Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 26(4): 1227 – 1246 (2022)
www.ejabf.journals.ekb.eg

Biological activities of secondary metabolites from *Turbinaria triquetra* (Phaeophyceae), *Hypnea cornuta* (Florideophyceae), and *Ulva prolifera* (Ulvophyceae) methanolic extracts

Mohamed S. M. Abd El Hafez[1,2,*], Miral G. Abd El-Wahab[2], Ali S. A . Abdel-Hamid[2], Doaa A. Ghareeb[2,3,4], Maha A. El Demellawy[2,5]

1. National Institute of Oceanography and Fisheries, NIOF, Cairo, Egypt.
2. Center of Excellence for Drug Preclinical Studies (CE-DPS), Pharmaceutical and Fermentation Industries Development Centre (PFIDC), City of Scientific Research and Technological Applications (SRTA-City), Egypt.
3. Bio-screening and Preclinical Trial Lab, Biochemistry Department, Faculty of Science, Alexandria University, Alexandria, Egypt.
4. Biochemistry Department, Faculty of Science, Alexandria University, Alexandria, Egypt.
5. Medical Biotechnology Department, Genetic Engineering & Biotechnology Research Institute, City of Scientific Research and Technological Applications (SRTA-City), Egypt.

[*]Corresponding Author: mohamedsaid80@yahoo.com

ARTICLE INFO

Article History:
Received: July 30, 2022
Accepted: Aug. 9, 2022
Online: Aug. 26, 2022

Keywords:
Marine algae,
Seaweeds,
Red sea,
Anti-cancer activity,
GC-MS,
Antioxidant activity

ABSTRACT

With respect to the potential natural resource in the marine environment, marine macroalgae or seaweeds are recognized to have health impacts. Three endemic marine algae, *Turbinaria triquetra* (brown algae), *Hypnea cornuta* (red algae), and *Ulva prolifera* (green algae) that are found in abundance in the Red Sea were collected. Antioxidant, anti-microbial, anti-cancer, anti-inflammatory, anti-diabetic, and anti-acetylcholinesterase activities of methanolic extracts of these algae were *in-vitro* evaluated. Fatty acids and their esters from the extracts were determined using GC-MS. Extracts of *T. triquetra* exhibited the highest antioxidant activity with a DPPH radical scavenging IC_{50} value of 1695.12±0.13 µg/ml and a ferric reducing power (EC_{50}) value of 574.71±0.15 mg/ml. The anti-diabetic and anti-acetylcholinesterase activities of *H. cornuta* extract were the highest, with IC_{50} values of 19.89 ± 0.03 µg/ml and 16.97 ± 0.03 µg/ml, respectively. Interestingly, all extracts exhibited anti-colon cancer activity (CACO), with IC_{50} values of 378.3±0.02, 276.88±0.08, and 274.32±0.05 µg/ml for *U. prolifera*, *H. cornuta*, and *T. triquetra* extracts, respectively. Moreover, the IC_{50} values to human gingival normal fibroblast cell lines were 783.37±0.07, 692.7±0.01, and 124.36±0.03 µg/ml, respectively. These findings highlight the potential of these seaweed species for cultivation as a sustainable and safe source of therapeutic compounds for treating human and fish diseases.

INTRODUCTION

With the increase in the spread of diseases and the harmful effects of synthetic medicines, exploring traditional medicine has become crucial. Finding a natural compound to treat different conditions as medicine or a supporting agent becomes a big

challenge. As a result, it is preferable to search for a novel, potent natural component derived from marine macroalgae **(Pushpa Bharathi *et al.*, 2019)**. Many marine algae have a unique chemical structure and characteristics that are not present in terrestrial organisms **(Khotimchenko *et al.*, 2020)**. Due to their ability to establish a defensive mechanism by releasing secondary metabolites, many species of macroalgae can grow in their habitats in extreme conditions **(Olasehinde *et al.*, 2019)**.

Macroalgae can be categorized according to nutrient and chemical composition into red algae (Rhodophyta), brown algae (Phaeophyta), and green algae (Chlorophyta) **(Rajasulochana *et al.*, 2009)**. Alkaloids, polyketides, cyclic peptides, polysaccharides, phlorotannins, diterpenoids, sterols, fatty acids, vitamins, minerals, quinones, lipids, and glycerols are among the biologically active secondary compounds developed by marine algae. These compounds demonstrate anti-microbial, anti-oxidant, anti-cancer, anti-helminthic, anti-inflammatory, anti-aging, and cancer-cytotoxic agents **(Al-Saif *et al.*, 2014)**. As a result, they have recently attracted much attention **(Rashedy *et al.*, 2021)**.

Antibiotic discovery and its improvement are among modern science and technology's most powerful and influential accomplishments in the fight against infectious diseases **(Chanda *et al.*, 2010)**. Because of the indiscriminate use of antibiotics, pathogenic bacteria have become resistant to the most commonly used antibiotics **(Lavanya *et al.*, 2011)**. Since several algal extracts and active constituents exhibited anti-gram positive and negative-bacterial activity, the isolation of marine natural products with antibacterial activities has received much attention in the last century **(Siddhanta *et al.*, 1997)**.

The ultimate objective of marine natural product drug development research is to discover compounds and extracts that can prevent the formation of free radicals and consequently quench the oxidative stress processes to reduce human diseases, such as neurological disorders, male infertility, heart disease, diabetes, inflammation, aging and cancer **(El Feky *et al.*, 2022)**. A wide variety of plants, fungi, and algae are recognized as essential sources of natural antioxidants **(Ebrahimzadeh *et al.*, 2018)**. Marine macroalgae are rich in phenolic and polyphenolic metabolites, including flavonoids and catechins, which have strong antioxidant properties capable of removing free radicals, scavenging reactive oxygen species, and inhibiting lipid peroxidation **(Vijayraja & Jeyaprakash, 2019)**.

Cancer is a severe global public health challenge; therefore, several trials are performed to discover cancer-fighting therapeutic compounds extracted from natural sources such as marine algae. Numerous studies have found that algal extracts exhibit anti-proliferative action *in-vitro* and tumor-inhibiting activities *in-vivo*. Several studies indicate that compounds isolated from algae, such as bromophenols, fucoidan, flavonoid, laminarin, carotene, and steroids could have anticancer activity **(Saadaoui *et al.*, 2020)**. This study evaluated the antioxidant, anti-microbial, anti-cancer, anti-inflammatory, anti-diabetic, and anti-acetylcholinesterase activities of the methanolic extracts of *T. triquetra*, *H. cornuta*, and *U. prolifera* that were abundant in the coastal region of the Red Sea in Egypt.

MATERIALS AND METHODS

Algae collection

Three algae species, *Turbinaria triquetra* (J. Agardh) Kützing, 1849; *Hypnea cornuta* (Kützing) J. Agardh, 1851, and *Ulva prolifera* O.F. Müller, 1778 were harvested from the tidal zone of the Red Sea shore at Hurghada between latitude 27º28.15'N and longitude 33º77.13'E, Egypt. The seaweed species were identified under microscope according to their morphological characteristics with taxonomic references following the descriptions of **Aleem (1978**). About 0.5kg of each alga was washed with water, shade dried, and powdered in a mixer grinder.

Preparation of algal extracts

One hundred grams from each algal powder was macerated in 400mL methanol for 48h. The solvent was filtrated and concentrated at 40°C under pressure in a rotatory evaporator. The extracts were then kept at -20°C for subsequent study **(Patra *et al.*, 2008)**.

Gas chromatography-mass spectrometer (GC-MS) analysis

GC-MS Agilent 7890A-5975C was utilized for the separation of different bioactive constituents. A capillary column (0.25 mm×30 mm×0.25 µm film thickness) was used. The temperature of the oven was kept at 70°C for 2min before being steadily increased from 20°C/min to 305°C and held there for 1.0 min. As a carrier gas, 1.2 mL/min helium gas was utilized, and the injected sample volume for the GC-MS analysis was 1.0µL **(Annegowda *et al.*, 2013)**. Based on the retention time, bioactive chemicals from algal extracts were identified. The National Institute of Standards and Technology (NIST) database was used to interpret the mass spectrum of the GC-MS.

Determination of total phenolic content

The total phenolic contents were estimated using the Folin-Ciocalteu reagent following the method of **Singleton *et al.* (1999**). A 0.1 mL aliquot of Folin-Ciocalteu reagent was added to 0.1 mL reconstituted extract. A 2.0 mL saturated sodium carbonate (2%) was added to the mixture after 15 min. For 30 min, the mixture was left to remain at room temperature. TPC was determined using a spectrophotometer (Labo America, USA) at 760nm with gallic acid as a reference. The linear regression equation generated from the standard gallic acid calibration curve (y= 0.0054x - 0.1) was used to calculate TPC as milligrams of gallic acid equivalent per gram sample. Each sample was evaluated in triplicate.

Determination of total flavonoid content

The total flavonoid contents were determined using a modified colorimetric method described in the study of **Sakanaka *et al.* (2005)**. A 1.0 mL of the sample and 4.0 mL of water were mixed in a flask. A 0.75mL of 5% sodium nitrite and 0.150mL of 10% of aluminum chloride were added to the mixture. After 5min at room temperature, 0.5mL of 1 M sodium hydroxide was added. A UV/VIS spectrophotometer (PG Instrument Ltd., UK) was used to measure the absorbance at 510nm. The results were expressed as

milligram catechol equivalent per gram sample from the standard catechol calibration curve (y= 0.0101x + 0.0275).

DPPH radical scavenging activity

The DPPH assay of **Brand-Williams *et al.* (1995)** was used to assess free radical scavenging activity of extracts. Ascorbic acid was used as a standard with different ranges (5-200 µg/ml) to generate the standard curve. The absorbance was measured at 517nm using a spectrophotometer. The activity of DPPH radical scavenging was determined as mg ascorbic acid equivalent (AAE)/g dry sample. The following equation was used to calculate the percent of DPPH radical-scavenging activity:

$$DPPH\ radical\ scavenging\ activity\ (\%\ inhibition) = \frac{\mathrm{Abs}_{\mathrm{control}} - \mathrm{Abs}_{\mathrm{sample}}}{\mathrm{Abs}_{\mathrm{control}}} \times 100$$

Ferric reducing power activity

The ferric reducing power was measured using a spectrophotometric method reported in the work of **Ferreira *et al.* (2007)**. The extracts reduced potassium ferricyanide to potassium ferrocyanide, which reacts with ferric chloride to form a ferric-ferrous complex. The absorbance of the generated complex was measured at 700 nm utilizing ascorbic acid as a standard. The extract concentration producing 0.5 of absorbance (EC_{50}) was established from the graph of absorbance versus extract concentration.

Anti-inflammatory activity

The percentage of hemolysis was performed in 2ml microtubes following the method described in the study of **Farias *et al.* (2013)** and was calculated as follows:

$$\%\ \mathrm{hemolysis} = \frac{\mathrm{Abs}_{\mathrm{test}}}{\mathrm{Abs}_{\mathrm{pc}}} \times 100$$

Where, Abs test = Abs 540 of the 1% cell suspension treated with sample test and Abs *pc* = Abs 540 of the 1% cell suspension treated with distilled water.

In-vitro anti-inflammatory activity of extracts was estimated by the human red blood corpuscles (HRBCs) membrane-stabilizing method **(Anandarajagopal**, **2013)**. The percentage membrane stabilization was calculated by using the following formula:

$$\%\ \mathrm{Protection} = 100 - \left(\frac{\mathrm{OD}_{\mathrm{test}}}{\mathrm{OD}_{\mathrm{control}}} \times 100\right)$$

Antimicrobial activity

Agar well diffusion assay of **Kadaikunnan *et al.* (2015)** was used for detecting the activity of the extract against five microbial species *Klebsiella pneumonia* (ATCC700603), *E. coli* (ATCC25922), *Staphylococcus aureus (*ATCC25923), *Streptococcus pyogenes* (EMCC1772), and *Candida albicans* (EMCC105), which

obtained from the city of Scientific Research and Technological Applications, using 100µl of the inoculums (1×10^8 CFU/ml).

Anti-cancer activity

The cytotoxicity of the algal extracts was determined using the methyl thiazolyl tetrazolium (MTT) assay following the method of **Abd El Hafez *et al.* (2022)**. Algal extracts were tested against colon human cancer cells (CACO) and normal human gingival fibroblast cell lines after dissolved in 10% DMSO. Cell lines were provided as a gift from the Faculty of Medicine, Alexandria University, Egypt.

Anti-diabetic activity

The inhibitory effect of algal extracts on α-glucosidase (EC 3.2.1.20) activity was determined according to the method described by **Abd El Hafez *et al.* (2021)**, with a slight modification. A 100mL aliquot, each extract (1.0 mg/mL) as a test, organic solvents as a control, and dist.H_2O as a blank, was diluted with 2.5mL of 0.1M phosphate buffer with a pH 7.4. An equivalent amount (100 mL) of the purified enzyme was added, thoroughly mixed, and the reaction mixture was incubated in a water bath at 30°C for 5min. Afterward, 500mL of PNPG (5 mM) was added, and the reaction was allowed to continue for 15min. The reaction was then stopped with 2mL of 1M Na_2CO_3, and the generated color was spectrophotometrically measured at 400nm. The released n moles of p-nitrophenol/min were used to establish a unit of enzyme activity.

$$\% \text{ Inhibition} = (1 - Absorbance\ of\ the\ test\ well\ /Absorbance\ of\ the\ untreated\ (control)) \times 100$$

Anti-acetylcholinesterase activity

The activity of acetylcholinesterase (AChE) was determined *in-vitro* following the method of **Ellman *et al.* (1961)**. A mixture of 20mL purified AChE enzyme, and 20mL extract (test) or organic solvent (control) was incubated for 45min at 37°C in phosphate buffer with a volume of 130 mL (0.1 M pH 7.4). Subsequently, 5 mL of ACTI (75 mM) substrate was added, mixed thoroughly, and incubated at 37°C for 15min. After that, 60 mL of DTNB (0.32 mM) was added and allowed to sit for 5min. The specific activity was estimated after measuring the absorbance at 405nm.

Statistical Analysis

All data collected were analyzed using one-way (ANOVA) by SPSS (n=3) and ($P< 0.05$) indicated a significant difference. Significant means were compared by Duncan post-hoc multiple comparison test.

RESULTS

GC-MS analysis

As shown in Fig. (1), the fatty acids and fatty acid esters components of the *U. prolifera*, *H. cornuta*, and *T. triquetra* extracts were identified by comparing their mass spectra to those of standard compounds stored in the GC-MS library. Table (1) highlights some significant chemicals that found in each algal extract, which may be involved in the

biological activities of these algae, in addition to other secondary metabolites such as phenolics and flavonoids.

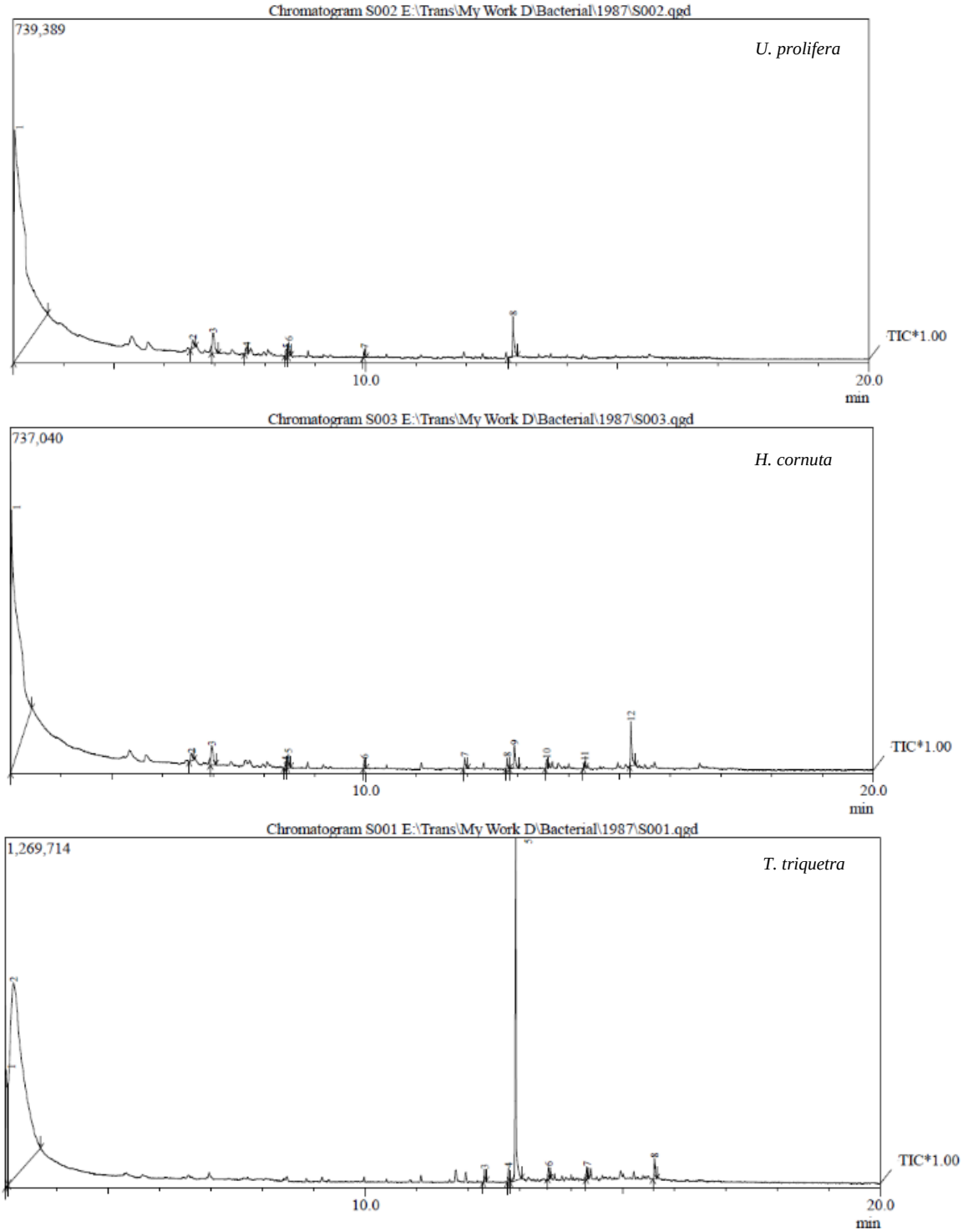

Fig. 1. Chromatogram of the three algal methanol extracts as investigated by GC-MS chromatography.

Table 1. The bioactive compounds identified by GC-MS that found in all the algal extracts.

Compound Name	Molecular Formula	Molecular Weight (m/z)
Cyanic acid, 2-methylpropyl ester	C_5H_9NO	99
Acetic acid, isopropyl ester	$C_5H_{10}O_2$	102
Cyclopenta[c]furo[3',2':4,5]furo[2,3-h][1]benzopyran-11(1H)-one, 2,3,6a,9a-tetrahydro-1,3-dihydroxy-4-methoxy	$C_{17}H_{14}O_7$	330
3-Isoxazolecarboperoxoic acid, 4,5-dihydro-5-phenyl-, 1,1-dimethylethyl ester	$C_{14}H_{17}NO_4$	263
4,8-Diacetyl-4H,8H-di[1,2,5]oxadiazolo[3,4-b:3,4-E]pyrazine	$C_8H_6N_6O_4$	250
N-Acetyl-N-(acetyloxy) acetamide	$C_6H_9NO_4$	159
N-Acetylacetamide	$C_4H_7NO_2$	101
n-Nonyl iodide	$C_9H_{19}I$	254
3-Methylbenzoic acid, 2,4,6-trichlorophenyl ester	$C_{14}H_9C_{l3}O_2$	314
m-Toluic acid, Cyclobutyl ester	$C_{12}H_{14}O_2$	190
p-Mentha-1,3,8-triene	$C_{10}H_{14}$	134
n-Tridecane	$C_{13}H_{28}$	184
1,2-Benzenedicarboxylic acid, diethyl ester	$C_{12}H_{14}O_4$	222
Phthalic acid, 4-bromophenyl ethyl ester	$C_{16}H_{13}BrO_4$	348
Ethyl 4-nitrophenyl ester	$C_{16}H_{13}NO_6$	315
2,6,10,14-Tetramethylheptadecane	$C_{21}H_{44}$	296
n-Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270
n-Tridecanoic acid methyl ester	$C_{14}H_{28}O_2$	228
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278

Antioxidant activity of algal extracts

The highest phenolic and flavonoid contents were demonstrated in extracts of *T. triquetra* (Table 2). The DPPH free radical scavenging activity assay revealed a reduction in the concentration of DPPH due to the scavenging ability of the different compounds present in the algal extracts. According to Table (3), *U. prolifera* extracts had the highest DPPH IC_{50} value of 1695.12±0.13 µg/ml, while *T. triquetra* extracts had the lowest IC_{50} value of 671.50±0.21 µg/ml. The highest DPPH scavenging activity was found in *T. triquetra* extracts, then *H. cornuta*, and finally, *U. prolifera* extracts. The *U. prolifera* extract had the highest ferric reduction capacity, then *H. cornuta* extracts and *T. triquetra* extracts using ascorbic acid as a reference at $p<0.05$ (Table 4).

Table 2. Total phenolic and total flavonoid contents of algal extracts.

Extracts	Total phenolic (mg/g)	Total flavonoid (mg/g)
U. prolifera	36.53 ± 0.21^{b}	18.16 ± 0.31^{b}
H. cornuta	32.49 ± 1.32^{c}	18.78 ± 0.70^{b}
T. triquetra	41.11 ± 0.40^{a}	32.07 ± 0.35^{a}

Reported values are the mean ± SD of three replicates. Means in the same column with different lowercase letters are significantly different at $p<0.05$.

Table 3. DPPH radical scavenging activity of algal extracts.

Extracts	DPPH (IC50) µg/ml
Ascorbic	5.41±0.01[a]
U. prolifera	1695.12±0.13[d]
H. cornuta	915.68±0.18[c]
T. triquetra	671.50±0.21[b]

IC_{50} (µg/ml): inhibitory concentrations at which 50% of DPPH radicals are scavenged. Means in the same column with different lowercase letters are significantly different at $p<0.05$.

Methanol extract of *T. triquetra* demonstrated the highest contents for total phenolic and total flavonoid 41.11±0.40 mg/g and 32.07±0.35 mg/g, respectively, as well as DPPH radical scavenging activity (IC_{50} value of 671.50±0.21 µg/ml, with 74.46 % inhibition at a concentration of 1000 µg/ml). The lowest flavonoid contents were 18.16±0.31 mg/g found in *U. prolifera* extract with the highest reducing power EC_{50} of 574.71±0.15 mg/ml, whereas extracts of *H. cornuta* revealed the lowest phenolic contents 32.49±1.32 mg/g.

Table 4. Ferric reducing power of algal extracts.

Extracts	Reducing power EC_{50} (mg/ml)
Ascorbic	16.82±0.01[a]
U. prolifera	574.71±0.15[d]
H. cornuta	432.91±0.19[c]
T. triquetra	265.96±0.11[b]

Means in the same column with different lowercase letters are significantly different at $p<0.05$.

Anti-inflammatory activity of algal extracts

The hemolytic activity of algal extracts on human RBC hemolysis is shown in (Table 5). Extracts of *H. cornuta* exhibited the highest hemolytic level, whereas *U. prolifera* and *T. triquetra* extracts had the lowest hemolytic level ($p<0.05$). The anti-inflammatory activity of algal extracts at different concentrations (10, 40, 100, 400, and 600 µg/ml) are presented in (Table 6). The *H. cornuta* extract showed the lowest IC_{50} value of 313.56±0.11 µg/ml, whereas extracts of *U. prolifera* and *T. triquetra* IC_{50} values were 315.79±0.03 µg/ml and 333.33±0.01 µg/ml, respectively. The IC_{50} value of the reference drug diclofenac was 336.29±0.18 µg/ml.

Table 5. Hemolytic activity of algal extracts.

Concentration (µg/ml)	% of Hemolysis		
	U. prolifera	*H. cornuta*	*T. triquetra*
10	3.45±0.02	3.24±0.22	3.38±0.08
40	3.78±0.14	3.31±0.08	3.58±0.12
100	3.85±0.06	3.58±0.04	5.14±0.18
400	4.12±0.18	3.65±0.13	8.65±0.02
600	4.32±0.24	4.12±0.06	10.00±0.05
IC_{50} (µg/ml)	**1298.25±0.11^{b}**	**1396.23±0.23^{a}**	**973.68±0.17^{c}**

Reported values are the mean ± SD of three replicates. Means in the same column with different lowercase letters are significantly different at $p<0.05$.

Table 6. Anti-inflammatory activity of algal extracts.

Concentration (µg/ml)	Human RBCs membrane stabilization			
	U. prolifera	***H. cornuta***	***T. triquetra***	**Diclofenac**
10	96.55±0.07	96.76±0.05	96.62±0.12	95.18±0.14
40	96.22±0.03	96.69±0.12	96.42±0.02	94.24±0.05
100	96.15±0.11	96.42±0.02	94.86±0.07	92.31±0.02
400	95.88±0.08	96.35±0.09	91.35±0.04	91.28±0.03
600	95.68±0.12	95.88±0.03	90.00±0.02	89.21±0.08
IC_{50} (µg/ml)	**315.79±0.03^{b}**	**313.56±0.11^{c}**	**333.33±0.01^{a}**	**336.29±0.18**

Reported values are the mean ± SD of three replicates. Means in the same column with different lowercase letters are significantly different at $p<0.05$.

Anti-microbial activity of algal extracts

The inhibition zones of algal extracts against different microbial strains were measured as shown in Table (7). The *H. cornuta* extract demonstrated moderate activity against *Staphylococcus aureus*, whereas *U. prolifera* extract had the highest action against *Streptococcus pyogenes*. The antifungal activity of *H. cornuta* extract showed a limited inhibition zone against *Candida albicans* (12±0.02 mm). In the current investigation, methanol extracts of all the examined marine algae exhibited antibacterial activities against all tested bacteria. The *U. prolifera* and *H. cornuta* extracts demonstrated a considerable inhibition zone diameter against *Streptococcus pyogenes*, 45±0.12 and 25±0.14 mm, respectively.

Table 7. Anti-microbial activity of algal extracts.

	Inhibition zone diameter (mm)*			MIC
Sample concentration %	0.1 mg/ml	0.05 mg/ml	0.025 mg/ml	(mg/ml)
Gram-negative Bacteria				
Klebsiella pneumonia ATCC700603				
H. cornuta	ND	ND	ND	ND
T. triquetra	ND	ND	ND	ND
U. prolifera	20±0.05	19±0.02	10±0.04	0.025
E. coli ATCC25922				
H. cornuta	13±0.06	ND	ND	0.1
T. triquetra	ND	ND	ND	ND
U. prolifera	ND	ND	ND	ND
Gram-positive Bacteria				
Staphylococcus aureus ATCC25923				
H. cornuta	20±0.04	12±0.06	10±0.08	0.025
T. triquetra	12±0.07	11±0.01	10±0.02	0.025
U. prolifera	12±0.03	ND	ND	0.1
Streptococcus pyogenes EMCC1772				
H. cornuta	25±0.14	17±0.11	ND	0.05
T. triquetra	ND	ND	ND	ND
U. prolifera	45±0.12	37±0.16	28±0.08	0.025
Yeast				
Candida albicans EMCC105				
H. cornuta	12±0.02	ND	ND	0.1
T. triquetra	ND	ND	ND	ND
U. prolifera	ND	ND	ND	ND

*Diameter includes 5 mm well diameter.
ND; Not detected. MIC; Minimum inhibition concentration (mg/ml).

Anti-cancer activity of algal extracts

The IC_{50} values for growth inhibition upon normal fibroblast cells and colon cancer (CACO) are given in (Table 8). The results indicated that *H. cornuta* was the safest and most potent anti-colon cancer extract, as it had the highest therapeutic index, while *T. triquetra* extract showed a therapeutic index lower than one, indicating its toxicity upon normal cells.

Table 8. Algal extracts activity against Colon cancer (CACO).

Extracts	Colon cancer (CACO) IC_{50} (µg/ml)	Normal fibroblast cells IC_{50} (µg/ml)	Therapeutic index
U. prolifera	378.3±0.02	783.37±0.07	2.068
H. cornuta	276.88±0.08	692.7±0.01	2.502
T. triquetra	274.32±0.05	124.36±0.03	0.543

Anti-diabetic activity of algal extracts

The three algal extracts exhibited remarkable α-glucosidase inhibition at all the concentrations examined (25, 50, 75, and 100 µg/ml). The inhibition effect was concentration-dependent, increasing as the concentration of the extract increased. The *U. prolifera* extract was the most effective inhibitor of α-glucosidase since it had the lowest IC_{50} value of 15.71 ± 0.05 µg/ml, compared to acarbose, which inhibited α-glucosidase activity with an IC_{50} value of 26.33 ± 0.02 µg/ml, as shown in Table (9).

Table 9. Anti-diabetic activity of algal extracts.

Extracts	(IC_{50}) µg/ml
Hypnea cornuta	19.89 ± 0.03^{b}
Turbinaria triquetra	17.06 ± 0.08^{c}
Ulva prolifera	15.71 ± 0.05^{d}
Acarbose	26.33 ± 0.02^{a}

Reported values are the mean ± SD of three replicates. Means in the same column with different lowercase letters are significantly different at $p<0.05$.

Anti-acetylcholinesterase activity of algal extracts

The results of the three algal extracts revealed that they had an inhibiting impact on Acetylcholinesterase (AChE). The inhibitory effects increased as the extract concentration raise. The extract from *U. prolifera* was the most effective AChE inhibitor, with the lowest IC_{50} value of 4.90 ± 0.01 µg/ml (Table 10).

Table 10. The anti-acetylcholinesterase activity of algal extracts.

Extracts	(IC_{50}) µg/ml
Hypnea cornuta	16.97 ± 0.03^{a}
Turbinaria triquetra	12.58 ± 0.04^{b}
Ulva prolifera	4.90 ± 0.01^{c}

Reported values are the mean ± SD of three replicates. Means in the same column with different lowercase letters are significantly different at $p<0.05$.

DISCUSSION

Three macroalgae collected from Egyptian Red Sea shores, *T. triquetra* (brown algae), *H. cornuta* (red algae), and *U. prolifera* (green algae), were studied for their antioxidant, anti-microbial, anti-cancer, anti-inflammatory, anti-diabetic, and anti-acetylcholinesterase activities. Marine algae are a rich source of biologically active chemicals with therapeutic effects, and their potential use in treating and preventing chronic diseases requires further exploration (**Abd El Hafez *et al.*, 2020**, **Alkhalaf**, **2021**). Because of the harsh conditions, where many macroalgae reside, powerful defensive mechanisms have evolved various sources of biologically active chemicals with structural and activity variations that differ from those existing in the terrestrial plants. Tetradecanoic acid, hexadecanoic acid, octadecanoic acid, and methyl ester were among the major constituents in many macroalgae species (**Mohy El-Din and El-Ahwany**, **2016**).

Heptadecanes, derived from fatty acids, are one of the principal components of the marine green algae *Ulva pertusa* and are the signature impact compound of edible kelp (**Yamamoto *et al.*, 2014**). Similarly, Hexadecanoic acid and 9,12,15-octadecatrienoic acid (α-linolenic acid) have been isolated from *U. prolifera* monomer compounds (**Gao *et al.*, 2020**). It has been recorded that the brown algae *Padina pavonica* (Dictyotales) and *Hormophysa triquetra* (Fucales) contain a high concentration of terpenes and sterols. Palmitic acid was the most abundant saturated fatty acid in *Hypnea cornuta*, while palmitoleic and stearic acids were the most abundant unsaturated fatty acids, according to (**Zhuang *et al.*, 2012**).

The activity of marine algae extracts can vary depending on the species, season, collection methods, extraction techniques, and solvents utilized. Screening for antioxidant activities in natural products is a rapidly growing area of research, and several antioxidant potentials have been tested using different methods. The DPPH test depends on the capability of the DPPH reagent to act as a stable radical to decolorize in the presence of antioxidants providing a quick, reliable, and low-cost method for determining the antioxidative potential of many natural products (**Molyneux**, **2004**). Total phenolic and total flavonoid contents estimation can be used as a rapid screening test for the

antioxidant activity of algal extracts. Marine algae are well known for synthesizing many secondary metabolites for free radical stabilization. It has been proposed that antioxidant action can use a variety of mechanisms to stabilize free radicals (**de Almeida *et al.*, 2011**).

According to (**Farasat *et al.*, 2014**), the green algae *Ulva clathrate* had the highest antioxidant activity, followed by *U. intestinalis*, *U. linza*, and *U. flexuosa*. Algae antioxidant activity is regulated by many processes and compounds, including lipophilic scavengers (carotenoids), enzymatic scavengers (catalase, superoxide dismutase, and peroxidase), and polyphenols (**Heim *et al.*, 2002**). Seaweeds are rich in phenolic compounds, which have been related to their anti-bacterial and antioxidant activity (**Yuan *et al.*, 2005**).

The existence of compounds possessing anti-microbial, antioxidant, and anti-cancer properties found in the bioactive secondary metabolites of concentrated extracts of the marine brown algae *Turbinaria conoides* and *Eucheuma cottonii* red algae was described by (**Kalaivani *et al.*, 2016**). The antioxidant capabilities of different algae species are attributable to the presence of flavonoids and polyphenols, and the concentration of these bioactive substances determines the intensity of antioxidant potential (**Zhang *et al.*, 2019**).

Sulfated polysaccharides isolated from the marine algae *Gracilaria caudata* were found to reduce tissue damage by inhibiting inflammation in an *in-vivo* study (**da Silva *et al.*, 2019**). Similarly, in LPS-stimulated cells, brown algae inhibited oxidative stress-induced inflammation (**Kim and Kim, 2010**). These findings clearly illustrate the anti-inflammatory ability of various marine algae species. In line with the previous studies, the methanolic algal extracts used in this investigation revealed a notable anti-inflammatory effect and were most likely obtained by inhibiting oxidative stress through free radical scavenging.

Our results were agreed with **Awad (2000)**, which reported that many *Ulva* extract products possess anti-bacterial activity, and most of these molecules are identified as fatty acids, hydroxyl unsaturated fatty acids, glycolipids, steroids, phenolic, and terpenoids. The anti-microbial activity of *Ulva* organic extract appears to be related to its lipophilic and phenolic content (**Abd El-Baky *et al.*, 2008**).

The brown algae *Cystoseira mediterranea* and *Cystoseira usneoides* were highly active against *S. aureus* (**Moreau *et al.*, 1984**). A similar observation was established by **Das *et al.* (2005)**, who studied methanol extracts of some algae and found that they had mild to high anti-bacterial activity. In disagreement with our findings, **Ibtissam *et al.* (2009)** found that methanol extracts of *Sargassum vulgare* had no antibacterial activity against *E. coli* and *S. aureus* growth.

This finding could be clarified by **Kantachumpoo and Chirapart (2010)**, who noticed that the crude extracts from most brown algae species lack anti-microbial activity. According to **Salvador *et al.* (2007)**, brown and red seaweeds harvested in the spring and

autumn inhibited microorganisms. Environmental factors are believed to affect the biological activities of marine algae (**Lobban and Harrison**, **1994**). The action of marine algae toward microorganisms can be attributed to reproductive status and seasonality (**Ely *et al.***, **2004**). Other significant reasons include the extraction method and the harvesting period (**Edwards *et al.***, **2006**).

Soluble bioactive metabolites in the solvent might explain the lower and higher antimicrobial activity of organic extracts towards microorganisms (**El Feky *et al.***, **2022**). *Turbinaria ornata* and *Sargassum wightii* marine algae extracts demonstrated significant efficacy against Gram-positive and Gram-negative bacteria and could be exploited as a potential source of antimicrobial drugs in the pharmaceutical industry (**Vijayabaskar and Shiyamala**, **2011**). In contrast to the current findings, **Wong and Cheung** (**2001**) reported that red seaweeds had weaker antibacterial activity than green seaweeds owing to the high phenolic content of green seaweeds.

In the current study, methanol extracts inhibited cell proliferation of human cancer cell lines at various doses. These findings agree with prior research that discovered anticancer effects in many algae species. Green, brown, and red algal bioactive lipids, were discovered to suppress the growth of MDA-MB-231 breast cancer cells (**Lopes *et al.***, **2020**).

The red alga *Porphyridium sordidum* contains a heteropolysaccharide that is cytotoxic to MCF-7 and MDA-MB-231 breast cancer cells (**Nikolova *et al.***, **2019**). Similarly, cytotoxic and antitumor effects of numerous algae species against various cancer cell lines were reported (**Zandi *et al.***, **2010**). Many research has linked the anticancer potential of algae to their polyphenol and flavonoid content (**Aravindan *et al.***, **2015**). Furthermore, the anticancer benefits of polyphenols are related to their capability to lower oxidative stress, which is thought to play a vital role in carcinogenesis (**Alkhalaf**, **2021**).

Polyphenols and polysaccharides derived from marine macroalgae exhibited remarkable anti-diabetic activity by interfering with the carbohydrate metabolism (**Zhao *et al.***, **2018**). Bioactive compounds extracted from edible marine algae are significant in glucose-induced oxidative stress regulation and the inhibition of starch digesting enzymes (**Unnikrishnan *et al.***, **2015**). The nature of the compounds found in the algal extracts of the present study is consistent with earlier research that found them to be effective inhibitors of α-glucosidases. Ethanolic extracts of *E. stolonifera* and *E. cava* marine algae had detectable AChE inhibitory activity as reported by (**Yoon *et al.***, **2008**), which is consistent with our findings. Seaweed extracts have also been tested against the AChE enzyme with positive results, according to Machado (**Machado *et al.***, **2015**).

CONCLUSION

The current study provides approximately data on the phytochemical constituents of three algal extracts for novel pharmaceutical compounds synthesis. Bioactive compounds found in algae are awaiting a significant breakthrough to be used as natural antioxidants in various food and pharmaceutical products. These algal extracts have antibacterial activity against bacterial infections, lending credence to their traditional use and implying a future role for these algae in combating microbial populations. Moreover, the algal extracts also exhibited significant anti-inflammatory, anti-diabetic, anti-acetylcholinesterase, and anti-tumor activities. The algal extracts examined in this study will require further fractionation and pharmacological evaluation to complete the preclinical investigations for their therapeutic uses in preventing and treating chronic diseases.

REFERENCES

Aleem, A.A. (1978).Contribution to the study of the marine algae of the Red Sea. III-Marine algae from Obhor, in the vicinity of Jeddah. Bull. Fac. of Sci. K. Abdle Aziz Uni., Jeddah,(2).

Abd El-Baky, H. H.; Baz, F. K. El and El-Baroty, G. S. (2008). Evaluation of marine alga *Ulva Lactuca* L. as a source of natural preservative ingredient. Electron. J. Environ. Agric. Food Chem., 7(11):3353–3367.

Abd El Hafez, M. S. M.; AbdEl-Wahab, M. G.;Seadawy, M. G.; El-Hosseny, M. F.;Beskales, O.; Saber Ali Abdel-Hamid, A.; Demellawy, M. A. El and Ghareeb, D. A. (2022). Characterization, *in-silico*, and *in-vitro* study of a new steroid derivative from *Ophiocoma dentata* as a potential treatment for COVID-19. Sci. Rep., 12(1):5846. https://doi.org/10.1038/s41598-022-09809-2.

Abd El Hafez, M. S. M.;Aziz Okbah, M. A. El;Ibrahim, H. A. H.;Hussein, A. A. E. R.;Moneim, N. A. A. El and Ata, A. (2021). First report of steroid derivatives isolated from starfish *Acanthaster planci* with anti-bacterial, anti-cancer and anti-diabetic activities. Nat. Prod. Res., 1–8. https://doi.org/10.1080/14786419. 2021. 2021200.

Abd El Hafez, M. S. M.;Elkomy, R. G.;Saleh, H. and Aboul-Ela, H. M. (2020). Extracts of the green algae *Ulva prolifera* possess antioxidant and antibacterial activities in vitro. Egypt. J. Aquat. Biol. Fish., 24(4). https://doi.org/10.21608/EJABF.2020. 98005.

Al-Saif, S. S. A. llah;Abdel-Raouf, N.;El-Wazanani, H. A. and Aref, I. A. (2014). Antibacterial substances from marine algae isolated from Jeddah coast of Red sea, Saudi Arabia. Saudi J. Biol. Sci., 21(1). https://doi.org/10.1016/j.sjbs.2013.06.001.

Alkhalaf, M. I. (2021). Chemical composition, antioxidant, anti-inflammatory and

cytotoxic effects of *Chondrus crispus* species of red algae collected from the Red Sea along the shores of Jeddah city. J. King Saud Univ. - Sci., 33(1). https://doi.org/10.1016/j.jksus.2020.10.007.

Almeida, C. L. F. de;Falcão, H. de S.;Lima, G. R. d. M.;Montenegro, C. de A.;Lira, N. S.;Athayde-Filho, P. F. de;Rodrigues, L. C.;Souza, M. F. V. de;Barbosa-Filho, J. M. and Batista, L. M. (2011). Bioactivities from marine algae of the genus Gracilaria. Int. J. Mol. Sci., https://doi.org/10.3390/ijms12074550.

Anandarajagopal, K. (2013). *In-vitro* Anti-Inflammatory Evaluation of Crude Bombax ceiba Extracts. European J. Med. Plants, 3(1). https://doi.org/10.9734/ejmp/2013/2113.

Annegowda, H. V.;Tan, P. Y.;Mordi, M. N.;Ramanathan, S.;Hamdan, M. R.;Sulaiman, M. H. and Mansor, S. M. (2013). TLC-Bioautography-Guided Isolation, HPTLC and GC-MS-Assisted Analysis of Bioactives of *Piper betle* Leaf Extract Obtained from Various Extraction Techniques: *In vitro* Evaluation of Phenolic Content, Antioxidant and Antimicrobial Activities. Food Anal. Methods, 6(3). https://doi.org/10.1007/s12161-012-9470-y.

Aravindan, S.;Ramraj, S. K.;Somasundaram, S. T.;Herman, T. S. and Aravindan, N. (2015). Polyphenols from marine brown algae target radiotherapy-coordinated EMT and stemness-maintenance in residual pancreatic cancer. Stem Cell Res. Ther., 6(1). https://doi.org/10.1186/s13287-015-0173-3.

Awad, N. E. (2000). Biologically active steroid from the green alga *Ulva lactuca*. Phyther. Res., 14(8). https://doi.org/10.1002/1099-1573(200012)14:8<641::AID-PTR668>3.0.CO;2-R.

Brand-Williams, W.;Cuvelier, M. E. and Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci. Technol. https://doi.org/ 10.1016/S0023-6438(95)80008-5.

Chanda, S.;Dave, R.;Kaneria, M. and Nagani, K. (2010). Seaweeds : A novel , untapped source of drugs from sea to combat Infectious diseases. Curr. Res. Technol. Educ. Top. Appl. Microbiol. Microb. Technol.

Das, B. K.;Pradhan, J.;Pattnaik, P.;Samantaray, B. R. and Samal, S. K. (2005). Production of antibacterials from the freshwater alga *Euglena viridis* (Ehren). World J. Microbiol. Biotechnol., 21(1). https://doi.org/10.1007/s11274-004-1555-3.

Ebrahimzadeh, M. A.;Khalili, M. and Dehpour, A. A. (2018). Antioxidant activity of ethyl acetate and methanolic extracts of two marine algae, *nannochloropsis oculata* and *gracilaria gracilis* - An *in vitro* assay. Brazilian J. Pharm. Sci., 54(1). https://doi.org/10.1590/s2175-97902018000117280.

Edwards, K. F.;Pfister, C. A. and Alstyne, K. L. Van. (2006). Nitrogen content in the brown alga *Fucus gardneri* and its relation to light, herbivory and wave exposure. J. Exp. Mar. Bio. Ecol., 336(1). https://doi.org/10.1016/j.jembe.2006.04.013.

Ellman, G. L.;Courtney, K. D.;Andres, V. and Featherstone, R. M. (1961). A new and

rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7(2). https://doi.org/10.1016/0006-2952(61)90145-9.

Ely, R.;Supriya, T. and Naik, C. G. (2004). Antimicrobial activity of marine organisms collected off the coast of South East India. J. Exp. Mar. Bio. Ecol., 309(1). https://doi.org/10.1016/j.jembe.2004.03.010.

Farasat, M.;Khavari-Nejad, R. A.;Nabavi, S. M. B. and Namjooyan, F. (2014). Antioxidant activity, total phenolics and flavonoid contents of some edible green seaweeds from northern coasts of the Persian gulf. Iran. J. Pharm. Res., 13(1). https://doi.org/10.22037/ijpr.2014.1448.

Farias, D. F.;Souza, T. M.;Viana, M. P.;Soares, B. M.;Cunha, A. P.;Vasconcelos, I. M.;Ricardo, N. M. P. S.;Ferreira, P. M. P.;Melo, V. M. M. I. and Carvalho, A. F. U. (2013). Antibacterial, antioxidant, and anticholinesterase activities of plant seed extracts from Brazilian semiarid region. Biomed Res. Int., 2013. https://doi.org/10.1155/2013/510736.

Feky, S. E. El;Abd El Hafez, M. S. M.;Abd El Moneim, N. A.;Ibrahim, H. A. H.;Okbah, M. A.;Ata, A.;Sedfy, A. S. El and Hussein, A. (2022). Cytotoxic and antimicrobial activities of two new sesquiterpenoids from red sea brittle star *Ophiocoma dentata*. Sci. Rep., 12(1):8209. https://doi.org/10.1038/s41598-022-12192-7.

Ferreira, I. C. F. R.;Baptista, P.;Vilas-Boas, M. and Barros, L. (2007). Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity. Food Chem., 100(4). https://doi.org/10.1016/j.foodchem.2005.11.043.

Gao, H.;Su, R.;Zhou, F.;Zhang, C. and Shi, X. (2020). Extraction and identification of toxic organic substances from decaying green alga *Ulva prolifera*. Harmful Algae, 93. https://doi.org/10.1016/j.hal.2020.101786.

Heim, K. E.;Tagliaferro, A. R. and Bobilya, D. J. (2002). Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. J. Nutr. Biochem. https://doi.org/10.1016/S0955-2863(02)00208-5.

Ibtissam, C.;Hassane, R.;José, M. L.;Francisco, D. S. J.;Antonio, G. V. J.;Hassan, B. and Mohamed, K. (2009). Screening of antibacterial activity in marine green and brown macroalgae from the coast of Morocco. AFRICAN J. Biotechnol., 8(7). https://doi.org/10.5897/AJB2009.000-9200.

Kadaikunnan, S.;Rejiniemon, S. S.;Khaled, J. M.;Alharbi, N. S. and Mothana, R. (2015). *In-vitro* antibacterial, antifungal, antioxidant and functional properties of *Bacillus amyloliquefaciens*. Ann. Clin. Microbiol. Antimicrob., 14(1). https://doi.org/ 10.1186/s12941-015-0069-1.

Kalaivani, G.;Hemalatha, N. and Poongothai, E. (2016). Screening of marine brown algae associated potential bacteria producing antagonistic bioactive compounds against uti pathogens. Int. J. Pharm. Bio. Sci., 7(2).

Kantachumpoo, A. and Chirapart, A. (2010). Components and antimicrobial activity of

polysaccharides extracted from thai brown seaweeds. Kasetsart J. - Nat. Sci., 44(2).

Khotimchenko, M.;Tiasto, V.;Kalitnik, A.;Begun, M.;Khotimchenko, R.;Leonteva, E.;Bryukhovetskiy, I. and Khotimchenko, Y. (2020). Antitumor potential of carrageenans from marine red algae. Carbohydr. Polym. https://doi.org/ 10.1016/ j.carbpol.2020.116568.

Kim, M. M. and Kim, S. K. (2010). Effect of phloroglucinol on oxidative stress and inflammation. Food Chem. Toxicol., 48(10). https://doi.org/10.1016/j.fct. 2010.07.029.

Lavanya, R.;Veerappan, N. and Nadu, P.-T. (2011). Antibacterial Potential of Six Seaweeds Collected from Gulf of Mannar of Southeast Coast of India. Adv. Biol. Res. (Rennes)., 5(1).

Lobban, C. S. and Harrison, P. J. (1994). Seaweed ecology and physiology. Seaweed Ecol. Physiol. https://doi.org/10.2307/2261617.

Lopes, D.;Melo, T.;Rey, F.;Meneses, J.;Monteiro, F. L.;Helguero, L. A.;Abreu, M. H.;Lillebø, A. I.;Calado, R. and Domingues, M. R. (2020). Valuing Bioactive Lipids from Green, Red and Brown Macroalgae from Aquaculture, to Foster Functionality and Biotechnological Applications. Molecules, 25(17). https://doi.org/10.3390/ molecules 25173883.

Machado, L. P.;Carvalho, L. R.;Young, M. C. M.;Cardoso-Lopes, E. M.;Centeno, D. C.;Zambotti-Villela, L.;Colepicolo, P. and Yokoya, N. S. (2015). Evaluation of acetylcholinesterase inhibitory activity of Brazilian red macroalgae organic extracts. Rev. Bras. Farmacogn., 25(6). https://doi.org/10.1016/j.bjp.2015.09.003.

Mohy El-Din, S. M. and El-Ahwany, A. M. D. (2016). Bioactivity and phytochemical constituents of marine red seaweeds (*Jania rubens* , *Corallina mediterranea* and *Pterocladia capillacea*). J. Taibah Univ. Sci., 10(4). https://doi.org/10.1016/j.jtusci. 2015.06.004.

Molyneux, P. (2004). The Use of the Stable Free Radical Diphenylpicryl-hydrazyl (DPPH) for Estimating Antioxidant Activity. Songklanakarin J. Sci. Technol., 26(December 2003). https://doi.org/10.1287/isre.6.2.144.

Moreau, J.;Pesando, D. and Caram, B. (1984). Antifungal and antibacterial screening of Dictyotales from the French Mediterranean coast. Hydrobiologia, 116–117(1). https://doi.org/10.1007/BF00027737.

Nikolova, B.;Semkova, S.;Tsoneva, I.;Antov, G.;Ivanova, J.;Vasileva, I.;Kardaleva, P.;Stoineva, I.;Christova, N.;Nacheva, L. and Kabaivanova, L. (2019). Characterization and potential antitumor effect of a heteropolysaccharide produced by the red alga *Porphyridium sordidum*. Eng. Life Sci., 19(12). https://doi.org/10.1002/elsc.201900019.

Olasehinde, T. A.;Olaniran, A. O. and Okoh, A. I. (2019). Macroalgae as a valuable source of naturally occurring bioactive compounds for the treatment of Alzheimer's disease. Mar. Drugs. https://doi.org/10.3390/md17110609.

Patra, J. K.;Rath, S. K.;Jena, K.;Rathod, V. K. and Thatoi, H. (2008). Evaluation of antioxidant and antimicrobial activity of seaweed (Sargassum sp.) extract: A study on inhibition of glutathione-S-transferase activity. Turkish J. Biol., 32(2).

Pushpa Bharathi, N.;Jayalakshmi, M.;Amudha, P. and Vanitha, V. (2019). Phytochemical screening and in vitro antioxidant activity of the seagrass *Cymodocea serrulata*. Indian J. Geo-Marine Sci., 48(8).

Rajasulochana, P.;Dhamotharan, R.;Krishnamoorthy, P. and Murugesan, S. (2009). Antibacterial Activity of the Extracts of Marine Red and Brown Algae. Marsl. Press J. Am. Sci., 5(3).

Rashedy, S. H.;Abd El Hafez, M. S. M.;Dar, M. A.;Cotas, J. and Pereira, L. (2021). Evaluation and Characterization of Alginate Extracted from Brown Seaweed Collected in the Red Sea. Appl. Sci., 11(14):6290. https://doi.org/10.3390/app11146290.

Saadaoui, I.;Rasheed, R.;Abdulrahman, N.;Bounnit, T.;Cherif, M.;Jabri, H. Al and Mraiche, F. (2020). Algae-derived bioactive compounds with anti-lung cancer potential. Mar. Drugs. https://doi.org/10.3390/md18040197.

Sakanaka, S.;Tachibana, Y. and Okada, Y. (2005). Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). Food Chem., 89(4). https://doi.org/10.1016/j.foodchem.2004.03.013.

Salvador, N.;Gómez Garreta, A.;Lavelli, L. and Ribera, M. A. (2007). Antimicrobial activity of Iberian macroalgae. Sci. Mar., 71(1). https://doi.org/10.3989/scimar.2007.71n1101.

Siddhanta, A. K.; Mody, K. H.; Ramavat, B. K.; Chauhan, V. D.; Garg, H. S.;Goel, A. K.;Jinandra Doss, M.;Srivastava, M. N.;Patnaik, G. K. and Kamboj, V. P. (1997). Bioactivity of marine organisms: Part VIII - Screening of some marine flora of Western Coast of India. Indian J. Exp. Biol., 35(6).

Silva, F. R. P. da;e Silva Conceição Pinto, M.;Carvalho França, L. F. de;Alves, E. H. P.;Santos Carvalho, J. dos;Lenardo, D. Di;Brito, T. V.;Medeiros, J. V. R.;Oliveira, J. S. de;Freitas, A. L. P.;Barros, F. C. N.;Reis Barbosa, A. L. dos and Vasconcelos, D. F. P. (2019). Sulfated polysaccharides from the marine algae *Gracilaria caudata* prevent tissue damage caused by ligature-induced periodontitis. Int. J. Biol. Macromol., 132. https://doi.org/10.1016/j.ijbiomac.2019.03.194.

Singleton, V. L.;Orthofer, R. and Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods Enzymol., 299. https://doi.org/10.1016/S0076-6879(99)99017-1.

Unnikrishnan, P. S.;Suthindhiran, K. and Jayasri, M. A. (2015). Antidiabetic potential of marine algae by inhibiting key metabolic enzymes. Front. Life Sci., 8(2). https://doi.org/10.1080/21553769.2015.1005244.

Vijayabaskar, P. and Shiyamala, V. (2011). Antibacterial Activities of Brown Marine Algae (*Sargassum wightii* and *Turbinaria ornata*) from the Gulf of Mannar

Biosphere Reserve. Adv. Biol. Res. (Rennes)., 5(2).

Vijayraja, D. and Jeyaprakash, K. (2019). Phytochemical analysis, in vitro antioxidant activity and anti-inflammatory activity of *turbinaria ornata* in human RBC model. Indian J. Geo-Marine Sci., 48(4).

Wong, K. H. and Cheung, P. C. K. (2001). Nutritional evaluation of some subtropical red and green seaweeds Part II. *In vitro* protein digestibility and amino acid profiles of protein concentrates. Food Chem., 72(1). https://doi.org/10.1016/S0308-8146(00)00176-X.

Yamamoto, M.;Baldermann, S.;Yoshikawa, K.;Fujita, A.;Mase, N. and Watanabe, N. (2014). Determination of volatile compounds in four commercial samples of japanese green algae using solid phase microextraction gas chromatography mass spectrometry. Sci. World J., 2014. https://doi.org/10.1155/2014/289780.

Yoon, N. Y.; Chung, H. Y.; Kim, H. R. and Choi, J. S. (2008). Acetyl- and butyrylcholinesterase inhibitory activities of sterols and phlorotannins from *Ecklonia stolonifera*. Fish. Sci., 74(1). https://doi.org/10.1111/j.1444-2906.2007.01511.x.

Yuan, Y. V.;Bone, D. E. and Carrington, M. F. (2005). Antioxidant activity of dulse (*Palmaria palmata*) extract evaluated *in vitro*. Food Chem., 91(3). https://doi.org/10.1016/j.foodchem.2004.04.039.

Zandi, K.;Tajbakhsh, S.;Nabipour, I.;Rastian, Z.;Yousefi, F.;Sharafian, S. and Sartavi, K. (2010). *In vitro* antitumor activity of *gracilaria corticata* (a red alga) against jurkat and molt-4 human cancer cell lines. African J. Biotechnol., 9(40). https://doi.org/10.5897/AJB10.602.

Zhang, X.;Wang, X.;Wang, M.;Cao, J.;Xiao, J. and Wang, Q. (2019). Effects of different pretreatments on flavonoids and antioxidant activity of *Dryopteris erythrosora* leave. PLoS One, 14(1). https://doi.org/10.1371/journal.pone.0200174.

Zhao, C.;Yang, C.;Liu, B.;Lin, L.;Sarker, S. D.;Nahar, L.;Yu, H.;Cao, H. and Xiao, J. (2018). Bioactive compounds from marine macroalgae and their hypoglycemic benefits. Trends Food Sci. Technol. https://doi.org/10.1016/j.tifs.2017.12.001.

Zhuang, Y.; Guo, J.;Chen, L.; Li, D.; Liu, J. and Ye, N. (2012). Microwave-assisted direct liquefaction of *Ulva prolifera* for bio-oil production by acid catalysis. Bioresour. Technol., 116. https://doi.org/10.1016/j.biortech.2012.04.036.