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Evaluation of Microalgae as Potential Sources of Antibacterial Activity

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ABSTRACT

Bioactive secondary metabolites are known to be present in marine microalgae. This study examined the antibacterial activity of three specific microalgae: *Oscillatoria acutissima*, *Oscillatoria simplicissima*, and *Spirulina platensis*, and assessed their production of antibacterial compounds against various microbes. We investigated the effects of temperature, light intensity, and pH on the generation of antibacterial activity. Hexane, chloroform, ethanol, and methanol were used to prepare extracts from the selected algae, which were then tested for antibacterial compounds against microorganisms, including *Aeromonas hydrophila*, *Micrococcus luteus*, *Salmonella* spp., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Vibrio* spp., and *Escherichia coli*, as well as the yeast *Candida albicans*. The findings revealed that, under conditions of pH 8.0, 30ºC, and 3000 lux, the methanol extract exhibited significantly greater effectiveness against both fungal and bacterial strains compared to other extracts for all three algal strains. No antibacterial activity was detected in water extracts. The highest production of antibacterial compounds occurred after incubation periods of 12, 14, and 12 days in an aerated culture for *Oscillatoria acutissima*, *Oscillatoria simplicissima*, and *Spirulina platensis*, respectively. These results suggest that these microalgae have potential as producers of antibacterial agents.

INTRODUCTION

The issue of bacterial resistance to antibiotics is getting worse these days, endangering the efficacy of treating infections and creating a problem for global public health. The rise in antibiotic resistance highlights the urgent need for new antibacterial sources. Exploring natural organisms and processes offers intriguing pathways to uncovering new solutions. Microorganisms, like microalgae containing abundant active compounds, hold promising potential for biotechnological applications. Such organisms might even be developed to create products with antibacterial properties **(Balasubramaniam *et al.*, 2021)**. Through the production of several metabolic substances, including carbohydrates, enzymes, lipids, and compounds with antimicrobial capabilities, these unicellular photosynthetic organisms are essential in maintaining the biosphere's ecological balance **(Sydney *et al.*, 2019)**. Microalgae are remarkably diverse and adaptable to several harsh environments. Under environmental stress, their internal

systems can shift, leading to the production of valuable metabolites with unique properties **(Heidar *et al.*, 2020)**. Furthermore, microalgae produce various antibacterial compounds, positioning them as promising agents against resistant infections. New strains could reveal even more potential, as research already highlights numerous antibacterial species, including the genera *Coelastrum, Coccomyxa, Cosmarium, Chlorella, Dunaliella, Scenedesmus, Spirulina, Nannochloropsis, Selenastrum* and *Synechococcus* **(Alsenani *et al.*, 2020; Balouch *et al.*, 2023)**. Nevertheless, the global development of antibacterial chemicals derived from microalgae is still relatively new. Low production of secondary metabolites within microalgal strains, driven by genetic and environmental influences, remains a key challenge. Further research is essential to identify strains that can thrive under harsh conditions and exhibit promising antibacterial activity. The goal of this project was to separate out microalgae strains that withstand various harsh environmental circumstances and to examine their biological characteristics to ascertain whether or not they are active against bacteria. The discovery of physiologically active substances in microalgae extracts that may lead to the creation of novel antibacterial medications constitutes scientific innovation. Consequently, this research advances our knowledge of the microalgae's potential as a reservoir of bioactive substances and could help create fresh approaches to the fight against infectious diseases.

MATERIALS AND METHODS

Algal isolation

The algae species used in this study were collected from the Gulf of Aqaba on the Red Sea coast of Alexandria. The samples were cultured in F/2 medium **(Guillard & Ryther, 1962; Guillard, 1975)**. The medium was then sterilized at 120°C in an autoclave for 30 minutes. The cultures were incubated at a pH of 8, a temperature of 30± 1°C, and a light intensity of 3000 lux. These optimal conditions were maintained for the algae. The isolated strain was identified morphologically based on available literature **(Prescott, 1968; Tomas *et al.*, 1996; Cronberg *et al.*, 2006)**.

Algal growth measurements

The procedure outlined by **Strickland and Parsons (1972)** was followed to measure the growth of algae using chlorophyll a. Centrifugation was performed for 15 minutes at 5000rpm to harvest the algae. The pigment concentration in the filtered extract was then assessed by spectrophotometrically comparing the absorbance at 663 and 645nm in a 1cm quartz cell to a blank of 80% aqueous acetone. The chlorophyll a concentration was calculated using the following formula:

Chlorophyll a = 12.7 × E663 - 2.69 × E645.

Test organisms

The test organisms included two Gram-positive bacteria: *Micrococcus luteus* and *Staphylococcus aureus*. The Gram-negative bacteria were *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Vibrio* spp., *Salmonella* spp., and *Escherichia coli*. The fungus *Candida albicans* is unicellular. These test organisms were obtained from the culture collection at the Microbiology Laboratory of the National Institute of Oceanography and Fisheries in Alexandria.

Preparation of the algal extracts

In F/2 media, three microalgae were cultivated under aeration. In order to extract antimicrobial compounds, microalgae pellets were collected for development during the stationary phase, followed by centrifugation of the culture. Then, the pellets underwent oven drying (60°C) until they reached a consistent weight. Each of the three microalgae's half-gram dry biomass was extracted using ten milliliters of hexane, chloroform, ethanol, and methanol. According to **Gonzalez Del Val *et al.* (2001)**, every extract was kept at -4°C.

Antimicrobial activity test

Using the agar diffusion technique, the evaluated algal extracts were screened for antibiotic activity by the **European Pharmacopoeia (1997)**. Each test organism was suspended, one loop at a time, in 3 milliliters of sterile NaCl solution (0.85%). This suspension of the relevant organism was added to nutrient agar (Difeco, UK), and then the mixture was transferred into a sterile Petri plate. 10µl of dimethyl sulfo-oxide (DMSO) containing 5mg from each extract was applied to a 6mm sterile paper disc based on the initial testing for optimal dosage. Aseptically, the loaded discs were positioned separately on the infected agar plate. Antibiotic discs (Cefprozil and Polymixin) represented the positive control, whereas DMSO-loaded, sterile functioned as the negative control. At 10°C, a 3-hour pre-diffusion was conducted **(Bansemir *et al.*, 2006)**. After incubating bacteria at 37°C for 24 hours and fungi at 30°C for 48 hours, the inhibition zones were assessed. Following incubation, calipers were used to measure the inhibitory zone's diameter, and the measurements were reported in millimeters (cm) **(Attaie *et al.*, 1987)**.

Impact of temperature, pH, and light intensity on the antimicrobial activity production

An Erlenmeyer flask (250ml) with 100ml of the F/2 medium was made. Independent optimization was done for the various growth parameters, which included temperature (25, 30, 35, and 40°C), pH (5, 6, 7, 8, and 9), and light intensity (1000, 2000, 3000, and 4000 lux). After that, 10 milliliters of a vigorously growing inoculum in the log phase was aseptically introduced into the culture flask and was kept there for 20 days in an aerated condition under fluorescent illumination. The antibacterial activity was determined utilizing the disc diffusion technique.

Statistical analysis

One-way ANOVA was utilized to statistically analyze the data for different biochemical parameters **(Duncan, 1957)**.

RESULTS

Microalgae isolated

The identified algal strains included *Oscillatoria acutissima*, *Oscillatoria simplicissima*, and *Spirulina platensis* (Fig. 1). At a temperature of 30± 2°C, pH of 8, and light intensity of 3000 lux, these algal strains were collected at the 14th day of their exponential growth phase (Fig. 2).

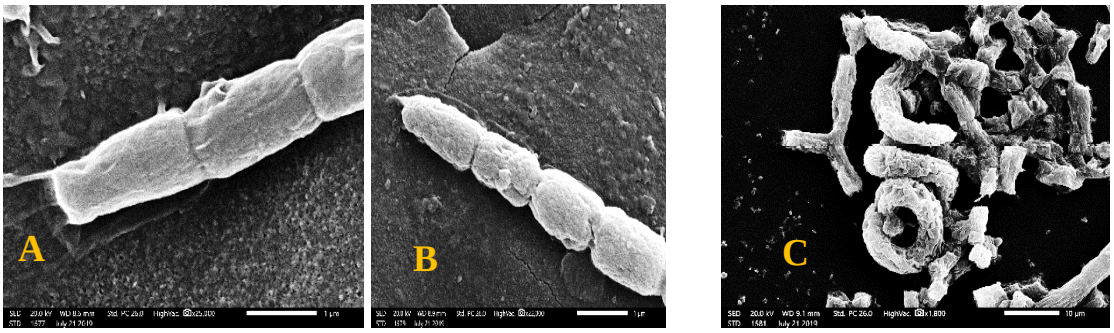

Fig. 1. Scanning electron microscope (SEM) images of **(A)** *Oscillatoria simplicissima* **(B)** *Oscillatoria acutissima* **(C)** *Spirulina platensis*

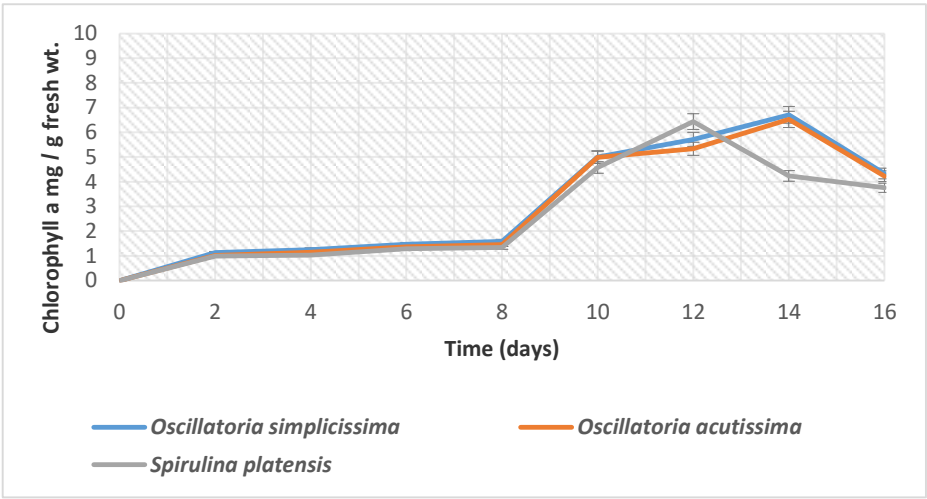

Fig. 2. The growth of the identified algal strains , quantified by chlorophyll (a) content in mg/g fresh wt

Antimicrobial activities

The sizes of the inhibition zones that resulted from the disc assay method for bacteria and one species of yeast were used to measure the antimicrobial activity. The hexane and chloroform for *Oscillatoria simplicissima* and *Oscillatoria acutissima* did not exhibit any antibacterial activity. Three microalgal methanol extract was more effective against the majority of the test species. In *Staphylococcus aureus*, the diameter of the inhibitory zones for *Oscillatoria simplicissima* methanol extract was measured to be 1.8cm. However, not all the examined microorganisms were susceptible to the effects of the hexane and chloroform extracts. *Oscillatoria acutissima* ethanol extract demonstrated greater efficacy against *P. aeruginosa*, with an inhibitory zone measuring 1.2cm in diameter. However, not all the examined microorganisms were susceptible to the effects of the hexane, chloroform, and water extracts. More action against *Pseudomonas aeruginosa*, *Escherichia coli*, *Vibrio* spp., and *Staphylococcus aureus* was shown by the methanol extract of *Spirulina platensis*. However, not every studied microbe was susceptible to the effects of the ethanol and water extracts (Table 1). Upon comparing the effects of marine microalgal extracts with reference antibiotics, it was observed that the antibiotics exhibited a stronger effect than the extracts from *Oscillatoria acutissima* and *Spirulina platensis*. Experimental results, based on inhibition zones, showed that the antibacterial drugs derived from *Oscillatoria simplicissima* extracts had a greater effect on the identical test bacteria than traditional antibiotics, aligning with the findings of **Ozdemir *et al.* (2004)** and **Tunney *et al.* (2006)**. Similarly, methanol extracts from three microalgae types produced the largest inhibition zones against bacterial pathogens.

Table 1. Assessment of antibacterial effectiveness of microalgal isolates against selected bacterial strains via the disk diffusion technique

Standard antibiotics		Diameter of inhibition zone(cm)								
		Gram (+V) bacteria		Gram (-V) bacteria						Fungal sp.
		S. aureus	*M. Luteus*	*S. marcescens*	*Salmonella spp.*	*Vibrio spp.*	*A. hydrophila*	*P. aeruginosa*	*E. coli*	*C. albicans*
Cefprozil		1.1 ± 0.003	1.2 ± 0.06	1.1±0.03	1.0 ± 0.03	0.8±0.03	2.1± 0.03	1.1± 0.03	1.4 ± 0.03	1.1±0.03
Polymixin		2.1 ± 0.03	1.3 ± 0.03	1.4±0.03	1.5± 0.03	1.1±0.03	2.2 ± 0.03	1.2± 0.03	1.5± 0.03	1.5±0.03
Microalgal sp.	**Solvent extracts**									
Oscilla	**Hexane**	-	-	-	-	-	-	-	-	-

	Chloroform	-	-	-	-	-	-	-	-	-
	Ethanol	**1.3 ± 0.03**	-	-	-	**1.2±0.03**	**1.1± 0.03**	**1.1 ± 0.03**	-	-
	Methanol	**1.8± 0.03**	**1.1± 0.03**	**1.2± 0.03**	-	**1.6±0.03**	**1.2± 0.03**	**1.4 ± 0.03**	**1.2±0.03**	**1.7±0.03**
Oscillatoria acutissima	**Hexane**	-	-	-	-	-	-	-	-	-
	Chloroform	-	-	-	-	-	-			-
	Ethanol	**1.2± 0.03**	-	**0.8±0.03**	-	**0.5±0.03**	-	**1.2± 0.03**	**0.7±0.01**	-
	Methanol	**0.8 ± 0.03**	**1.1± 0.03**	-	-	**1.0±0.03**	**1.1± 0.03**	-	**1.0±0.03**	**0.6±0.03**
Spirulina platensis	**Hexane**	**0.8± 0.03**	-	-	-	**1.2±0.01**		**0.8 ± 0.01**	-	-
	Chloroform	**1.0 ± 0.01**	**0.9 ± 0.03**	**0.9±0.03**	-	**1.1±0.01**	-	**0.9± 0.01**	**0.9±0.01**	**1.0±0.03**
	Ethanol	-	-	-	-	-	-	-	-	-
	Methanol	**1.5± 0.03**	**0.8 ± 0.01**	**1.0±0.01**	-	**1.4±0.03**	-	**1.6± 0.03**	**1.4±0.06**	**0.5±0.01**

- = No inhibitory effect; width 0.1 to 0.8cm = week activity; width 0.8 to 1.0cm = moderate activities; width >1.0cm = strong activity. Data are presented as mean ±SD Impact of varying initial pH, temperature and light intensities on antimicrobial activities production by *Oscillatoria acutissima*, *Oscillatoria simplicissima*, and *Spirulina platensis.*

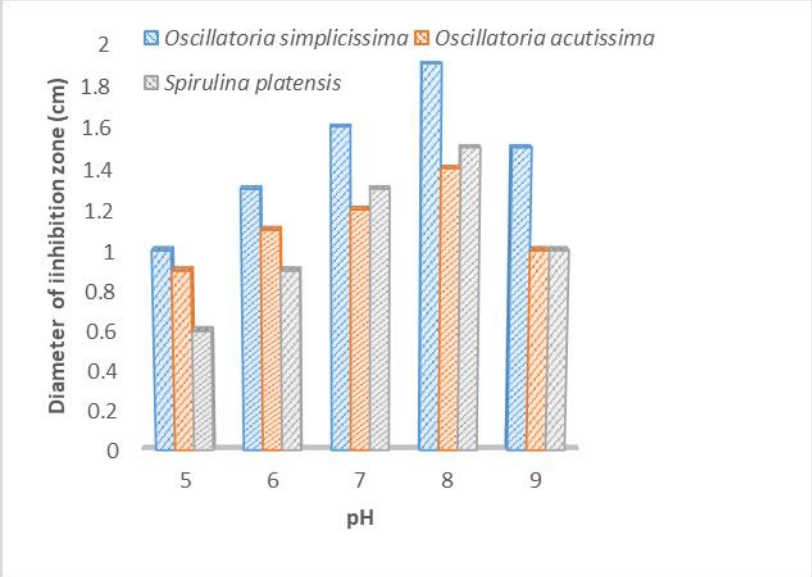

Fig. 3. Impact of varying pH levels on the generation of antimicrobial activity by *Oscillatoria acutissima*, *Oscillatoria simplicissima*, and *Spirulina platensis*

Three microalgae were found to produce antimicrobial agents at one optimal pH of 8.0 (Fig. 3). The diameter of the inhibitory zone was measured as 1.8 cm for Oscillatoria simplicissima against S. aureus, and 1.2 and 1.6cm, respectively, for Oscillatoria acutissima and Spirulina platensis against *P. aeruginosa*.

As an environmental variable, temperature indirectly influences microalgae development and antibacterial activity, according to **Huang *et al.* (2008)**. The findings displayed in Fig. (4) demonstrate that the inhibitory zone's diameter (1.8cm) was determined after *Oscillatoria simplicissima* was incubated for 14 days. Following a 14-day incubation period, *Oscillatoria acutissima*'s inhibitory zone diameter (1.2cm) was determined at the same temperature.

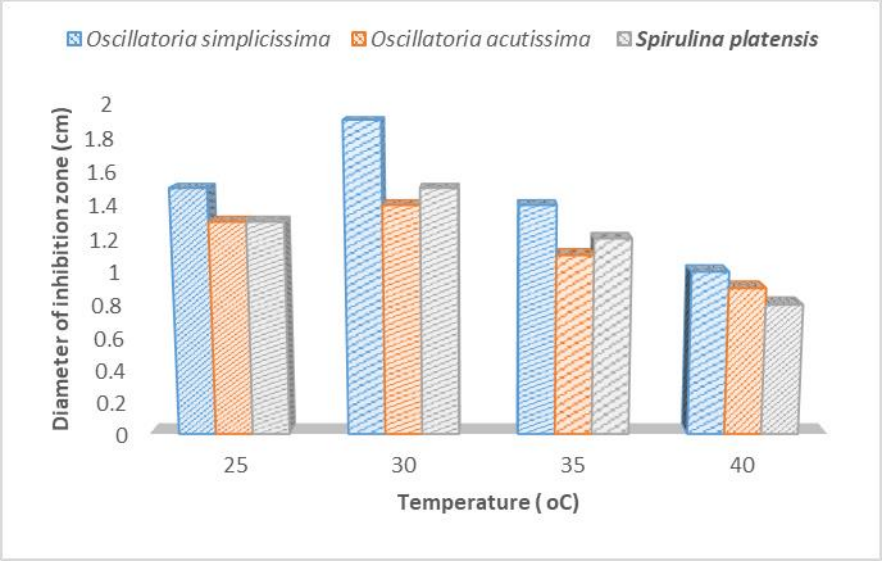

Fig. 4. Impact of varying temperatures on the generation of antimicrobial activity by *Oscillatoria acutissima*, *Oscillatoria simplicissima*, and *Spirulina platensis*

Light is a vital component for microalgae growth. The efficiency of bioactive chemicals also varied with changes in light intensity. Fig. (5) made it evident that, at a light intensity of 3000 lux, the three cyanobacterial strains generated the most potent bioactive chemicals against all microorganisms tested. The diameter of the inhibitory zones for *Spirulina platensis*, *Oscillatoria simplicissima,* and *Oscillatoria acutissima* were measured at 1.6, 1.2, and 1.8cm, respectively, during the stationary phase.

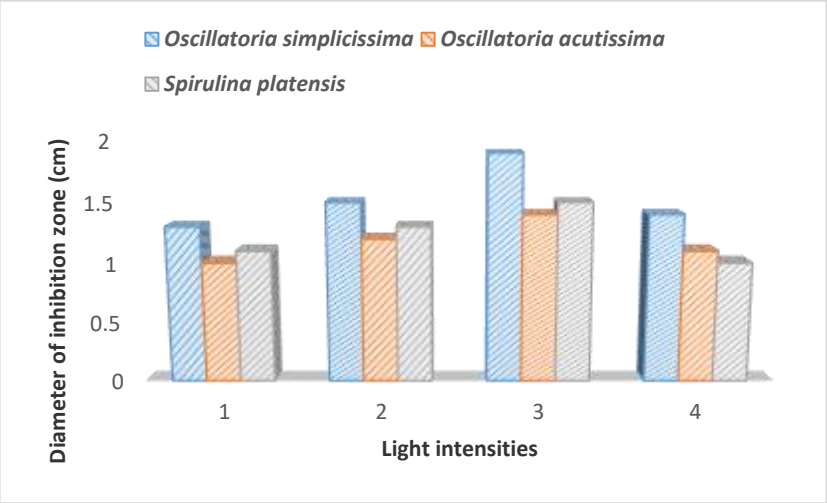

Fig. 5. Impact of varying light intensity on the generation of antimicrobial activity by *Oscillatoria acutissima, Oscillatoria simplicissima,* and *Spirulina platensis*

DISCUSSION

The majority of research on algal biochemical synthesis and analysis has been conducted during the stationary phase of growth **(Becker, 1994)**. **Prakash *et al.* (2011)** demonstrated the antibacterial effectiveness of *Oscillatoria sancta* and *Lyngbya* against *S. aureus* in acetone and methanol extracts. Additionally, **Ostensvik *et al.* (1998)** and **Rao *et al.* (2007)** found that *Microcystis aeruginosa* aqueous extracts inhibited *B. subtilis*. Notably, the extracts from the different solvents used in the present work exhibited both antifungal and antibacterial properties, suggesting they may be more potent than conventional antibiotics. The rise in antibiotic resistance has sparked critical inquiries into the origin of new chemical compounds capable of addressing this growing challenge. Despite significant advancements in chemical synthesis and biosynthesis engineering, natural sources remain the most abundant and adaptable source of novel antibiotics **(Pina-Pérez *et al.*, 2017)**. Recent studies, including those by **Alghazeer *et al.* (2013)**, **Mendes *et al.* (2013)** and **Al-Saif *et al.* (2014)**, further highlight algae and seaweed as promising sources for new antibacterial agents. These results align closely with findings from other microalgae species **(Aksoy & El Hind, 2021; Shevelyuhina *et al.*, 2022; Vahdati *et al.*, 2022)**. Moreover, they are comparable to those obtained from methanol extracts of larger biomasses of macroalgae **(Singh *et al.*, 2020; Silva *et al.*, 2020)**. Previous studies **(Borowitzka *et al.*, 1990; Renaud *et al.*, 1991, 1995; Richmond, 2000)** have thoroughly documented this.

The development of microorganisms, the nature of their metabolism, and, consequently, the production of secondary metabolites—such as antimicrobial compounds—are all highly dependent on the pH of the medium. For instance, it was found that *Scytonema ocellatum* produced the most scytophycin at pH 8.0–8.5 **(Patterson**

& Boils, 1995). **Ame *et al*. (2003)** reported that although the cyanobacterium *Cylindrospermopsis raciborskii* achieved its maximal synthesis of cylindrospermopsin at 30°C, higher temperatures promoted greater production of microcystin, a bioactive toxin **(Griffiths & Saker, 2003)**. According to **Lehtimäki *et al*. (1997)**, cyanobacteria produced the highest levels of nodularin at elevated temperatures, while lower temperatures (7, 10, and 16°C) resulted in reduced levels. Temperature and microalgal development are linearly correlated **(Takemura *et al.*, 1985)**. Temperature influences intracellular enzyme activity and response rates, subsequently affecting algal photosynthesis, respiration, growth, and distribution **(Tan *et al.*, 2009)**.

Microalgae utilize light for photosynthesis, but since they cannot retain light energy, it must be provided sustainably. Microalgae are unable to absorb all the photons they are exposed to, and an excess of light can lead to light inhibition in the algae's surface layer. Through photosynthesis, visible light serves as the primary energy source for autotrophic microalgae, enabling them to convert atmospheric carbon dioxide into organic molecules **(Carvalho *et al.*, 2011)**. The visible light spectrum allows microalgae to absorb chlorophylls, phycobilins, and carotenoids. Excessive stimulation of the photochemical system in cells can result in substantial detrimental effects due to high light intensity **(Skjånes, Rebours & Lindblad, 2013)**. Reactive oxygen species (ROS) can be produced when chlorophyll interacts with oxygen under extreme light stress. The most common defense mechanism in algae against ROS is the increased production of carotenoids. Alternatively, **Seepratoomrosh *et al.* (2016)** found that *D. tertiolecta* had a higher chlorophyll concentration under low light compared to high light. **Simionata *et al.* (2011)** reported that *Nannochloropsis gaditana* cells increased their chlorophyll content more under low light than in high light, as light harvesting requires chlorophyll **(Li, Wakao, Fischer & Niyogi, 2009)**. Thus, the increase in pigment production under low light was expected. Carotene has been recognized for its antioxidant qualities, and phenolic compounds are effective antimicrobials as part of the photoprotective response **(Skjånes, Rebours & Lindblad, 2013)**.

CONCLUSION

One crucial area of research in addressing the growing problem of bacterial antibiotic resistance is the exploration of microalgae's antibacterial properties. The current study demonstrated that four strains of microalgae exhibit significant antibacterial potential, highlighting the importance of understanding the bioactive chemical composition of these species. These findings pave the way for the development of effective antibacterial agents derived from microalgae, which are essential for combating microbial infections and tackling antibiotic resistance. Further investigation into the mechanisms by which the bioactive compounds in microalgae exert their effects could provide a foundation for innovative approaches to infection treatment and prevention. Such research not only expands our understanding of microalgae as a valuable resource

but also underscores the critical role of biotechnology in developing novel treatments for modern industries and medicine.

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