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Bioactivity of natural compounds extracted from *Oedogonium cilitum* isolated from Qalachwalan pond

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ABSTRACT

Natural extracts of microalgae are widely recognized for their biologically active compound. They represent an untapped supply of pharmaceutical substances. In this study, the freshwater macroalgae (*Oedogonium cilitum)* was collected from Qalachwalan Pond in Sulaimani City. The sample was identified, authenticated, and investigated for their chemical composition and pharmacological properties. the GC/MS was used to identify and quantify using methanol extract of *O. cilitum* macroalgae. The GC/MS results of (*Oedogonium cilitum*) revealed the existence of various significant chemicals, such as long chain fatty acids (saturated fatty acid, unsaturated fatty acids), ester, volatile organic compounds(saturated aldehyde, polyunsaturated aldehydes(PUAs), terpene Accordingly, palmitic acid(12.71 %),9-Octadecenoic acid,(E) (9.59 %),9- Octadecenoic acid,(Z) (5.67 %), Acetic acid, propyl ester(24.93 %), Acetic 2-methylpropyl ester(13.64 %), Acetic acid, butyl ester(8.75 %), Thiosulphuric-(2-aminoethyl)ester(2.15 %), Hexanal (5.58 %), Nonanal (1.61 %),2-Decenal,(E)-(3.23 %),2-Undecenal(2.71 %),2-Pentene,4,4-dimethyl-(2.32 %), Neophytadiene (5.58 %) respectively. macroalgae extract was tested utilizing the DPPH radical scavenging assay for its antioxidant properties, with a comparatively low IC_{50}(0.538 mg/ml), extract bioactive components demonstrated enhanced antioxidant activity, by increasing concentration (4.8 μg/ml to 1250 μg/ml) Antioxidant activity (Radical scavenging activity) increase. This suggests that antioxidant activity and concentration are related. On several microorganisms, the antimicrobial effects of (*Oedogonium cilitum*) extract were studied using a well diffusion method. Three organic extracts (Dioxane, chloroform, Dichloromethane) were used against three Gram-negative bacteria (*Escherichia coli, Acinetobacter species, and Enterobacter species*), and one Gram-positive bacteria (*Staphylococcus aureus*). The highest zone of inhibition was observed in Dioxane extract which showed the maximum antibacterial activity against *Staphylococcus aureus* (12.4 mm), *Escherichia coli* (10.6 mm), and *Acinetobacter species*(9.9 mm) respectively. The present finding revealed that freshwater macroalgae is more effective against Gram-positive bacteria than Gram-negative germs. Our findings highlight the value of bioactive compounds as potential antibacterial agents and suggest that they might be used in place of traditional antibiotics.

1. Introduction

Algal flora is one of the most important organisms that are found in the aquatic ecosystems especially which is the main source of oxygen and food chain. Recently algae entered nanotechnology and microtechnology for industrial purposes, antibiotics, toxicity, productivity, water quality assessment, and water pollution indicators (Dash et al.,2021). A wide variety of chemicals from several metabolic pathways, including amino acids, fatty acids, polysaccharides, and carotenoids are produced by microalgae (Martínez-Ruiz, 2022). Numerous ecologists and scientists have studied the importance of naturally occurring algae in various ecosystems, including freshwater, because they may have biological effects on microorganisms like bacteria and fungi (Thomas et al.,2021). Among the reported pharmacological effects of bioactive substances are their anti-inflammatory, antibacterial antioxidant, antiviral, and anthelmintic properties. (El-Sheekh et al.,2021). Both marine and freshwater algae are a good source of pharmacologically active metabolites (Gullon, 2020) and(Pulz and

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Gross,2004]. The presence of bioactive secondary metabolites with antitumor activity, antibacterial (Shamsabadi et al.,2013) antioxidant, and anti-inflammatory (Zbakh et al.,2020) activities has been recognized in various macroalgae as they contain active biological phytochemicals, such as fatty acids, sterols, peptides, proteins, polysaccharides, heterocyclic carbons and terpenes, activities have been identified in several macroalgae because they include biologically active phytochemicals like fatty acids, sterols, peptides, proteins, polysaccharides, heterocyclic carbons, and terpenes (Wu et al.,2013). Recently, the possibility of discovering medications made from natural sources rather than synthetic compounds has received attention (Blunt et al.,2017). Marine algae, often known as seaweeds, are one of the natural resources primarily used for the production of a variety of bioactive secondary metabolites with potential use as new medicinal and industrial agents (Rico et al.,2017). According to several studies, algae's capacity to create secondary metabolites with a range of pharmacological actions, including anti-inflammatory, antioxidant, antibacterial, cytotoxic, antiviral, and anticoagulant effects, macroalgae plays an important role in the pharmaceutical business (Al-Enazi et al.,2018b). Natural antioxidants found in terrestrial plants have been extensively researched, as well as their uses in nutraceuticals and food preservation. Tertiary butyl hydroquinone, propyl gallate, butylated hydroxytoluene, and butylated hydroxyanisol (BHA, BHT, and BHT) are some examples of synthetic antioxidants that have been used in food, cosmetic, and pharmaceutical products (Ahn et al.,2007). Scientists are looking for natural antioxidants that can be utilized in food and medicine without posing any safety or toxicity risks as the safety and toxicity of these synthetic antioxidants have been questioned (Wang et al.,2009). Natural antioxidants like Oedogonium algae are widely used and could take the place of synthetic antioxidants. Antioxidants found in algae and lichen extracts are particularly attractive for usage in nutraceuticals, cosmetics, and nutraceutical goods since their potential toxicity and health risks are fewer than those of synthetic antioxidants (Thomas and Kim, 2013). By delaying oxidation, the antioxidant qualities of natural algal chemicals can lengthen the shelf life of foods and cosmetics (Chintale et al.,2013). Numerous chemicals have been isolated and identified as antimicrobial agents, including chlorellin derivatives, acrylic acid, halogenated aliphatic compounds, terpenes, sulfur-containing heterocyclic compounds, phenolic inhibitors, alkaloids, etc. (Espeche et al.,1984) and (Etahiri et al., 2001; 2003 and 2007). Extract of *Oedogonium capillary*, a freshwater green alga, has a potent antibacterial agent against 23 different Enterobacteriaceae bacterial species., Pseudomonadaceae, Aeromonadaceae, and Vibrionaceae families; a strong association between the effectiveness of algal extract and antibiotics such as kanamycin, tetracycline, and chloramphenicol has been found (Rosell and Srivastava, 1987) and (Rosell and Srivastava, 2006). Algae are used in traditional medicine to treat a variety of human disorders, including dysentery, diarrhea, and thrush on infants' tongues, and to heal wounds and treat other skin conditions as an antiseptic (Pérez-Gutiérrez, 2016). The present study is aimed to assess the biological and chemical characteristics of the alga *Oedogonium cilitum*. from Sulaimani city that has not yet been described, it is hoped that this would serve as an illustration for describing the potentially beneficial of *Oedogonium cilitum* strain for medicinal use.

2. Material and methods:

2.1. Algal sample collection

Algal bloom samples were collected from Qalachwalan pond freshwater in Sulaimani district, Kurdistan region of Iraq. Fig. 1 during October 2021, and December 2021. After being carefully chosen from a blooming of algal biomass, samples were cleansed of epiphytes, extraneous matter, and necrotic particles. They were then put in clean plastic containers with their original water and taken to the laboratory. The sample was then dried at 40C in an oven for 2–3 days until the dry weight was constant after being rinsed with distilled water, shade-dried, chopped into small pieces, and powdered. (Elsie and DhanaRajan,2010).

Fig. 1. Oedogonium cilitum Bloome at Qalachwalan open pond.

2.2. Morphological identification of algal

The algae sample was identified as *Oedogonium cilitu*m according to the keys of identification (Prescott,1982). The filamentous green alga belonging to the genus Oedogonium featured cylindric cells, some of which were ring-shaped at the end of each cell had pyrenoids and partial chloroplast. Oedogonium species are often found in ponds, lakes, and rivers. Fig. 2.

2.3. Extract preparation

According to (Wong and Shahirah,2019) instructions were followed while making the organic extraction, with some modifications. Soxhlet extraction was performed using 20 g of powdered algae and 200 ml of methanol. The samples were then incubated for 96 h at 56 °C.

1- **Gas Chromatography-Mass Spectrometer (GC MS) analysis**:

Freshwater microalgae were analyzed using a gas chromatograph using a Carbowax capillary column-equipped quadrupole gas chromatography-mass spectrometer (GC–MS) from Shimadzu (30 m 0.25 mm ID; 0.25im film thickness) (intercut DB5MS. Japan). The capillary column was filled with a sample volume of two microliters. As a carrier gas, helium was used. Temperatures were set at 210 °C for the injector and detector. Split mode was used to inject the fluid (1:30). The column's temperature was programmed to begin at 50 °C for 1 min before rising by 3 °C each minute to 210 °C at the end. NIST Library 2008′s mass spectral database is used to compare the mass spectra, peaks

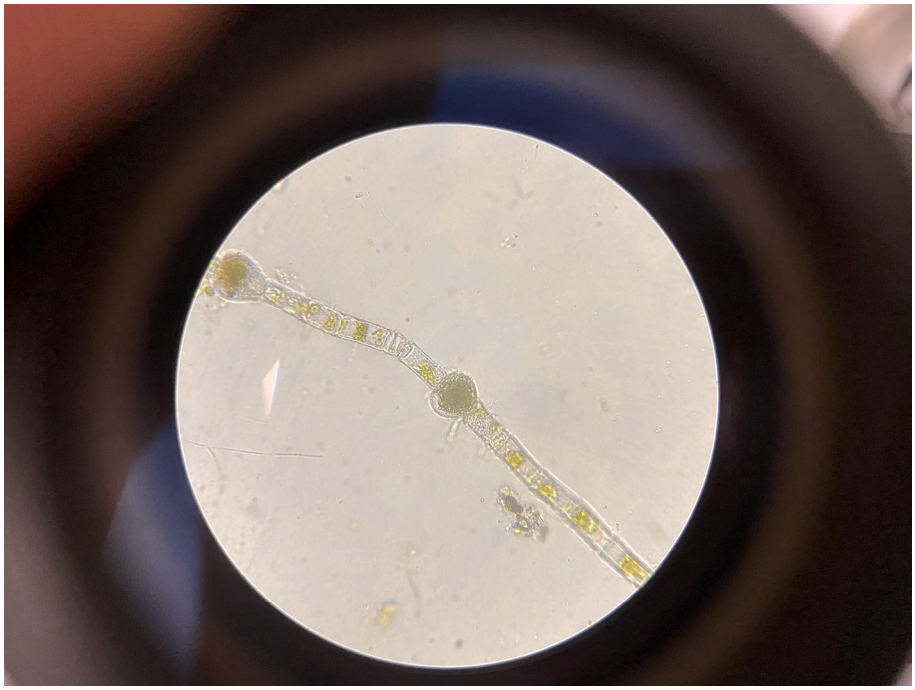

Fig. 2. Oedogonium cilitum.

in the separation of bioactive chemicals at constant pressure (100 kPa) were found. The components of the test materials' names, molecular weights, and structures were determined. (Toshihiro Obata.,2013; Saeed et al., 2020; Palaniappan, 2022).

2- **Free radical-scavenger activity assessment (DPPH° assay)**:

A method for conducting the DPPH assay. Extreme potential for scavenging was calculated according to the recorded technique (Blois, 1958). At different concentrations, 1.5 ml of 0.25 m DPPH mixture and 1.5 ml of extract were diluted in methanol. For a constant state, this combination was vigorously shaken at ambient temperature. After thirty minutes DPPH decolorization was measured by taking a spectrophotometer (UVMini-1240 model, Shimadzu, Columbia, USA). to measure the absorbance at 517 nm. Then, the process of scavenging DPPH radicals was calculated using the following equation. By using a linear regression analysis, the half-maximal inhibitory concentration (IC_{50}) was determined and expressed as the mean of three measurements. A positive control was ascorbic acid.

$$\text{Scavenging activity equation} = [\frac{\boldsymbol{A}0 - \boldsymbol{A}1}{\boldsymbol{A}0} \times 100].$$

A0 denoted the control absorbance (unqualified, with no extraction). A1 Denoted the absorbance when the excerpt or regular example is present.

2.4. Antimicrobial bioassays

Dioxane, Chloroform, and Dichloromethane were used to extract Oedogonium cilitum from three different organic solvents. These solvents were utilized to treat one Gram-positive bacteria, Staphylococcus aureus, as well as three Gram-negative bacteria, Escherichia coli, Acinetobacter species, and Enterobacter species. Muller Hinton agar was used for the bacterial culture's incubation (24 hr. at 37°C). With the help of a cork borer, four wells measuring 6 mm each were created into the medium to assess the extracts' antimicrobial activity using the Agar well diffusion method. After allowing bacteria to acclimate to medium on Petri dishes for 15 min, extracts (50 l) were added to the wells. After 24 h, the agar plates were incubated at 37 °C for the negative control well (which contained only the necessary solvent) and the positive control well (15 g) of erythromycin, and the inhibition zones were measured using a ruler. Tests were carried out in duplicate. Following incubation, the inhibition zones surrounding the wells were measured underneath and represented in millimeters as proof of antibacterial activity (Abdo et al.,2012).

3. Result and discussion

3.1. Chemical composition of algal extracts

By using GC/MS analysis, a total of fifteen bioactive chemicals were discovered in the peaks of methanolic extracts of fresh (*Oedogonium cilitum*). Table 1. Contains information on the bioactive chemicals in tabular form. The chromatogram of the chemicals found by GC/MS is shown in Fig. 3. The compounds' mass spectra were described and identified by comparison with the databases from the standard library. There were obviously several types of chemical compounds in (*Oedogonium cilitum*) such as fatty acids, esters, terpenes, hydrocarbons, and aldehydes.

Fatty acids like (n-Hexadecenoic acid %12.71,9-Octadecenoic acid, (E) % 9.59,9-Octadecenoic acid, (Z) 5.67 %), another class of aroma compounds like esters was abundant in this algae as (Acetic acid propyl ester %24.93, Acetic acid, 2-methyl propyl ester %13.64, Acetic acid butyl ester %8.75, Thiosulphuric acids-(2-aminoethyl) ester %2.15). By peak percentage in all three fractions, the total quantity of ester compounds in *Oedogonium cilitum* was about (47.32 %). For algae to have order and flavor, volatile chemicals are essential. Consequently, it is not surprising that researchers have looked at the volatile chemicals in algae. volatile compounds classed as alkanes, alkene, aldehyde, sulfur compounds, alcohol, and ester were identified in the freshwater macroalgae (Table 1).

Hydrocarbons, including alkane, and feasible. euro −2-methyl cyclopentene 2.71 %, Neophytadiene 2.06 %,2-pentene,4,4-dimethyl 2.32 %. The relative concentration of saturated aldehydes like hexanal has been observed to follow an opposite trend 5.58 %, Nonanal 1.61 %,

Table 1
List of bioactive compounds identified through GC/MS analysis of methanol extract *Oedogonium cilitum* algae.

Serial N.	Compounds	Area %	Retention time	Molecular weight	Molecular structure
1	(1S,2S)-1-fluoro-2-methyl cyclopentane	1.54	5.362	102.15	$C_6H_{11}F$
2	Acetic acid, propyl ester	24.93	6.099	102.13	$C_5H_{10}O_2$
3	Acetic acid, 2-methyl propyl ester	13.64	7.408	116.16	$C_6H_{12}O_2$
4	Hexanal	5.58	8.197	100.16	$C_6H_{12}O$
5	Acetic acid, butyl ester	8.75	8.540	116.16	$C_6H_{12}O_2$
6	Nonanal	1.61	21.216	142.23	$C_9H_{18}O$
7	2-Pentene, 4,4-dimethyl-	2.32	28.074	98.18	C_7H_{14}
8	2-Decenal, (E)-	3.23	28.806	154.25	$C_{10}H_{18}O$
9	2-Undecenal	2.71	33.447	168.28	$C_{11}H_{20}O$
10	Neophytadiene	3.52	51.816	278.5	$C_{20}H_{38}$
11	Neophytadiene	2.06	53.290	278.5	$C_{20}H_{38}$
12	n-Hexadecanoic acid	12.71	56.148	256.4	$C_{16}H_{32}O_2$
13	9-Octadecenoic acid, (E)-	9.59	61.680	282.5	$C_{18}H_{34}O_2$
14	9-Octadecenoic acid (Z)-	5.67	61.823	282.5	$C_{18}H_{34}O_2$
15	Thiosulphuric acid (H2S2O3), S-(2-aaminoethyl) ester	2.15	62.372	157.22	$C_2H_7NO_3S_2$

the first identification of 2-Decenal (E), 3.23 %,2-Undecenal 2.71 %. Unsaturated aldehyde in *Oedogonium cilitum* extracts shows that parallel-focused and untargeted investigation of previously unidentified chemicals is feasible.

3.2. Antioxidant activity

In recent years, many samples have been investigated using a variety of approaches due to the existence of various bioactive components with antioxidant potential. The DPPH radical scavenging method was chosen in the current investigation to assess the antioxidant potential of the algal extracts since it provided an easy, quick, and practical way to test the antioxidants and radical scavengers (Nickavar et al.,2007). Antioxidants were considered to have a scavenging impact on DPPH radicals because of their capacity to donate hydrogen (Ilhami et al.,2004). The ability of the DPPH radical to reduce was evaluated using the reduction caused by antioxidants. Results are shown in (Table 2), and (Figs. 4 and 6). Antioxidant activity is increased when the IC50 value is reduced. In this study, the standard antioxidant ascorbic acid's IC50 was calculated to be 0.021 mg/ml, which was much lower than the IC50 of the algal extract. Ascorbic acid's scavenging power ranged from 11.44 % at a concentration of 8.25 µg/ml to 80.54 % at a concentration of 66 µg/ml as shown in (Table 3), (Figs. 5 and 7). Extract of *Oedogonium cilitum* exhibited high antioxidant activity with a relatively low IC_{50} (0.538 mg/ml). The scavenging effect of the tested extracts at 4.88 µg/ml to 1250 µg/ml increased with increasing concentration as shown in (Fig. 4) which showed higher scavenging activity (56.68 %)on DPPH free radical.

3.3. Antimicrobial activity

As a potential source of bioactive chemicals, freshwater *Oedogonium cilitum* is one of the primary topics for the development of many medicinal drugs. In this work, three agal extracts made from three organic solvents and tested against four microorganisms were found to have antibacterial properties. (Table 4). The results showed that algal extracts included many antibacterial substances. The finding demonstrated that the Dioxane extract provided stronger antimicrobial activity than the Dichloromethane and chloroform extract. The present study investigated that microalgae exhibited the strongest antibacterial activity against *Staphylococcus aureus* (12.4 mm) microalgae extract, and reported the lowest antibacterial activity against Enterobacteria's(4.4 mm), The results of this study showed that the macroalgae Dioxane extracts had the greatest level and widest range of antibacterial activity against the tested microorganisms. Results show Dioxane extract exhibited strong inhibition against *Staphylococcus aureus* (12.44 mm) followed by *E.coli* bacteria (10.6 mm) *Acinetobacter sp*. (9.9 mm) and *Enterobacter sp*.(8.4 mm).In contrast, Dichloromethane extract showed a strong inhibition zone against *Staphylococcus aureus* (10.2), followed by *Acinetobacter* sp. (6.9 mm)and *E.coli* (4.8 mm), from the result seen that Dichloromethane extract showed no inhibition zone against *Enterobacter* sp., chloroform extract showed high inhibition zone (7.2 mm)against Acinetobacter species, followed by *E.coli* (5.6 mm) and showed lowest inhibition zone against Enterobacter species(4.4 mm).chloroform extract showed no inhibition zone against *Staphylococcus aureus*, also, investigated that chloroform extract showed least efficiency antimicrobial activity compared to two the extracts. These variations could be related to the different solubility behavior of bioactive compounds. The efficiency of extraction, the solvent used, and the resistance of the tested bacteria are only a few of the variables that may have affected the antibacterial potency of freshwater macroalgae and caused this variation in the results (Seenivasan et al.,2010). Gram-negative bacteria were resistant to the mild antibacterial activity displayed by all three macroalgae extracts, Dioxane extract, and Dichloromethane extract showed high activity against Gram-positive bacteria. While chloroform extract shows no activity against Gram-positive bacteria. The role of bacteria and algae as antibacterial agents, as well as their structure were dependent upon the processes by which the freshwater algae, including both macro and microalgae, were used as antimicrobial agents (Pina-Pérez et al., 2017) and (Breijyeh et al.,2020). Antibiotics were typically less successful against Gram-negative bacteria because of their stiff cell walls, which make them more complex than Gram-positive bacteria. Because of this, it was challenging for active compounds like -lactams, quinolones, and other antibiotics to penetrate bacteria and exert their antibacterial effects. (Breijyeh et al.,2020) The results demonstrated that Gram-positive bacteria were much more resistant to the antibacterial effect than Gram-negative bacteria due to their cell wall structure and components as well as the presence of short-chain fatty acids (Santoyo et al.,2009). Although it is still unclear how the fatty acids were able to block the entry of the active chemicals from algal extracts, numerous studies have found that fatty acids and lipids were the reason for cellular membrane breakdown (Leflaive 2009) and (Al-Saif at el.,2014). The study concluded by stressing the importance of naturally occurring freshwater algae as a possible antibiotic against a variety of harmful and disease-causing bacteria. However, more research is necessary to fully understand the mechanism by which they fight germs and if they could replace traditional antibiotics.

4. Conclusion

The selected freshwater green macroalgae's phytochemical screening using GC/MS analysis revealed significant components such (as aldehyde, terpenes, and esters of fatty acids, fatty acid, unsaturated fatty acid, and Hydrocarbons), the chemical structure of these compounds was identified using GC/MS. The research clearly shows that

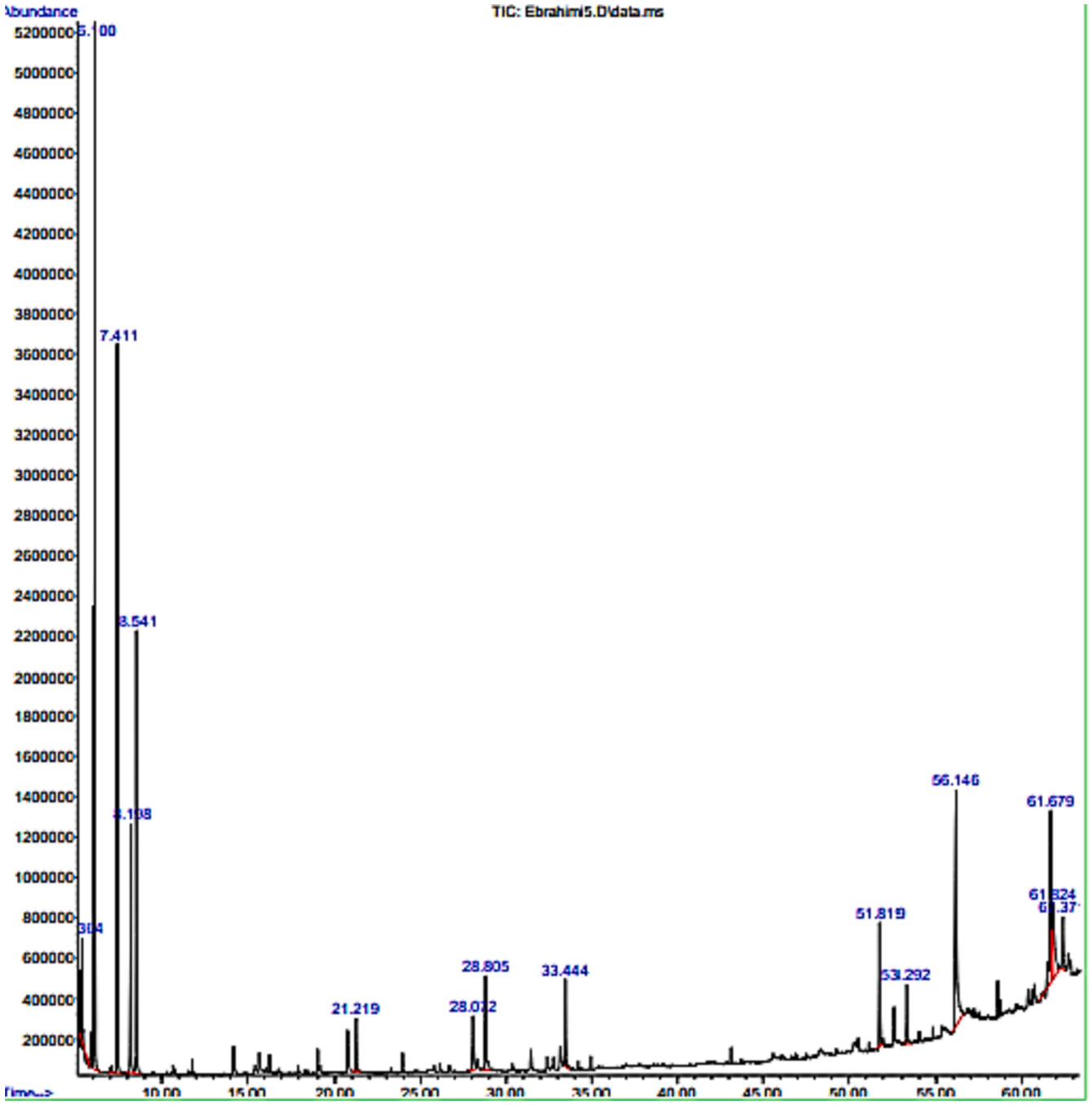

Fig. 3. Chromatograms of GC/MS of (Oedogonium cilitum).

Table 2
Antioxidant capacity of *Oedogonium cilitum* extract using DPPH.

Concentration (μg/ml)	1250	625	312.5	156.25	78.125	39.0625	19.53125	9.765625	4.882813	Control
OD1	0.426	0.418	0.538	0.685	0.823	0.856	0.912	0.993	0.984	0.992
OD2	0.432	0.42	0.551	0.672	0.811	0.861	0.904	0.982	0.97	0.975
OD3	0.422	0.426	0.529	0.668	0.809	0.847	0.919	0.98	0.978	0.988
Average	0.426	0.421	0.5393	0.675	0.814	0.8546	0.911	0.985	0.977	0.985
RSA% 1	56.751	57.563	45.380	30.456	16.44	13.096	7.411	−0.812	0.101	−0.710
RSA% 2	56.142	57.360	44.060	31.776	17.664	12.588	8.223	0.304	1.522	1.015
RSA% 3	57.157	56.751	46.294	32.182	17.868	14.010	6.7005	0.507	0.710	−0.304
Average	56.683	57.225	45.245	31.472	17.326	13.231	7.445	0	0.778	0.00
STDEV	0.5109	0.4226	1.122	0.902	0.7687	0.720	0.761	0.710	0.713	0.902

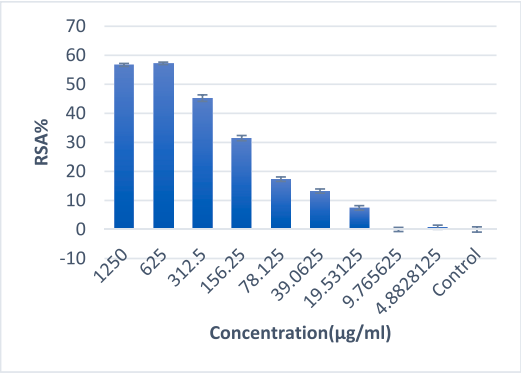

Fig. 4. The percentage of Radical Scavenging Activity of *Oedogonium cilitum* algae on DPPH radicals.

Table 3
Ascorbic acid's ability to scavenge DPPH radicals.

concentration (µg/ml)	66	33	16.5	8.25	Control
	0.171	0.298	0.455	0.785	0.886
	0.173	0.298	0.456	0.784	0.885
	0.173	0.3	0.453	0.784	0.886
Average	0.172333	0.298667	0.454667	0.784333	0.885667
RSA1	80.69251	66.35303	48.62627	11.3662	−0.03764
RSA2	80.46669	66.35303	48.51336	11.47911	0.075273
RSA3	80.46669	66.12721	48.85209	11.47911	−0.03764
RSA%	80.54196	66.27776	48.66391	11.44148	4.18E-15
STDEV	0.130376	0.130376	0.172472	0.065188	0.065188

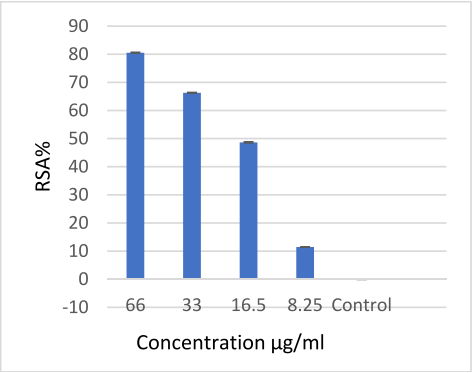

Fig. 5. The percentage of Radical Scavenging Activity of Ascorbic acid.

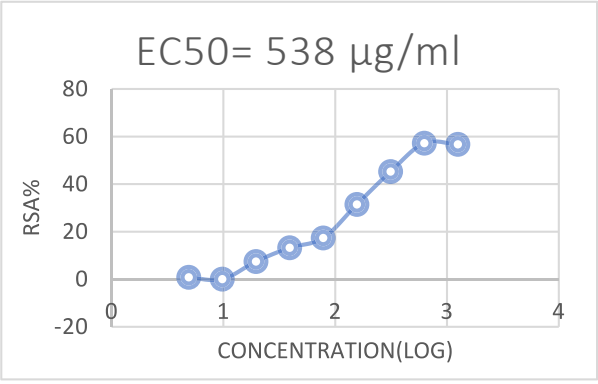

Fig. 6. The DPPH radical scavenging activities of Oedogonium cilitum extract.

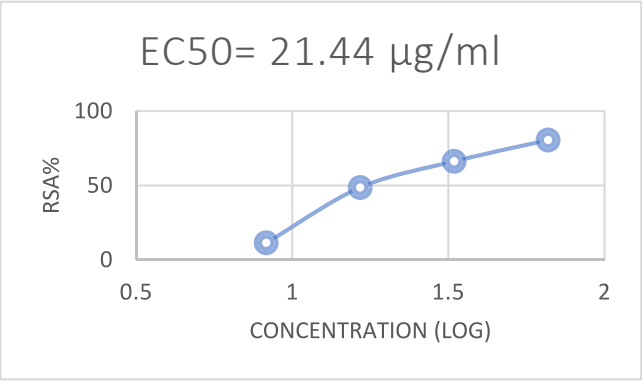

Fig. 7. The DPPH radical scavenging of Ascorbic acid.

Table 4
Antimicrobial Activity of (*Oedogonium cilitum*) extracts.

Bacterial species	Control	Dioxane	chloroform	Dichloromethane
Staphylococcus aureus	–	12.4	–	10.2
Acinetobacter species	–	9.9	7.2	6.9
Enterobacter species	–	8.4	4.4	–
E -Coli	–	10.6	5.6	4.8

Oedogonium cilitum is a rich potential source of many bioactive compounds. For this reason, we assessed the antioxidant and antibacterial activity of the extract *Oedogonium cilitum*, which is rich in biologically active compounds, making it a rich source of natural antioxidants and antimicrobial compounds. Important applications for these substances include medicine, nutraceuticals, cosmeceuticals, and agriculture. In the current study, the antioxidant activities of *Oedogonium cilitum* were evaluated. The results indicated that the microalgae possess high antioxidant activity with low IC_{50} (0.538 mg/ml), the strong positive significant correlation between DPPH radical scavenging and concentration of extracts, so (*Oedogonium cilitum)* can be used as a natural antioxidant source. This study describes the existence of antibacterial chemicals in algae gathered from ponds in Qalachwalan. Depending on the class of algae, algae have different antibacterial properties; regarding the extraction method, dioxane, dichloromethane, and chloroform were the solvents that, in most cases, increased the activity of the extracts toward the bacterial strain utilized for the antibiotic test. algae extract showed high activity against Gram-positive and Gram-negative. Despite the fact that Gram-positive bacteria were more vulnerable. As a result, it can be concluded that macroalgae from sulaimani should be researched for the isolation of natural antibiotics as they are potential sources of bioactive chemicals. From the result, we suggest that *Oedogonium cilitum* could be very beneficial to pharmaceutical and therapeutic applications in the future. Therefore, after confirming their safety, these species can be raised in captivity or in the wild for use as food by humans, farm animals, or as a nutritional supplement.

Declaration of competing 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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2023.102984.

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