-inflammatory
21	Aromadendrene oxide-(1)	1.002	$C_{15}H_{24}O$	Antioxidant
23	2-Myristynoyl pantetheine	0.970	$C_{25}H_{44}N_{2O}5S$	Antioxidant
24	Heptadecane	3.080	$C_{17}H_{36}$	Antioxidant, Anticancer, Anti-inflammatory
25	1-Dodecanol, 3,7,11-trimethyl	0.577	$C_{15}H_{32}O$	Antioxidant
26	1-Dodecanol, 3,7,11-trimethyl-	0.444	$C_{15}H_{32}O$	Mentioned before
27	2-Hexadecene,3,7,11,15-tetramethyl	0.932	$C_{20}H_{40}$	Antioxidant
28	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	36.822	$C_{20}H_{40}O$	Anticancer, Antioxidant, Anti-inflammatory
29	Phytol	8.579	$C_{20}H_{40}O$	Antimicrobial, Anticancer, Antioxidant, Anti-inflammatory
30	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	5.371	$C_{20}H_{40}O$	Mentioned before
31	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	8.854	$C_{20}H_{40}O$	Mentioned before
32	Cyclopropanepentanoic acid, 2-undecyl-, methylester, trans-	0.542	$C_{20}H_{38}O_2$	Antioxidant, Anti-inflammatory

* (Source: Dr. Duke's Phytochemical and Ethnobotanical Databases).

MF: Molecular Formula.

DISCUSSION

Because of the use of artificial antioxidants is being questioned, natural antioxidants appear to be promising, and finding novel antioxidants is of great interest. When seaweeds are fresh, they are known to contain reactive antioxidant molecules like glutathione (GSH) and ascorbate as well as secondary metabolites like carotenoids and tocopherols **(Frazzini *et al*., 2022).** Algae are well-known for their strong antioxidant activity, but this activity is affected by a number of factors, including conditions of growth, collecting time, and activity determination method (**Cmiková *et al*., 2022**). Our findings confirm that the extracts have a high proton donating capacity and can act as free radical scavengers or inhibitors, acting as primary antioxidants. The type of solvent used to extract the seaweed also affects its antioxidant activity. According to the findings, the method of preparation of the extracts is one of the most important factors influencing antioxidant activity. Further testing and comparison of the extracts efficacy with different solvents is required. Through their capacity to scavenge the DPPH radical, different seaweeds with different solvents were examined for their antioxidant potential. Depending on the species and solvent, the majority of the examined algal extracts were able to convert the stable DPPH radical to diphenylpricrylhydrazine (the yellow-coloured). In the present study the most potent scavenging of DPPH radicals is *Sargassum lacerifolium* ethanolic extract (60.17±0.4%) followed by *Ulva compressa, Amphiroa rigida* and *Ulva fasciata* (56.25±0.27, 54.39±0.25 and 52.56±0.26%, respectively). The IC_{50} of *S. lacerifolium,* which was determined as the minimum concentration, revealed inhibition percentages greater than 50%, whereas *U. compressa* IC_{50} was 18.1 g/ml. These results agree with **Gheda *et al*. (2023b)** who reported that *Sargassum linifolium* phlorotannin extract displayed the highest level of DPPH maximum inhibition at the concentration 50 µg/ml and IC_{50} being 50.1 µg/ml. Also, the acetone extract of *Sargassum wightii* and the benzene extract of *Sargassum polycystum* exhibited DPPH antioxidant activity with 43% and 22%, respectively **(Unnikrishnan *et al*., 2015).**

The antioxidant assay by ABTS (2,2–azinobis (3-ethylbenzthiazoline-6-sulfonic acid) free radical scavenging is one of the most used methods measured the antioxidant potential of hydrophilic and lipophilic substances **(Torres *et al.,* 2017).** This study demonstrated that ethanolic extract of *S. lacerifolium* demonstrated stronger antioxidant activity with ABTS, and the inhibition reached maximum at the concentration of 60 µg/ml and IC_{50} 48.25 µg/ml. Comparable results were introduced by **Gheda *et al*. (2023b)**, who declared that phlorotannin extracts of *S. linifolium* showed increasing of antioxidant activity with ABTS assay, and the inhibition increased as the extract concentration increased. The maximum inhibition of *S. linifolium* phlorotannin extracts was at the concentration of 125 µg/mL and IC_{50} was 85.4 µg/ml. The total antioxidant capacity method of *Sargassum lacerifolium* ethanolic extract showed the highest antioxidant activities (40.19±0.27 mg ascorbic acid equivalents/g crude extract) between

the tested seaweeds followed by the total antioxidant *Ulva compressa* and *Ulva fasciata*, respectively, while the lowest antioxidant activities was observed in *Amphiroa rigida.*

These seaweeds antioxidant activity could be attributed to their ability to act as free radical scavengers or to supply the molecule with a hydrogen atom (**Boonchum *et al.,* 2011).** The provided finding was similar to those published by **Mohy El-Din and El-Ahwany** (**2018)** for the total antioxidant capacity of methanolic extract of *Pterocladia capillacea*, with values of 0.940 mg AAE/g DW. The enhanced antioxidant activity in the present work may result from the synergistic interactions of several bioactive chemicals found in the algal extracts. These are confirmed with numerous earlier researches **(Shanab *et al*., 2012; Rajishamol *et al*., 2016 and Abd El Sadek *et al.,* 2017)** who suggested that the antioxidant activity caused by a synergistic action amongst secondary metabolites, particularly phenolic compounds, flavonoids, polyunsaturated fatty acids, pigments, alkane and polysaccharides.

One of the most prevalent families of phytochemicals in algae, phenolic components have potential antioxidant properties through their ability to scavenge singlet oxygen, superoxide, and hydroxyl radicals, as well as through their ability to chelate metals, donate electrons or hydrogen, and stabilise lipid peroxidation. Additionally, algae are renowned for having the capacity to withstand oxidative stressors by activating both enzymatic and non-enzymatic antioxidants as a defence mechanism **(Gheda &Ismail, 2020).**

Algae have been considered as a source of new anticancer drugs **(Montuori, 2022; Romano, 2022 and Sugumaran *et al.,* 2022).** They have a wide range of favourable biological activities, including antioxidant, antitumor, immunomodulatory, antiviral, antithrombotic, anticoagulant, antithrombotic, antidiabetic and lipid-lowering activities **(AboShady *et al*., 2023 and Li *et al.* 2023).** Marine seaweeds have been proposed as major sources of new anticancer medications (**Kwak, 2014**; **Murphy *et al*., 2014 and Newman and Cragg, 2014).**

Jin *et al.* (2022) recorded that the anticancer mechanism shown by marine drugs commonly involves regulation of signal transduction, cell cycle arrest, cell apoptosis, and inhibition of migration and neo-angiogenesis, and also stimulates the immune responses and antioxidant system to prevent cancer. In this work, *In vitro* cytotoxic effectiveness of *Sargassum lacerifolium* ethanolic extract was determined versus four cell lines (WI38, HepG2, Hela, and MCF7).

Results indicated that they have high cytotoxic effect on the investigated cell lines. The algal extracts tested showed varying inhibitory activity levels against the human tumour cell lines tested. Weak cytotoxic activity against the WI38 and MCF7 cell line (IC_{50} 76.11± 3.9 and 56.54±3.2 μg/ml) respectively. *S. lacerifolium* showed moderate

cytotoxic activity against the HepG2 and Hela cell line (IC_{50} 33.60 ± 2.3 and 47.71±2.8 µg/ml), respectively. This was consistent with the findings of (**Mashjoor *et al.,* 2016; Deviyani *et al.,* 2018; and Saeed *et al.,* 2020),** who reported cytotoxic potential and activity of extracts of algae against three cell lines: MCF7, HepG2 and Hela. The mechanism(s) of action by which algae extract causes tumour cell death remains unknown. As explained by **(Dai *et al.*, 2013)**, the activity of fatty acids stopped by tumour may be due to: (a) increased production of ROS (b) activation of caspase enzymes (c) toxic products of lipid peroxidation accumulate, leading to cell death (d) stimulation of peroxisome. proliferator-activated receptors (PPARs) (e) changing gene/anti-oncogene expression, and (f) Cancer cells are activated due to chromosomal damage.

In the current study, bioactive compounds from *S. lacerifolium* ethanolic extract were identified using analytical tools such as (UV) spectroscopy, (IR) infrared spectroscopy, and Gas Chromatography-Mass spectroscopy analysis (GC-MS) , which confirmed the presence of bioactive substances and potential antioxidant, and antitumor compounds. To detect the active groups of algal extracts that responsible for antioxidant and anticancer activities, the purified fraction's infrared (IR) absorption spectra was examined **(He *et al*., 2016).** The spectrum of infrared (IR) absorption detect broad band of OH group, CH sharp band, sharp band of C=O, sharp band of C=C and C-O respectively. These results confirmed by GC-MS. The GC-MS profiles indicated that *S. lacerifolium* ethanolic extract was a consistent source of bioactive chemicals and mass spectrometry (GC-MS) and demonstrated the occurrence of compound having antioxidant, antitumor and anticancer properties **(Kalaivani *et al.,* 2016).** The component proportions differed across various species. However, basic chemicals including alcohols, fatty acids, phytol, hydrocarbons and phthalate were prevalent and might be the cause of the observed biological function. Several studies are in accordance with the studied work. 3,7,11,15-Tetramethyl-2-hexadecen-1-ol was the main compound found in *S. lacerifolium* in the current research, and these are similar with the majority of studies **(Famuyide *et al.,* 2020; El-Sheekh *et al*., 2021).**

CONCLUSION

The four seaweed extracts used for the current study were *Ulva fasciata*, *Ulva compressa*, *Sargassum lacerifolium*, and *Amphiroa rigida*, all of which had excellent antioxidant characteristics. Among the seaweeds examined, *S. lacerifolium* ethanolic extract had the highest antioxidant activity. In addition, *S. lacerifolium* exhibits significant cytotoxic activity against the Hela and HepG2 cell lines while exhibiting only modest activity against the MCF7 cell line. The presence of the bioactive chemicals responsible for these antioxidant and cytotoxic effects was confirmed by GC/MS analysis of *S. lacerifolium* extract. This seaweed extract was suggested by the study as a

component of natural medicines. Future study is necessary, nonetheless, in order to foresee seaweed crude extract as a supplier for the pharmaceutical industry after thorough clinical testing.

Acknowledgment

Authors would like to express our gratitude to Prof. Dr. Mona M. Ismail (Assistant Professor of taxonomy and biodiversity of aquatic biota, National Institute of Oceanography and Fisheries, Alexandria, Egypt) for her assistance in identification of the seaweeds.

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