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Research Article

Evaluation of antioxidant and antibacterial activities of bioactive compounds extracted from the green microalgae (*Dunaliella salina*) on *Enterococcus faecalis, Bacillus cereus*, and *Pseudomonas aeruginosa*

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Abstract

The marine microalgae are producing modern technological compounds. Nowadays, algae are the most important natural resource of carotenoids. The production of strategic products in the modern pharmaceutical industry depends on these compounds. In this study, ethanol, aqua, and ethanol extracts: aqua (50:50) *Dunaliella salina* were extracted under different temperature (10, 30, and 50°C) and times (15 and 30 min) with ultrasound waves. Also, the minimum inhibitory concentration (MIC) of antibiotics and the minimum bacterial concentration (MBC) of the extracts were obtained on Gram-positive and Gram-negative bacteria using the microdilution broth method. The results of antioxidant activity of *D.salina* showed that the highest antioxidant capacity was based on DPPH and IC50 tests for microalgae (D. Salina) in ethanol and ethanol: water extraction (15 minutes at 30°C). ethanol (30 min / 30°C), ethanol: water and water (15 min / 10°C) and water (30 min / 10°C), respectively. IC50 values of 0.045, 0.096, 0.45, 0.48, 0.48 and 0.481 were measured as the highest antioxidant activity. MIC in Gram-positive bacteria is related to ethanolic extract (15 min/30°C) and ethanol/water extract (30 min/30°C). MIC was obtained in gram-negative bacteria in ethanol/water (30 min/30°C) and water extract (30 min/10°C). The results of DPPH inhibition in the studied treatments (temperature and time) showed that the antioxidant activity in ethanol, ethanol: water (15 minutes / 30°C) and ethanol: water (30 minutes / 30°C) showed the best results A significant difference was observed in the MIC obtained from the aqueous ethanol treatments compared to other treatments ($p \leq 0.05$). Also, no significant difference was observed in the minimum MIC levels in the water treatments ($p \geq 0.05$). The concentration of MIC and MBC in this bacterium in all extracts are equal to each other. The best MIC in Gram-negative bacteria (P. aeruginosa) was observed in AE (30 min/30°C), and EWE (30 min/10°C) extracts at the rate of 6.25 µg/ mL; investigations showed that equal times in the extraction process Extracts with different temperatures do not have significant differences. According to the results of this study, the halophyte green microalga *D. salina* can be used as a potential source of antioxidant and antibacterial activity in the food and pharmaceutical industries.

Introduction

Microalgae are a group of microorganisms that account for 40% of global production and 50% of carbon fixation annually. They produce organic matter through photosynthesis (Dolganyuk *et al.,* 2020). Microalgae are present in all biotopes of the biosphere and play an important role as producers in food chains. At aquatic ecosystems, they create the aquatic food web (Martinez *et al.,* 2018; Levy *et al.,* 2000). Marine microorganisms are new rich sources of secondary metabolites bioactive which have high potential in the development of pharmaceutical industry (Singh *et al.,* 2016; Gautheir *et al.,* 2020., Dolganyuk *et al.,* 2020). One of the advantages of algae is their flexible metabolism. Based on this, the production of marine medicines leads to the production of various compounds with different applications in the food, pharmaceutical, health, medical, and environmental science (Martinez *et al.,* 2018; Farasat *et al.,* 2023). Algal are also an important source of vitamins, minerals, antioxidant and natural colors. Changing consumer attitudes have increased global demand for superfoods (Glasso *et al.,* 2019). Algae primary and secondary metabolites can be used as active substances in biological compounds. Various applications of these compounds in the pharmaceutical, medical and food industries with antiviral, antibacterial, antifungal and anticancer effects have been identified and extracted in many studies on algae species (Candan *et al.,* 2007., Al-Haj *et al.,* 2009; Havas *et al.,* 2022). Microalgae are rich in phytochemical compounds, phytonutrients, carotenoids, and polysaccharides. Polysaccharides include beta-glucan, antioxidants, vitamins, enzymes, phenols, sterols, and long-chain unsaturated fatty acids (Assant *et al.,* 2018; Harvy *et al.,* 2020; Mousavi Nadushan and Hosseinzadeh, 2020). *D. salina* belongs to the Chlorophyceae family, order Volvocales, and phylum Chlorophyta. The microalgae *D. salina* is a primary producer saline and hypersaline environments (Hosseini Tafreshi and Shariat., 2009). The green microalgae can live in 45% salinity (450,000 mg/ L) (Cakmak *et al.,* 2014).

Dunaliella is one of the richest natural sources of beta-carotene and contains various carotenoids. Beta-carotene is a terpenoid pigment. Its use as a precursor of vitamin A, its antioxidant and anticancer properties, and free radical scavenger is valuable in treating diseases (Barkia *et al.,* 2019; Hosseini Tafreshi and Shariat., 2009). Beta-carotene biosynthesis in the membrane of chloroplasts is performed through a series of consecutive reactions from the degradation of photosynthetic products (starch). It turns into dihydroxyacetone phosphate (DHAP) and 2-phosphoglyceraldehyde (PGA2) (Dolganyuk *et al.,* 2020). Microalgae are excellent sources of antioxidant vitamins; for example, D. salina contains 3.46% carotenoids (based on dry weight) and 12.1 mg/ L vitamin E (Ambrico *et al.,* 2020). *Dunaliella* contains high amounts of two types of cis and trans isomers of beta-carotene, astaxanthin, and alpha-carotene. Research shows that the consumption of two substances, beta-carotene and astaxanthin, and the combined consumption of beta-carotene and alpha-carotene can reduce the risk of various cancers and heart diseases (Gurlek *et*

al.,2020). Also, research has shown that D. salina extract, rich in colorless carotenoids, is an active anti-inflammatory and anti-aging substance (Suh *et al.,* 2017a; Glasso *et al.,* 2019; Havas *et al.,* 2022). Also, extracts from different microalgae species have obtained new treatments for all cancers (liver, breast, intestine, and lung) (Martinez *et al.,* 2018). In addition, clinical studies have been conducted on humans. Cis-9 beta-carotene in *Dunaliella* extracts effectively treats arteriosclerosis, psoriasis (Harvey and Ben-Amotz*.,* 2020) and hyperlipidemia in male smokers (Levy *et al.,* 2020). The antioxidant property of *D. salina* increases under stress conditions compared to normal conditions. *Dunaliella* green algae start synthesizing beta-carotene when stressed (decrease in nitrogen concentration, increase in salt concentration, and high temperature). In this case, the water turns red due to beta-carotene accumulation in the cell (Widowati *et al.*, 2021). At high salinity, *D. salina* increases the production of specific secondary metabolites, such as beta-carotene, by up to 14%, indicating the production of antibacterial metabolites (Cakmak *et al.,* 2014; Kilic *et al.,* 2019). In recent years, the widespread use of antibiotics has caused the emergence of resistant strains of microorganisms worldwide. Therefore, obtaining new marine resources is critical (Little *et al*., 2021). The study of the antibacterial property of *D. salina* on the Gram-positive bacterium that causes tooth decay (*Streptococcus muntas*) showed that MIC = 6.25 mg /mL has a suitable antibacterial effect (Jafari *et al.,* 2018). Gram-positive and Gram-negative bacteria are responsible for diseases in plants that cause significant economic losses in crop production. The cause of speck spot disease on tomato plants was studied and showed that hexane extract with MIC = 0.3 mg /mL effectively controlled the disease (Ambrico *et al.,* 2020). The antibacterial activity was performed on aquatic pathogens (fish, shrimp, oysters) to hold two species of *Psedomonas fluorescence* and *Vibrio harveyi*. The results showed that using bioactive compounds in microalgae is very effective in producing ultra-beneficial foods in the development of the aquaculture industry and disease control (Farasat *et al.,* 2015; Widowati *et al.,* 2021). High beta-carotene and phenolics of *D. salina* enhance the immune system and provide optimal growth conditions for aquatic life. Green microalgae in stressful environments can produce compounds with antibacterial activity (Challouf *et al.,* 2012; Senhulinho *et al.,* 2018). Also, the possibility of comparing the bioactive compounds extracted from the studied microalgae with changes in the type of solvents, extraction time, and temperature can provide the best effect. This study aims to the impact of changes (temperature and time) on DPPH extracted from green microalga *D. salina* and MIC and MBC on three types of bacteria (*Enterococcus faecalis*, *Bacillus cereus*, and *Pseudomonas aeruginosa*).

Materials and methods

Culture of microalgae

The microalgae *D.sallina* was isolated from Urmia lake, Iran. Individual cells were morphologically studied under a light microscope (Labo America, NC-USA) and confirmed by sequencing 18s r RNA gene

using the PCR method. The single colonies of *D. salina* were maintained on the BG-11 and Johnson at 25±2°C , under low illumination (37µmol m^2/s) and unlimited aerated condition during 21-31 days. After colonization, pure cultures were prepared by sub culturing on the agar plate and then transferring to broth mediums

Extraction

Algae powder prepared, weighed (5 gr) and added 75 mL of solvent [E, A, and EA (50:50)]. Glass containers were subjected to ultrasound treatment with 40 kHz, 400 W conditions at (10, 30, and 50°C) for (15 and 30 min). After the extraction process, the extracts were filtered with filter paper (Watman 42). Then 18 treatments (for each solvent, six treatments based on time and temperature) were transferred to dark glass containers and placed in a refrigerator at 4°C (Borowitzka *et al.,* 1990).

DPPH radical scavenging activity

The scavenging effects of samples for DPPH radical were determined by the method of Von gadow *et al.* (1997). Briefly, 2.0 ml of aliquot of test samples was added to 2.0ml of 0.16 Mm DPPH methanolic solution. The mixture was vortexed for 1 min and then left to stand at room temperature for 30 min in the dark. The absorbance of all the sample solutions was measured at 517 nm. The scavenging effect (%) was calculated by using the formulae given by *Dunan et al.,* (2006).

% RSA= (A blank- A sample)/A blank*100

A sample is the sample's absorption after the desired time; A blank is the absorption of the DPPH solution and no model. Ascorbic acid (0.2 mg/ mL) was used as a standard sample. The experiment was performed in three replicates, (Taheri *et al.,* 2017).

Collection of bacteria

The human pathogenic bacterial strains Enterococcus faecalis, Pseudomonas aeruginosa and Bacillus cereus were used for this experiment. The human pathogenic bacteria were obtained from the Pasteur Institute of Iran. Muller-Hinton broth (HMB)was obtained from Hi-Media while solvents used were HPLC grade. The bacterial strains used in this study are shown in (Table 1).

Table1: Bacteria used in this study

Bacteria species	Bacterial code	Germs' reaction
Enterococcus faecalis	ATCC 1237	Germ-positive
Pseudomonas aeruginosa	ATCC1430	Germ-negative
Bacillus cereus	ATCC 1247	Germ-positive

Minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration was measured according to the methods of National Committee for Clinical Laboratory Standards (NCCLC). The extracts were selected for the solvents aqueous, ethanol, aqueous/ ethanol. The initial test concentration of extract was 1mg/ml. Each tube containing 2 mL of broth was inoculated with 5µ of bacterial suspension containing 108 CFU/mL of bacteria. The test tubes were incubated for

24 h at 37°C.MIC was determined as the lowest concentration of extract showing OD of 600nm of spectrophotometer. All the data were statistically analyzed. The wells, in which no growth occurred, were noted to specify the Minimum Bactericidal Concentration (MBC), 24 hours light wells were used to culture in Mueller Hinton agar plates, and incubated overnight at 37 °C. Bacterial colony observation, demonstrated the inhibitory effect of extracts on bacterial growth while absence of bacterial colony supported mortal effect (Rahman *et al.,* 2004; Ravi *et al.,* 2019).

Statistical analysis

All the values were expressed as Mean±standard Deviation (SD). The statistical significance was evaluated by Shapiro-Wilk and one-way Analysis of Variance (ANOVA) using SPSS version 13 and the individual comparisons were obtained by Duncan (Zar, 2007).

Results

Investigation of the biochemical composition of microalgae

Microalgae produce chemical molecules, including carbohydrates, proteins, lipids, nucleic acids, essential vitamins, and minerals. The cell content of each part depends on the specific strain of algae and their physiological responses to biological factors, and non-biological factors such as light intensity, light period, temperature, food, and growth phase are different. The primary chemical components of *D. salina* are shown in Table 2.

Table 2: Biochemical composition of *D. salina* (Barkia *et al.*, 2019).

Species	Class	Protein %(w/w)	Carbs %(w/w)	Fat %(w/w)
Dunaliella salina	Chlorophyceae	57%	32%	6%
Dunaliella sp.	Chlorophyceae	17-34%	14-57%	14-36%

DPPH radical scavenging activity

The results of neutralizing activity of free radicals (DPPH) and Ethanol extracts, of *D. salina* algae in concentrations of (0.01-0.05-0.1-0.5-1) %, in sound and thermal treatments are determined in Table 3 is provided. According to the obtained results, increasing the engagement of EAE increases the amount of DPPH. The concentration of 1% of EAE in the six thermal-acoustic treatments provided has the highest amount of DPPH compared to other engagements. Also, the acoustic-thermal treatment (15 min/30°C) in the five concentrations provided is 48.30, 55.90, 64.49, 69.97, and 72.58, respectively. That is having the highest level of neutralization activity (Table 3).

Table 3: The results of investigating the free radical inhibition (DPPH) of *D. salina* (EE).

solvent (%)	15 min/10°C	30 min/10°C	15 min/30°C	30 min/30°C	15 min/50°C	30 min/50°C
0.01	5.48	10.44	48.30	12.01	14.09	22.71
0.05	7.31	7.39	55.09	15.66	16.97	25.58
0.1	16.71	19.06	64.49	21.14	27.93	32.37
0.5	19.32	22.80	69.97	43.08	41.25	42.29
1	48.82	60.31	72.58	59.00	69.97	50.99

The results of neutralizing activity of free radicals (DPPH), AE *D. salina* algae in concentrations of (0.01, 0.05, 0.1, 0.5, and 1) % in sound and thermal treatment are presented in Table 4. According to the obtained data, the amount of DPPH increases with the increase in the concentration of EAE. The concentration of 1% of EAE in the six treatments (thermal-sonic) presented has the highest amount compared to other engagements. Also, thermal-sound treatments were respectively (15 min/10°C) with 0.01 concentration with 16.33%, (30 min/30°C) with 0.05 concentration, with 33.87 %, (30 min/10°C) with 0.5 and 0.1 with 52.01 and 39.51% respectively, and (15 min/10°C), at a concentration of 1% with 71.64%, had the highest level of neutralization activity. Also, the concentration of 0.01% in the acoustic-thermal patient (30 min/10°C) had the lowest neutralization activity with 2.06% (Table 4).

Table 4: The results of investigating the free radical inhibition (DPPH) of *D. salina* (AE).

solvent (%)	15 min/10°C	30 min/10°C	15 min/30°C	30 min/30°C	15 min/50°C	30min /50°C
0.01	16/33	2/06	6/65	21/97	5/24	4/03
0.05	22/37	19/75	19/95	33/87	6/65	11/29
0.1	27/62	39/51	34/27	36/89	15/92	29/23
0.5	51/81	52/01	44/55	45/36	25/00	30/04
1	71/64	57/86	50/20	52/21	46/37	43/34

The results of neutralizing activity of free radicals (DPPH), EAE extracts, *D. salina* algae in concentrations of (0.01, 0.05, 0.1, 0.5, and 1) % in sound and thermal treatments are determined in Table 5. The results showed that the amount of DPPH increases with the increase in the concentration of EAE. Treatment (15 min/10°C) in (0.01, 0.05, 0.5, and 1) % including (38.02, 46.81, 72.74 and 75.38%), respectively, gave the highest level of neutralization activity. The treatment (30 min/10 °C) at a concentration of 0.01 has the lowest neutralization activity with 2.63% (Table 5).

Table 5: The results of investigating the free radical inhibition (DPPH) of *D. salina* (EAE).

solvent (%)	15 min/10°C	30 min/10°C	15 min/30°C	30 min/30°C	15 min/50°C	30 min/50°C
0.01	38.02	2.63	36.92	14.72	22.63	19.78
0.05	46.81	12.96	46.59	25.93	32.30	37.58
0.1	51.20	46.61	51.64	36.92	39.34	47.03
0.5	72.74	45.49	61.09	50.76	44.83	47.91
1	75.38	52.52	69.01	55.38	55.02	51.42

Determination of IC50

The results of the percent inhibition IC50 of *D. salina* extracts are presented in Table 6. The comparison between their data shows that in extracts of EE, and EAE (15 min /30°C), with IC50, is equal to 0.045, and 0.096, EAE (30 min/30°C), IC50 is equal to 0.45, EAE, AE (15 min/10°C), IC50 for both cases is equivalent to 0.48, and AE (30 min/10°C), IC50 is equal to 0.481. The highest IC50 values were obtained, respectively (Table 6).

Table 6: The results of the IC_{50} values in *D. salina* extracts.

IC_{50} extracts	30 min/50°C	15 min/50°C	30 min/30 °C	15 min/30 °C	30 min/10°C	15 min/10°C
EE	0.78[c]	0.66[c]	0.71[c]	0.045[a]	0.83[c]	1.15[d]
AE	1.15[d]	1.07[d]	0.96[d]	0.99[d]	0.481[b]	0.48[b]
EAE	0.97[d]	0.89[c]	0.4۰[b]	0.096[a]	0.95[d]	0.48[b]

Ethanol extract (EE), Aqueous extract (AE), and ethanol/Aqueous extract (EAE). *The non-similar letters in each column and row indicate a significant between (p≤0.05).

Antibacterial activity of different extracts of D. salina

The results of the MIC of the growth of different hydrolysates extracts of *D. salina* against *B. cereus* are presented in Table 7. As a result of the comparison of varying EE, AE, and EAE, extracts of *D. salina* in different extraction conditions. The MIC for *B. cereus* bacteria in the EAE and EE in the treatment (15 min/30°C) equals 12.5 and 6.25 mg/ mL. The EAE in the treatment (30 min/30°C) equals 6.25 mg/ mL (Table 7).

Table 7: The MIC of growth of different *D. salina* extracts against *B. cereus* bacteria.

Sample	MIC	(600 OD)	MIC (mg /mL)
EAE, 15 min/30°C	5	0.54±0.04[b]	12.5
EE, 15 min/30°C	6	0.53±0.05[c]	6.25
EAE, 30 min/30°C	6	0.68±0.05[c]	6.25
EAE, 15 min/10°C	4	0.56±0.1[a]	25
AE, 15 min/10°C	4	0.58±0.07[a]	25
AE, 30 min/10°C	4	0.49±0.80[a]	25

Ethanol extract (EE), Aqueous Extract (AE), Ethanol extract (EE), and ethanol/Aqueous extract (EAE). *The non-similar letters in each column and row indicate a significant between (p≤0.05).

The results showed the MBC of different *D. salina* extracts against B. cereus bacteria, EE, AE, and EAE recorded the best results at a temperature of (15 min/30°C). The concentration of MIC and MBC in this bacterium in all hydrolysates are equal to each other. The results of the MIC of the growth of different extracts of *D. salina* against P. aeruginosa bacteria are presented in Table 8. The results of the microbial tests of other hydrolysates of *D. salina* prepared against *P. aeruginosa* bacteria have been different after the dilution of each sample. The minimum dilution required to inhibit the bacteria or individual MICs has been shown (Table 8).

Table 8: The MIC of growth of different *D. salina* extracts against *P. aeruginosa* bacteria.

Sample	MIC	(600 OD)	MIC (mg /mL)
EAE, 15 min/30°C	4	0/66±0/08[a]	25
EE, 15 min/30°C	4	0/65±0/06[a]	25
EAE, 30 min/30°C	6	0/58±0/03[c]	6.25
EAE, 15 min/10°C	5	0/57±0/07[b]	12.5
AE, 15 min/10°C	5	0/55±0/04[b]	12.5
AE, 30 min/10°C	6	0/50±0/03[c]	6.25

Ethanol extract (EE), Aqueous extract (AE), and ethanol/Aqueous extract (EAE). *The non-similar letters in each column and row indicate a significant between (p≤0.05).

Comparison of AE, EE, and EAE extracts of D.salina

In different extraction conditions, the lowest MIC for *P. aeruginosa* (6.25 mg/ mL) was in the AE extracts in the treatment (30 min/10°C) and the EAE in the treatment (15 min/30°C). The AE extracts in the treatment (15 min/10°C) and treatment (30 min/30°C), with the concentration (12.5 mg/ mL), the lowest MIC was obtained (Table 8). The results show that the MIC and MBC in this bacterium are equal in all extracts. The results of the MIC of the growth of different *D. salina* hydrolysates against *E. faecalis* bacteria are presented in Table 9. As a result of the comparison of different AE, EE, and EAE extracts of *D. salina* in other extraction conditions, the lowest MIC in *E. faecalis* was found in the treated EE (15 min/30°C), and the treated EAE (15 min/10°C). EAE extracts of treatment (15 min/30°C) and AE hydrolysate of treatment (30 min/10°C) were obtained at the rate of 6.25 mg mL^{-1}, the lowest MIC. The highest inhibitory concentration was observed in the aqueous extracts of the treatment (15 min/10°C) with the attention of 100 mg/ mL (Table 9).

Table 9: The MIC of the growth of different *D. salina* extracts against *E. faecalis* bacteria.

Sample	MIC	(600 OD)	MIC (mg mL^{-1})
EAE, 15 min/30Ċ	6	0.56±0/05^{c}	6.25
EE, 15 min/30Ċ	6	0.63±0/06^{c}	6.25
EAE, 30 min/30Ċ	3	0.58±0/07^{b}	50
EAE, 15 min/10Ċ	6	0.66±0/05^{c}	6.25
AE, 15 min/10Ċ	2	0.48±0/2^{a}	100
AE, 30 min/10Ċ	6	0.59±0/02^{c}	6.25

Ethanol extract (EE), Aqueous extract (AE), and ethanol/Aqueous extract (EAE), *The non-similar letters in each column and row indicate a significant between (p≤0.05).

The results of MBC show different extracts of *D. salina* versus *E. faecalis*. The concentration of MIC and MBC in this bacterium are equal to each other in all extracts. EE extracts treatment (15 min/30°C) and EAE treatment (30 min/30°C) on Gram-positive bacteria *E. faecalis* and *B. cereus* obtained the best results in MIC. *E. faecalis* and *B. cereus* (Gram-positive bacteria) to *P. aeruginosa* (Gram-negative bacteria) MIC values showed significant differences (p<0.05). The best MIC in *P. aeruginosa* bacteria was observed in EWE extracts during treatment (30 min/30°C) and treatment (30 min/10°C).

Discussion

The amount of primary biochemical compounds in the species (*D. salina*) was recorded with 70% protein, 32% carbohydrate, and 6% fat. *Chlorella vulgaris,* with 14-22% fat, and *Spirulina maxima,* with 60-71% protein, are richer in fat and protein compared to the species studied in this research (Becker, 2007). Marine resources have attracted worldwide attention due to their various biological activities. Proteins play an essential role in the structure and metabolism of microalgae cells. The protein content of microalgae can compete qualitatively and quantitatively

with conventional protein sources (Moheimanian *et al.,* 2021).

Quantitatively, reports have shown several species of microalgae have a very high protein content. This amount can vary from 42% to more than 70% in certain cyanobacteria and up to 58% in *Chlorella* based on the dry weight. The use of microalgal proteins in foods has been limited so far, mainly due to the presence of non-protein components that can affect the color and taste of products. In addition, the rigid cell wall of some strains of green microalgae can reduce the extraction efficiency of intracellular proteins, and whole biomass applications can lead to low digestibility in humans (Barkia *et al.,* 2019). Due to increasing concerns about diseases caused by oxidative stress, many therapeutic approaches and herbal medicines have been developed to identify natural compounds rich in nutritional antioxidants. Seaweeds have been proposed as essential producers of antioxidants since the discovery of new compounds with biological activities. The ability to survive oxidizing conditions increases the cell's antioxidant content. It causes the production of secondary antioxidant metabolites. Despite various antioxidants in plasma, the body's defense system alone cannot eliminate the free radicals created in the body. Therefore, it needs to provide antioxidants from external sources, which are supplied through food and pharmaceutical sources. Thus, the demand for natural antioxidant compounds has increased as an alternative to synthetic antioxidants. Artificial antioxidants such as butylated hydroxyl toluene and anisole have carcinogenic and toxic effects. So the need for solid antioxidants with less toxicity and greater effectiveness is inevitable (Barbagallo *et al.,* 2015; Coulombier *et al.,* 2021)

One part of prooxidants is reactive ROS produced during normal metabolism through the respiratory chain during cellular immune system function. Cellular components can trigger a free radical chain reaction if they do not effectively neutralize ROS which is responsible for cell, tissue, and gene damage. Lipid membrane peroxidation, mitochondrial swelling, and post-translational protein modifications affect oxidative stress. This oxidative damage is associated with several degenerative diseases such as Alzheimer's, Parkinson's, atherosclerosis, rheumatoid arthritis, cancer, and premature aging (Barkia *et al.,* 2019 ; Cezare *et al.,* 2019). The results, showed that the highest antioxidant capacity based on the DPPH and IC_{50} the extracts of EE and EAE (15 min/30°C), EAE (30 min/30°C), EAE and AE (15 min/10°C) and AE (30 min/10°C), respectively. The IC_{50} values equal to 0.045, 0.096, 0.45, 0.48, 0.48 and 0.481 were measured the highest antioxidant activity. The results show that the EE hydrolysates at (15min/30°C) had the highest antioxidant activity. Also, the results show that increasing the temperature to 50Ċ has a significant decrease in the antioxidant properties of the studied algae ($p \leq 0.05$). Dichloromethane extracts from algae *Chaetoceros sp*. 1029 µmol TEg-1 and methanolic extract of *Nanno chloropsis* sp. were reported in some species. The 3224 µmol TEg-1 and *Chlorella* and *Phaeodactylum tricornutum* Aqueus extract with IC50 of 48.37 and 68.61,

respectively, show an excellent ability to neutralize superoxide radicals (Guzman *et al.,* 2001). In a study, Farasat *et al.* (2012) investigated the antioxidant activities of the methanolic extract of the green algae *Caulerpa sertularioides f.farlowii* from the northern coast of the Persian Gulf. Their results showed that the methanolic extract prepared from the algae has specific characteristics. In their research, they obtained the IC50 between 0.797 and 14.13 mg g in the methanolic extract of the studied algae. Hemalatha *et al.* (2013) investigated the antioxidant properties of different extracts from *D.salina* . Their results showed that the maximum antioxidant activity was recorded in the methanolic extract. According to previous studies, the methanolic extract of green microalgae showed good antioxidant potential. The antioxidant properties of the methanolic extract of *Colpomenia sinousa* seaweed collected from the coast of Chabahar showed that the concentration of 1 $mgml^{-1}$ recorded the highest amount of free radical removal (DPPH) of 55.45% (Taheri and Moradi, 2018). High concentration increases the number of hydroxyl groups in the reaction medium due to more antioxidant compounds, including phenolic compounds. In this situation, the possibility of donating hydrogen to free radicals and increasing the inhibitory power of the extract increases (Taheri *et al.,* 2017). In a similar study optimizing the extraction of antioxidants from *Dunaliella salina* microalgae was done by pressurized fluids, results were obtained: the hexane extract has recorded a high activity of up to 1118 µmol TEg-1 (Herrero *et al.,* 2006; Hemalatha *et al.,* 2013). A study of Ethanol extracts, *Cholromomas* sp. and *Botryidiopsidaceae* sp. recorded the ability to neutralize free radicals of DPPH, with IC_{50} of 0.97 and 1.53 µg/ mL, respectively (Suh *et al.,* 2017 a; Suh *et al.,* 2017 b). Also, the antioxidant activity of *Scenedesmus obliques* (IC_{50} =41 µg /mL) was recorded (Aremu *et al.,* 2015). Results by measuring DPPH in the species: *Galdieria sulphuraria, Ettlia carotinosa, Neochloris texensis, Chlorella vulgaris, Schizochytrium limacinum, Stichococcus bacillaris, Crypthecodinium cohnii* with an inhibition percentage between 89% and 95% with Aqueous or methanolic extract with a concentration of 250 µg/ mL was obtained (Gurlek *et al.,* 2020). In similar study,*Isochrysis galbana, Nannochloropsis oculata*, *Chaetoceros calcitrans, Scenedesmus qtadircauta,* and *Tetraselmis tetrahele* have a solid ability to inhibit lipid peroxidation. The inhibition percentage was 88 to 98% for methanolic extracts with 80 µg/ mL (Natrah *et al.,* 2007). The ability of the *Tetrasemis* genus has been reported at 3.4 µg/ mLfor inhibiting lipid peroxidation (CH_3OH and CH_2Cl_2 extracts) (Coulombier *et al.,* 2021). The antioxidant activity of the genus *Chlorella* has been confirmed by several researchers with different antioxidant assay methods (Aremu *et al.,* 2016).

Few links have been established between antioxidant activities and active metabolites, such as carotenoid content, phenolic compounds, and flavonoids. There is a positive correlation between gallic acid and vitamin E (Gurlek *et al.,* 2020). Also, the extracts extracted in different solvents have different degrees of antioxidant power, which is consistent with the results

of the present study. These compounds can be used as a practical therapeutic tool. Also, antioxidant activity was studied in six types of brown algae. The results showed that *Hormosira banksi* and *Sargassum vestium* have the highest inhibitory and antioxidant activity among the studied species. It is suggested that the bioactive compounds of these two species be used in the food and pharmaceutical industries (Dang *et al.,* 2018). The main active compounds with antioxidant properties in brown algae are phlorotannins and fucoxanthins (Imbs and Ermakova, 2021). An essential antioxidant is extracted from green microalgae; *Chlorella vulgaris* has significant benefits and is required for high performance worldwide. Algae need vitamins to grow; thiamine is the best adequate vitamin. The antioxidant has been identified. The antitumor effects of methanol extracts of *Chlorella vulgaris* and vitamin thiamine have been observed on prostate cancer (PG-3), hepatocellular carcinoma (HEPG-2), Colorectal carcinoma (HTC-116)and epithelioid carcinoma (HeLa) are other cases (Hamouda *et al.,* 2022).

Determining the inhibitory concentration (IC_{50}) of antioxidant ethanolic extract of red algae (*Eucheuma spinosum*) compared to vitamin C antioxidant showed. The level of antioxidant inhibition of vitamin C is more significant with 2588 µg mL^{-1}. Increased antibiotic resistance is a vital issue that increases the demand for new antibacterial compounds. Antibacterial activity in green microalgae extracts based on MIC and MBC evaluation of green microalgae (*D. salina*) extracts in a solvent. AE, EE, and EAE were performed. According to the obtained results, the best MIC in gram-positive bacteria (*B. cereus*) was observed in EE (15 min/30°C) and EWE (30 min/30°C); these two treatments showed the best temperature. It has been recorded in the extraction of active antibacterial metabolites at 30°C. The number of active metabolites decreases with increasing and decreasing temperature—more polar compounds were in EAE due to the presence of (aqua and alcohol). The polar compounds in the extracts with a minimum concentration of 6.25 µg/ mL quickly passed through the Gram-positive bacteria cell membrane and destroyed *B. cereus*.

A significant difference was observed in the MIC obtained from the aqueous ethanol treatments compared to other treatments ($p \leq 0.05$). Also, no significant difference was observed in the minimum MIC levels in the water treatments ($p \geq 0.05$). The concentration of MIC and MBC in this bacterium in all extracts are equal to each other. The best MIC in Gram-negative bacteria (*P. aeruginosa*) was observed in AE (30 min/30°C), and EWE (30 min/10°C) extracts at the rate of 6.25 µg/ mL; investigations showed that equal times in the extraction process Extracts with different temperatures do not have significant differences. Although the types of solvents are different, the two treatments with the lowest inhibitory error recorded a substantial difference from the other treatments ($p \leq 0.05$). Two EWE and EE treatments (15 min/30°C) have recorded the highest inhibition rate of 25 µg /mL. The lowest MIC value in Gram-positive bacteria (*E. faecalis*) in AE (30 min/10°C), EAE (15 min/30°C), EAE (30 min/30°C), and EE (15 min/30°C) in the amount of mg

6.25 µg/ mLwas observed. Also, the four treatments with the lowest inhibitory error recorded significantly different from the other treatments ($p \leq 0.05$). The temperature of 30°C has recorded the MIC in three treatments, indicating a high antibacterial activity suitable for stopping the movement of Gram-positive bacteria. The antimicrobial activity of liquid extracts under pressure from *D. salina* microalgae against several vital microorganisms for the food industry (*E. coli*, *Staphylococcus aureus, Candida albicans, Aspergillus nigers*) was studied in solvents (hexane, water, and ether) at temperatures of (40, 100, 160°C). Each solvent was obtained for best antimicrobial activity at the highest extraction temperature of 160Ċ. The extraction efficiency increased when ethanol was used as a solvent, and the aqueous extract recorded the lowest extraction efficiency—reporting antimicrobial activity by hexane extract. At the same time, ethanolic extracts also showed good antimicrobial activity (Herrero *et al.,* 2006). Research has highlighted the importance of microalgae in the production of antibiotics (Little *et al.,* 2021). *Chlorella vulgaris* (green microalgae) is capable of producing Chloralin. An antibacterial activity compound can prevent the growth of Gram-positive and Gram-negative bacteria. Studies have shown that some green microalgae produce substances that prevent the development of human pathogens (Mezzari *et al.,* 2017). The antibacterial activity of *Chlorella vulgaris* and *Dunaliella* was studied based on the extracts obtained in solvents (methanol, acetone, and diethyl ether) in a volume ratio (1:2:5).

The results showed that they could inhibit the growth of Gram-positive and negative bacteria, which is consistent with the results of this research. Several studies have shown that the type of solvent used for extraction has the most significant effect on the antibacterial activity of the obtained extract. Solvents with less polarity have better results than solvents with more polarity. The reason is more polarity. Most algal compounds (plenty of chlorophyll) are extracted along with bioactive substances. Marine algae's primary and most ingredients are sulfated compounds and sugary substances. According to these results, the desired bioactive components should be compounds with low polarity and lipophilic (Little *et al.,* 2021). Studies have shown that Gram-positive bacteria are more sensitive to raw algal extracts (Ismet, 2012). The greater sensitivity of Gram-positive bacteria to algae quotes is caused by differences in the structure of the cell wall and its composition (Taskin *et al.,* 2007). Gram-negative bacteria involve a lipopolysaccharide layer in the outer membrane and channels transporting substances. It can be more resistant to toxic substances, hydrophilic paints, and antibiotics (Moubayed *et al.,* 2017).

The results showed a significant difference between the inhibitory properties of different extracts in three bacteria ($p<0.05$). In the present research, extracts of *D. salina* antimicrobial and inhibitory properties were proven.

Lipids and some long-chain free unsaturated fatty acids, including Docosahexaenoic acid, Stearidonic acid

(18:4n-3), create the antibacterial activity of algae (Engler *et al.,* 2000). The antibacterial activity of unsaturated fatty acids was determined, and linoleic acid showed the highest activity against *Staphylococcus aureus* (10 µg per disc). Unsaturated fatty acid aldehydes of microalgae species, *Coeiastrum* sp., *Scenedesmus, Quadricauda*, & *Selenastrum* sp., were obtained. A wastewater treatment plant inhibited the Gram-negative bacterium *Serratia marcescens* in the disc diffusion method (Little, 2020). In a particular study, the extraction of aldehydes from unsaturated fatty acids is targeted. The results have shown that aldehydes from the degradation of unsaturated fatty acids have antibacterial activity. Instead, they reduce the proliferation of cancer cells and the growth of fungi (Rouhi *et al.,* 2020) . The present research showed that the AE, EE, and EAE from *D. salina* have antioxidant activity and growth inhibitory properties in Gram-negative and Gram-positive bacteria. It is a potential source of antioxidant and antibacterial activity compounds. It can be considered a suitable substitute for synthetic antioxidants and antibiotics and can be used in various fields, such as medicine and the food industry.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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