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The Contribution of Algal Extracts as Antibacterial Agents Used to Reduce Bacterial Contamination

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ABSTRACT

The relevance of looking for novel natural sources of antimicrobial chemicals is highlighted by the ongoing appearance of new harmful strains of bacteria that are resistant to antibiotics. Natural algae extracts are seen as a hopeful antibacterial option that could potentially replace strong chemical antiseptics and modern antibiotics since they are natural and almost non-toxic. This study examined the antibacterial properties of algal extracts that were isolated from Iraq's aquatic environment, including *Coelastrella* sp., *Chlorella pyrenoidosa*, and *Spirogyra fluviatilis*. Ethanol was used to extract active compounds at varying concentrations; compared to water or Dimethyl Sulfoxide (DMSO), this organic solvent was more successful in extracting antibacterial substances. According to the results of bacterial susceptibility testing, the extracts of *Coelastrella* sp. and *Chlorella pyrenoidosa* had the strongest inhibitory effect against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *E. coli*, and *Staphylococcus aureus*. These algae species have different amounts of proteins, lipids, carbohydrates, polyphenols, carotenoids, and flavonoids, which are chemicals that contribute to their antibacterial activity, according to the chemical examinations of some extracts. The effectiveness of these substances was also discovered to be affected by growing conditions, including temperature, pH, light intensity, and culture medium type. According to the findings, algae, especially *Chlorella pyrenoidosa*, may offer a viable substitute source for the synthesis of organic antibacterial, where these compounds provide a good alternative to conventional antibiotics, particularly in view of the growing problems brought on by drug resistance. Therefore, more research is advised to accurately identify the active ingredients, assess their modes of action, and consider their possible uses in industrial and medical domains such as food preservation, water purification, and improving the health of humans and animals.

INTRODUCTION

The use of algal extracts as antibacterial agents and their contribution to reduce bacterial contamination has attracted a lot of attention lately. Research indicates that

certain algal extracts possess bioactive compounds that inhibit the growth of various pathogenic bacteria, making them promising candidates for natural antimicrobial agents in food preservation and medical applications **(Shartooh, 2017)**. One of the biggest problems in the world is antimicrobial resistance (AMR) **(Al-Anzy *et al.*, 2023; Yousif *et al.*, 2024a)**. The ability to cure common infectious diseases is in danger due to the global emergence and dissemination of new resistance mechanisms and an increasing number of organisms resistant to antibiotics. The decreased efficacy of antibiotics is making it harder to treat an expanding variety of infections **(Frazzini *et al.*, 2022)**. However, some bacterial based infectious diseases still require the use of antibiotics. Therefore, it is necessary to identify substitutes that can reduce human use of antibiotic drugs **(Amezcua *et al.*, 2002)**. As photosynthetic organisms, algae exhibit a broad spectrum of adaptability to unfavorable environmental circumstances, such as high or low salinity, photo-oxidation, osmotic stress, and temperature extremes **(Afzal *et al.*, 2023)**. Large quantities of secondary metabolites that are effective against a variety of harmful microorganisms are the main characteristic of algae. There have been reports of antibiotic action against bacteria in various algae, including micro- and macro-algae from rivers, lakes, and the ocean **(Yousif *et al.*, 2024b)**. Additionally, several algae compounds with antibacterial and antifungal properties have been identified, including polysaccharides, sulfated polysaccharides, phycocyanobilins, polyphenols, lectins, proteins, lutein, vitamin E, B_{12}, and K_1, peptides, polyunsaturated fatty acids, and pigments **(Kilic *et al.*, 2018)**. The biological activity of these derived compounds as antibacterial agents and their most potential uses will be covered in this study **(Jusidin *et al.*, 2022)**. Since ancient times, people have utilized natural products as therapeutic agents to treat a variety of ailments, and they have played a significant part in providing the basic needs of human communities. Infectious agents have threatened contemporary medicine's progress and effectiveness for the past seven to eight decades **(Levy & Marshall, 2004)**. Antibiotics have completely changed the medical system and have been shown to be very successful in both treating and preventing a wide range of infectious diseases **(Ahmed *et al.*, 2021)**. Nevertheless, the use of antibiotics had a negative effect on the harmful bacteria since it made them more resistant to the antibiotics **(Levy & Marshall, 2004; Ahmed *et al.*, 2021)**. This would not be an issue in and of itself if germs were not able to quickly develop resistance to once-debilitating substances. Thus, there is an urgent need for novel antibiotics. Additionally, many developing and underdeveloped nations have experienced economic and health challenges because of this type of antibiotic resistance development **(Tillotson, 2015; Tolpeznikaite *et al.*, 2021)**. Antimicrobial-antimicrobial, antimicrobial-adjuvant, and drug cocktail therapies were employed instead of mono-therapy to prevent the development of drug resistance in the microorganisms **(WHO, 2016)**. Drug-resistant microorganisms of any infectious disease were prevented and treated with its help **(Worthington & Melander, 2013)**. However, the impact of medication resistance was not anticipated to be overcome by such therapeutic therapy

(Ismail *et al.*, 2022). Natural products have demonstrated their effectiveness against a variety of ailments since the prehistoric era **(Cui *et al.*, 2015)**. The development of antimicrobial drugs has benefited greatly by the use of natural products **(Pradhan *et al.*, 2022)**. Because of their ethnopharmacological qualities, which offered a foundational platform for drug development, plants and plant-derived compounds have been utilized as the source of therapies from ancient times **(Dawd *et al.*, 2025)**. Over the past few decades, the market for and usage of herbal medications has grown rapidly. Approximately 80% of the 122 plant-derived medicines are used for ethnopharmacological purposes, according to the WHO **(Fabricant & Farnsworth, 2001)**. Its low cost, easy accessibility in the area, minimal side effects, and numerous other alluring features are the primary reasons for its widespread use in society. Less than 10% of the world's biodiversity is now thought to be used medicinally, and there are still a lot of various natural compounds out there waiting to be discovered **(Teasdale *et al.*, 2012)**.

MATERIALS AND METHODS

Unless otherwise noted, all of the reagents used came from Acros Organics (USA) and Sigma-Aldrich (Germany).

1. Isolation, identification, and cultivation of algae

The algae used in the current study were isolated from the Iraqi aquatic environment, and the algal isolates were identified using the diagnostic methods including the shape of the algae, the amount of chlorophyll, in addition to the color of the water and the odor produced **(Wehr *et al.*, 2015)**. The algal isolates were grown on a local NPK culture medium under controlled laboratory conditions, including light intensity, temperature, and pH.

2. Extract preparation and antibacterial activity

The process outlined by **Santoso *et al.* (2004)** was used involving combining 10g of lyophilized powder of algae with 250mL of ethanol in distilled water at various concentrations (0, 30, 50, or 80% v/v) to create the algae extracts utilized in antibacterial activity assessments. A magnetic stirrer was used to agitate the mixtures for 24 hours at room temperature (22 C) after they had been vigorously shaken for two minutes. Following a 20-minute centrifugation at 8000 r/m (Sigma, Germany), the mixtures were filtered using Whatman #1 paper (20-µl pore size) to remove any remaining contaminants. Three duplicates of each extraction were performed. Extracts were kept at 4°C for the next 24 hours or until they were needed again.

3. Extraction of active compounds from biomass

Active compounds were extracted from algal biomass after propagation and growth. Proteins were extracted according to **Van Dijk and Houba (2000)**, anthocyanins according to **Sutharut and Sudarat (2012)**, carotenoids according to **Kumar and Sharma (2014)**, lipids and carbohydrates according to **Baba and Malik (2015)**, phenols

according to **Laouini and Ouahrani (2017)**, and flavonoids according to **Al-Qaisi *et al.* (2019)**. The optical characteristics of the produced material were assessed using a Shimadzu 1800 UV-visible spectrophotometer in order to characterize the algal extracts. An FTIR spectrophotometer (Bruker, Alpha II, Germany)was used to perform functional group analysis.

4. Bacterial isolates

Samples were collected from several sources of hospital wastewater, including Al-Yarmouk, Al-Karama Teaching Hospital, and Al-Karkh Maternity Hospital, which were selected for the following bacterial isolates: *Bacillus subtilis*, *Pseudomonas aeuroginosa*, *E. coli* and *Staphylococcus aureus*. Samples were diluted to 10^3 CFU/ml, and cultured on nutrient agar. After colonies appearance, new plates of nutrient agar were used for the next isolation and then on differential media, including mannitol salt agar (MSR) which was used to isolate *Staphylococcus aureus*. After incubation for 24 hours at 37°C, the color of the medium changed from red to yellow **(George, 2018)**. Pseudomonas agar was used to the growth of *Pseudomonas aeuroginosa.* After incubation, the plate turned from white to olive green **(Darweesh & Luti, 2024)**. Eosin-methylene blue (EMB) was determined for *E. coli* as the colonies appeared bright blue on it **(Nada *et al.*, 2023)**. Tryptone soy agar (TSA) medium was used to isolate *Bacillus subtilis* **(Fahim *et al.*, 2022)**. Then, all the bacteria were identified using a VITEK-2 compact system (French company).

5. Disc diffusion antibacterial assay

The antibacterial activity of the different microalgae extracts was evaluated against strains of the pathogenic microorganisms *Bacillus subtilis*, *Pseudomonas aeruginosa*, *E. coli*, and *Staphylococcus aureus*. The antibacterial activity of algal extracts was examined using a modified agar disc diffusion assay technique (George, 2018). The bacterium inoculum was planted at three different concentrations (10^5 CFU mL^{-1}, 10^6 CFU mL^{-1}, and 10^7 CFU mL^{-1}) in petri dishes that contained TSA and 2% (w/v) NaCl. Sterile filter paper discs (6 mm) were impregnated with 20 µL of the different algal extracts in order to examine the extracts' activity. Before being put on test plates that had been infected with *B. subtilis*, *P. aeruginosa*, *E. coli*, and *S. aureus*, the discs were left to dry at room temperature. Discs containing the same amount of extract (20µL) were prepared as a control. At 35°C, the plates were incubated for 48 hours. By measuring the growth inhibition zone (mm) surrounding the filter discs after 24 hours and assigning a score based on the diameter of the inhibition zone, extracts with antibacterial components were able to produce clear, distinct, and circular zones of inhibition around the discs **(Darweesh & Luti, 2024)**. All information was computed using three replicates and the arithmetic mean and standard deviation. The one-way ANOVA test was used for statistical analysis.

RESULTS

Antibacterial compounds are among the many pharmacologically and bioactive chemicals found in microalgae. FTIR analysis was used to identify a number of biomolecules in the extracts, revealing distinctive peaks of functional groups in algal crude extracts (Fig. 1). Several biomolecules in the extract may serve as antibacterial and stabilizing agents throughout the extraction process, as indicated by the characteristic peaks of several functional groups in the FTIR spectrum of extracted of algal.

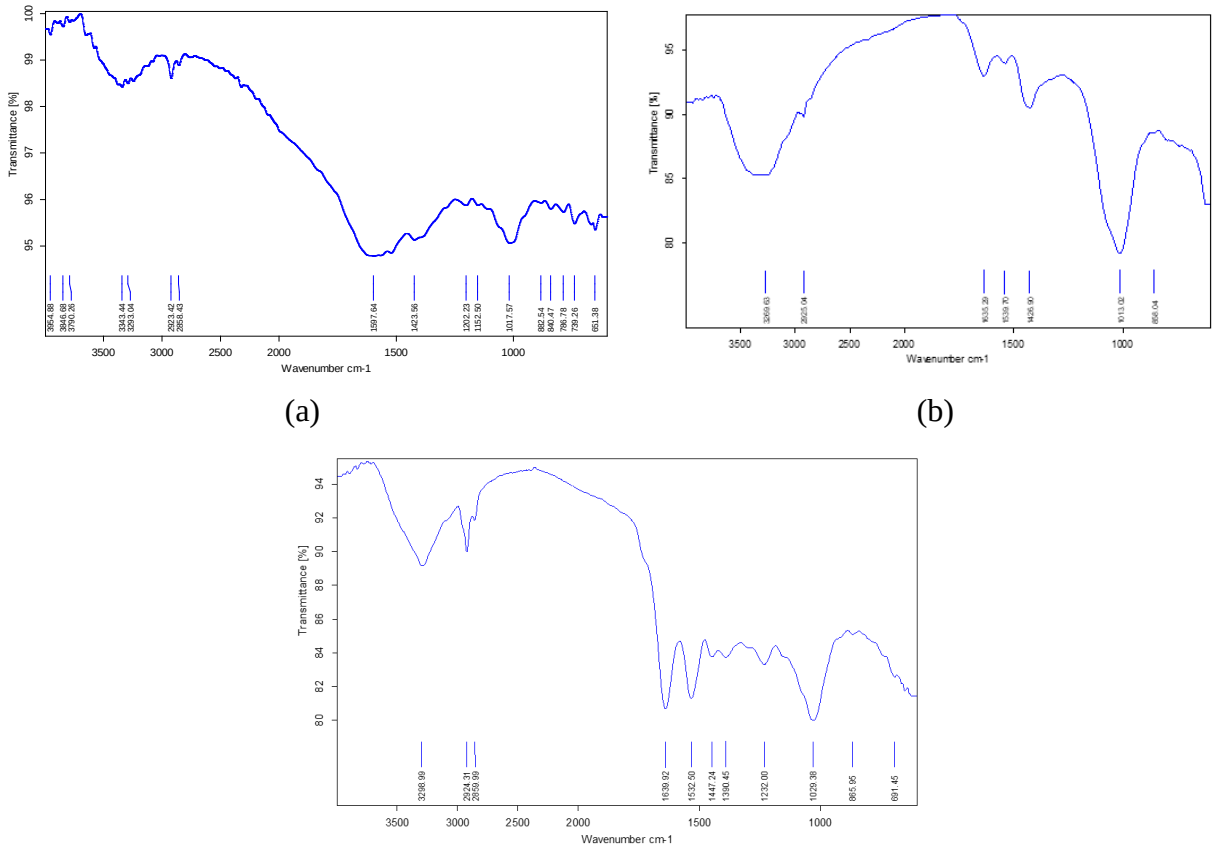

Fig. 1. FTIR spectra of the crude algal extract. (a) *Spirogyra fluviatilis* (b) *Coelastrella* sp. (c) *Chlorella pyrenoidosa*

The biochemical makeup of the investigated algal isolates is presented in Table (1). Protein (47.08%), fat (34.05%), total phenolic content (18.94 mg GAE/g), and total flavonoid content (13.98 mg Rutin/g) were all at their highest in *Chlorella pyrenoidosa*, which was followed by *Coelastrella* sp. and *Spirogyra fluviatilis*.

Table 1. Active compounds in algal isolates by alcoholic extraction

Name	Protein %	Lipid %	TPC mg Gallic / gm	TFC mg Rutin / gm	Carbohydrates CHO %	T. Zantho. (ppm)	T. Carotene. (ppm)
Spirogyra fluviatilis	39.8	23.3	14.6	8.0	8.8	0.71	14.5
Chlorella pyrenoidosa	47.08	34.05	18.94	13.98	15.6	2.89	13.58
***Coelastrella* sp.**	38.45	30.59	15.66	11.25	11.7	1.32	12.58

The results of the antimicrobial susceptibility test are displayed in Table (2). *Spirogyra fluviatilis* demonstrated the least amount of inhibition, while *Chlorella pyrenoidosa* showed the strongest antibacterial activity against all tested bacteria, particularly *Staphylococcus aureus* and *Pseudomonas aeruginosa*. On the other hand, *Coelastrella* sp. came in second.

Table 2. Antibacterial susceptibility test of the algal extract against bacteria

Types of bacteria / Algae	Extract of Algae Mg/l.	Bacteria			
		Negative		Positive	
		P. aeuroginosa (mm)	*E. coli* (mm)	*Staphylococcus aeurus* (mm)	***Bacillus subtilis* (mm)**
Chlorella pyrenoidosa.	30	15	12	20	**12**
	40	20	20	22	**15**
Coelastrella sp.	30	21	19	12	**11**
	40	21	24	20	**18**
Spirogyra fluviatilis	30	14	8	13	**10**
	40	21	21	23	**17**

DISCUSSION

Microalgae are becoming more and more well-known because of their capacity to produce bioactive metabolites that have antioxidant, antibacterial, anti-inflammatory, and anticancer properties **(Barone *et al.*, 2021; Khavari *et al.*, 2021)**. Previous research has indicated that the antibacterial action in microalgae is caused by fatty acids **(Desbois *et***

***al.,* 2009)**, terpenoids, carbohydrates, peptides, polysaccharides, and alkaloids. Additionally, new antibacterial compounds were found in microalgae, with fatty acid extracts from *Coccomyxa onubensis* inhibiting *Pseudomans aeuroginosa* and *E. coli* **(Falaise *et al.,* 2016)**. Furthermore, other substances with antioxidant or anti-inflammatory qualities as well as antimicrobial activity, such as against *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA), such as cyanovirin, oleic acid, linoleic acid, palmitoleic acid, beta-carotene, or phycocyanin, have been used more and more to treat microalgae **(Falaise *et al.,* 2016)**. Several species of microalgae extract from this study exhibit varying degrees of inhibition against bacteria (Table (1) provides a summary of the results).

Consequently, microalgal species play a major role in the production of antibiotics **(Falaise *et al.,* 2016)**. Additionally, depending on which ecotypes are adapted to particular conditions, the availability of antibiotic drugs can differ significantly between species within the same class or within a single species **(Farisa *et al.,* 2019; Prasetiya *et al.,* 2020)**. For instance, compared to ecotypes derived from less polluted waters, the green microalga *Chlorella pyrenoidosa* isolated from highly polluted waters was found to be more active against bacteria and that results agree with previous study **(Lustigman, 1988)**. While the microalgae strain of *Chlorella pyrenoidosa* was found to be high in anthocyanins and carotenoids **(Sun *et al.,* 2019)**, microalgae of the genus *Nannochloropsis* have recently been found to be rich in polyunsaturated fatty acid compounds (PUFAs), carotenoids, polyphenols, and vitamins, and have been utilized in aquaculture **(Zanella & Vianello, 2020)**. However, the *Chlorella pyrenoidosa* high concentration of compounds including lipids, phospolipid, polyunsaturated fatty acid, fucoxanthin, and eicosapentaenoic acid (EPA) gives it antibacterial qualities **(Marella & Tiwari, 2020)**. Compared to other microalgae extracts, especially at higher concentrations of *Spirogyra fluviatilis* had a smaller inhibited zone. This might be as a result of low compound content, which only includes non-essential amino acids **(Merchie *et al.,* 1997)** (Table (2) provides a summary of the results of the antibacterial test of the algal extract).

In addition to microalgae species, the extraction method and solvent used have a significant impact on whether antibacterial compounds are present in microalgae extracts. Since ethanol showed the broadest inhibition against *E. coli* in the disc diffusion method, the results of this experiment indicate that it is the best extractant. According to earlier studies, the thalli of *Gracilaria fisheri* from India was likewise extracted using ethanol, and the resulting extract showed a strong inhibitory action against *V. harveyi* **(Kanjana *et al.,* 2011)**. It was shown that an Indian ethanol extract of *Gracilaria corticata* was very effective against *V. cholerae* and *V. parahaemolyticus*, but less effective against *Shigella flexneri* and *Pseudomonas aeruginosa* **(Padmakumar & Ayyakkannu, 1997)**. Another investigation found that *Spiriluna*'s ethanolic extract was more efficient against two *Vibrio* species **(Pradhan *et al.,* 2012)**. According to these results, antimicrobial

compounds found in microalgae are often hydrophobic and may even be easier to extract using organic solvents. This may suggest that ethanol is an effective extraction technique that can remove lipids when a particular combination of compounds is present.

In the present investigation, microalgae extraction by saline water exhibited a comparable, less efficient antibacterial activity against *E. coli* based on the concentration of active compound. Nevertheless, it was found that the extraction of antimicrobial substances from *Spirogyra fluviatilis* using water extraction reduced the growth of *Pseudomans aeuroginosa, Staphylococcus aeurus* and *E. coli*. According to earlier research, *Dunaliella* sp. water extracts exhibited less bioactive qualities than those derived from organic solvents **(Sharqi *et al.*, 2024)**. Therefore, choosing the right solvent is crucial for optimizing the extraction process and producing its bioactive components, which have a variety of uses in aquaculture as well as in the fight against human infections. The low polarity of the active ingredients is likely the reason why no antibacterial action was found in the aqueous extracts **(Pradhan *et al.*, 2012)**. Short-chain fatty acids, which are linked to antibacterial action, have been shown to be synthesized by the microalgae species *Spirogyra fluviatilis*, *Chlorella pyrenoidosa* and *Coelastrella* sp. **(Defoirdt *et al.*, 2007)**.

The fact that antibacterial activity is correlated with the microalgae cultures' growth phase and culture conditions is another factor that might account for these differences. The growth phase for each microalgae culture may have varied, which could have affected the results, even though all microalgae species were present at the same concentration in every trial in this investigation. According to earlier research, altering the culture conditions of green microalgae may have caused variations in their antibacterial activity **(Hamouda & Abou-El-Souod, 2018)**. However, altering the culture conditions of microalgae may encourage the production of secondary metabolites with antibacterial activity and possibly greater amounts of these secondary metabolites **(Ruffell *et al.*, 2016)**. This could involve adjustments to the temperature, light, pH, and media composition **(Santhakumaran *et al.*, 2020)**. For instance, *Chlorella pyrenoidosa* efficiently produced antibacterial compounds when exposed to the maximum light intensity (4800 lux), indicating that the amount of bioactive compounds produced under stress circumstances was linked to the greater antimicrobial impact. The duration of microalgae cultivation is another crucial factor in the production of bioactive compounds, in addition to the culture environment **(Kilic *et al.*, 2018)**. Some species exhibit positive activity against at least one bacterial strain. The antibacterial activity within the chlorophyceae class reveals a particularly marked interest. Sixty-seven percent of the algal extracts showed antimicrobial activity **(Farid *et al.*, 2024)**. Therefore, it may be possible to artificially alter the culture environment to enable microalgae to produce more antibacterial chemicals.

CONCLUSION

Several algal extracts have bioactive chemicals that prevent the growth of different harmful bacteria, which makes them attractive options for natural antimicrobial agents in medical and food preservation applications. The use of algal extracts has the potential to completely transform methods for infection control and food safety as scientists continue to identify the precise substances causing these antibacterial qualities. Furthermore, including algal extracts in preservation techniques may result in more environmentally friendly procedures by lowering the need for artificial preservatives. This strategy could encourage customers to choose healthier options while also improving the general quality and safety of food goods. The future of food preservation and healthcare may become more and more entwined with nature-inspired solutions as scientists explore the potential of algae extracts. These developments may result in the creation of sustainable methods that promote biodiversity and ecological balance in addition to improving public health.

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