Heatmap Analysis of Summer Environmental Physicochemical Factors and Their Correlation with Antibacterial Activity of *Symbiodinium* sp. in the Persian Gulf and Gulf of Oman

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Abstract

This study investigates the impact of physicochemical parameters on the antibacterial activity of *Symbiodinium* sp. in the Persian Gulf and the Gulf of Oman during the summer season. *Symbiodinium* sp., a crucial symbiont in coral ecosystems, plays an essential role in marine environments, contributing significantly to the health and stability of coral reef ecosystems. Samples of *Symbiodinium* sp. were collected from the anemone *Stichodactyla haddoni* at three locations: Qeshm Island, Hormuz Island, and Chabahar Bay. Additionally, laboratory-cultured *Symbiodinium* sp. was included for comparative analysis. Physicochemical parameters, including temperature, salinity, and pH, were assessed, revealing significant differences across the sampling sites. Specifically, the Persian Gulf exhibited higher temperatures and salinity levels compared to the Gulf of Oman. The antibacterial activity of the algae extracts was evaluated against *Escherichia coli* and *Staphylococcus aureus* using the Kirby-Bauer disk diffusion method. The results indicated that extracts of *Symbiodinium* sp. isolated from Hormuz Island exhibited the most potent antibacterial activity when compared to laboratory-cultured samples. However, the overall antibacterial efficacy of these extracts was found to be significantly weaker than that of established antibiotics, such as penicillin. These findings underscore the influence of environmental factors, particularly temperature and salinity, on the antibacterial properties of *Symbiodinium* sp. and offer valuable insights into its potential for biotechnological applications. Moreover, the study highlights the untapped potential of *Symbiodinium* sp. as a source of bioactive compounds with antimicrobial properties, which could pave the way for the development of novel antimicrobial agents. This research also emphasizes the importance of sustainable environmental management strategies to protect coral reef ecosystems, particularly in the Persian Gulf and Gulf of Oman, regions of high ecological and economic importance.

Keywords: *Symbiodinium* sp., Persian Gulf, Gulf of Oman, *Stichodactyla haddoni*, Environmental stress, Bioactive compounds

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Introduction

Symbiodinium sp. is a significant dinoflagellate alga, which primarily supports the coral reef ecosystems found chiefly in the tropics and subtropics of the Persian Gulf and the Gulf of Oman (Rawat et al., 2024; Khalfeh Nilsaz et al., 2024). As a photosynthetic organism, *Symbiodinium* is hardly tolerant to environmental variability brought about by physicochemical changes in such ecosystems (Jiang et al., 2021). These algae have symbiotic relationships with corals and other marine invertebrates, with their health being directly related to the stability and resilience of coral reef ecosystems (Adeniran-Obey et al., 2024). Hence, especially considering the myriad threats posed by increasing climate change and anthropogenic activities like coastal development and pollution, understanding the ecological and physiological responses of *Symbiodinium* sp. to environmental stressors has become highly important (Shafiqa-Yusof et al., 2022). The Environmental Microbiology Section of the Gulf of Oman and Persian Gulf is indeed unique in the characteristics and conditions that they embrace (Muteeb et al., 2023). Their waters are closed semi-warm areas that experience acute seasonal variations in temperature, salinity, and other physicochemical factors. For example, sea surface temperatures can reach values as high as 35°C and more during summer months, just below the thermal limits for many marine organisms, including *Symbiodinium* sp. (Shemesh et al., 2024). Prolonged exposure to stress of this magnitude by *Symbiodinium* sp. produces physiological and ultimate results in bleaching events, loss of symbiotic algae, and corals subsequently undergoing degradation in health (Lesser, 2021). Therefore, studying the effect of such fluctuations directly on the *Symbiodinium* sp. is important because it helps understand the guidelines about coral health and eventually gives hints about long-term future impacts concerning the biodiversity of coral reef ecosystems (Papke et al, 2024).

The ecological role of *Symbiodinium* sp. in coral ecosystems has great holistic biotechnological potential. Research identified that, besides its ecological importance, the different species of *Symbiodinium* sp. produce a variety of bioactive metabolites, several of which have antibacterial, antifungal, and antiviral activities (Anitha et al., 2024). Most of these metabolites, especially those synthesized under environmental stress conditions, have gained immense interest in pharmaceutical and biotechnological applications (Wadhwa et al., 2024). *Symbiodinium* sp. metabolites exhibited antibacterial activity, for instance, including the inhibition against both Gram-positive and Gram-negative organisms (Raimundo et al., 2018). According to the growing interests in antibiotic resistance and the decreasing number of new antibiotics, *Symbiodinium* is likely to pitch forth new antimicrobial agents (Lewbart, 1998).

The Persian Gulf and the Gulf of Oman are extreme environments, making these two areas particularly interesting for research into *Symbiodinium* sp. and its sources of antimicrobial activity (Aeby et al., 2024). Their harsh environments serve as homes for numerous marine organisms, including various endemic species, all of which have experienced adaptation to high salinity levels, temperature multidirections, and poor nutrient availability (Donelson et al., 2019). The bioactive compounds produced by *Symbiodinium* sp. in these regions may have special properties that cannot be found in organisms more temperate or stable (Raimundo et al., 2018). There is here a justification for studying *Symbiodinium* sp. from these ecosystems, both from the ecological point of view and for the contribution to the potential creation of new biotechnological products (Mutalipassi et al., 2021).

A comprehensible understanding of how environmental alterations affect the processes inside the unicellular algal organism *Symbiodinium* sp. is a significant step toward exploiting these organisms in biotechnology applications (Jebali et al., 2022). The Heatmap technique is one of the most powerful tools for determining the interaction of biological functions with variables of environmental change (Whiting et al., 2024). Heatmaps graphically represent multivariate complex data that enable the identification of patterns or correlation among variables such as temperature, salinity, and bacterial activity. For example, in the era of *Symbiodinium* sp. research, a Heatmap can be used to determine how physicochemical changes in environmental factors affect the production or antimicrobial activity of *Symbiodinium* sp. under different conditions, pinpointing factors that are relevant for the bioactivity and valid conditions for *Symbiodinium* sp. cultivation for biotechnological purposes (Bigham Soostani et al., 2022).

In addition to ecological and biotechnological considerations, the study of *Symbiodinium* sp. is also of significant economic importance (van de Water et al., 2022). The Persian Gulf and the Gulf of Oman are crucial areas for both local economies and global biodiversity. Coral reefs in these regions support a vast array of marine life, including commercially important species such as fish, mollusks, and crustaceans (Zhang et al., 2024). The vitality of these ecosystems is intrinsically linked to the economic prosperity of the communities that rely on them (Davids et al., 2021). The deterioration of coral reefs, which may be intensified by the reduction of *Symbiodinium* sp., poses a significant threat not only to biodiversity but also to the livelihoods of millions who depend on these ecosystems for sustenance, income, and tourism opportunities (Shah and Shah, 2021). Given the growing challenges posed by climate change and overfishing, efforts to understand and mitigate the effects of environmental stressors on *Symbiodinium* sp. and coral reefs are essential for the long-term sustainability of these valuable ecosystems (Rawat et al., 2024).

Symbiodinium sp. shows really promising commercial activities in terms of antimicrobial properties (Orefice et al., 2023). Marine microorganisms have been in the spotlight for the last few years for their use in natural antimicrobial agent development (Gomes et al., 2021). With the increasing challenge from antibiotic-resistant bacterial strains and a rise in the demand for more sustainable alternatives to chemical disinfectants, *Symbiodinium* sp. indeed might be viewed as one of the promising sources of bioactive compounds that could be included into the formulation of next-generation antimicrobial products (Stoskopf et al., 2021). Not only this, but also *Symbiodinium* sp. would be found producing a vast collection of secondary metabolites, such as pigments, lipids, and polysaccharides, for which applications have been sought in the food, cosmetic, and pharmaceutical industries (Abril et al., 2024). The research on *Symbiodinium* sp. in the Persian Gulf and the Oman Gulf has great prospects for the coral reef ecology developement and new biotechnological products. It also deals with the effects of environmental changes on the biological and metabolic activity of *Symbiodinium* sp., especially its potential as an antimicrobial, thereby possibly bringing new dimensions in the use or optimization of marine resources for commercial and therapeutic purposes. The findings of such studies will perhaps be focused on discovering entirely new bioactive compounds for numerous industries because of the unique mixed biodiversity and environmental conditions of the Persian Gulf and Gulf of Oman.

Materials and methods

Sampling of *Stichodactyla haddoni* (during the summer season) was conducted along the northern coast of the Persian Gulf at three locations and three stations: Qeshm Island at the Dukohak station (26°59'40.4"N 56°11'39.9"E), Hormuz Island at the Khezr station (27°04'50.1"N 56°29'25.9"E), and Chabahar Bay at the Tis station (25°21'49.6"N 60°36'22.1"E). Each sample was identified based on morphological characteristics and identification keys (Zarei Darki and Krakhmalnyi, 2017). The samples were then transported in liquid nitrogen tanks to the laboratory at Tarbiat Modares University for analysis and purification of symbionts (Fig 1).

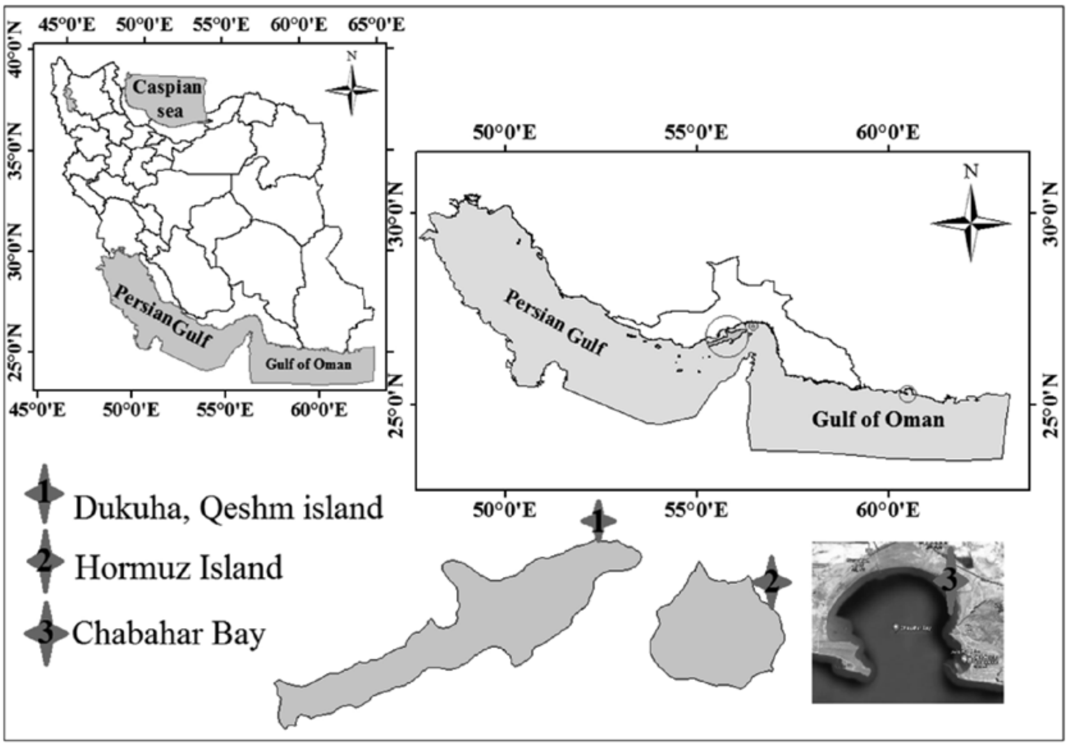

Fig 1. Sampling locations at the three stations in the Persian Gulf and the Gulf of Oman

The *Symbiodinium* sp. dinoflagellates were cultured using the ASP-12 medium, which is one of the most commonly used media for culturing dinoflagellates. The required volume of the medium was sterilized using an autoclave. Subsequently, the *Symbiodinium* sp. microalgae were transferred to the liquid medium for mass cultivation. The cultures were maintained in the liquid medium at a temperature of 27°C±1°C and a 14-hour light cycle until they reached the desired growth phase. After 30 days, the cultures reached the target growth phase (McLachlan, 1973; Benstein et al., 2014). The samples used for extraction included those cultured under laboratory conditions and *Symbiodinium* sp. extracted from the tissue of *Stichodactyla haddoni*. To initiate the extraction process, the samples were subjected to drying through a freeze-dryer (Model OPR-FDU-7012, Korea). Subsequently, one gram of the powdered microalgae was added to 50 mL of methanol and incubated in the dark for 24 hours. The supernatant was decanted, and the residue was re-extracted with an additional 50 mL of methanol for 24 hours in the dark. The extract obtained from the second extraction was filtered using filter paper and combined with the extract from the first extraction. Finally, the solvent was removed using a rotary evaporator at room temperature, and the dried extracts were stored at -18 °C until further use. To ensure that the antimicrobial properties observed were solely attributed to *Symbiodinium* sp., we carefully controlled for the presence of other microbial species by culturing the dinoflagellates in sterile conditions and comparing the antimicrobial activity of *Symbiodinium* sp. extracts from both cultured and natural environments. Our results indicate that the observed antimicrobial activity is primarily due to the *Symbiodinium* sp. species, as no significant contribution from other microbial species was detected under the experimental conditions.

The effects of *Symbiodinium* sp. extracts on the human pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus* were assessed using the Kirby-Bauer disk diffusion method (NCCLS, 1997) on Mueller-Hinton agar. The inhibition zones for each plate were measured using a caliper. Three replicates of blank paper discs were performed for each treatment, of 6.4 mm diameter. On the agar plates, one streak of E. *coli* and S. *aureus* was streaked. Further, sterile single bacterial colonies of each bacterium were picked and cultured in tubes containing 5 mL of liquid lactose broth (LB) medium under sterile conditions. The tubes were incubated for 6 h at 37°C ± 1°C in a Memmert INB200 incubator, Germany. The turbidity of the cultured bacteria was adjusted according to the McFarland standard and the bacterial suspension was inoculated onto the agar plates. Sterile forceps were used to gently press the discs onto the agar surface, ensuring full contact with the medium. Then, 15 µL of *Symbiodinium* sp. extracts was applied to each disc with a micropipette, with three replicates per treatment on each plate. A blank control was prepared by adding 15 µL of methanol (the solvent for the *Symbiodinium* sp. extracts) onto the discs in three replicates. Besides that, commercial antibiotic discs containing ampicillin were used as a positive control. The plates were then incubated upside down at 37°C ± 1°C for 24 hours. After incubation, the inhibition zone diameter was measured using a caliper (Yousefzadi et al., 2014).

Results and discussion

Table 1 provides a list of abbreviations for place names. The Mean and standard deviation, as well as comparison of the means of physicochemical factors across sampling stations in the Persian and Oman Gulf, and in laboratory-cultivated *Symbiodinium* sp. are detailed in Table 2. . The water temperature in the Persian Gulf was higher in the summer, whereas the lowest temperature was recorded in the Oman Gulf during the same season.

The assessment of the antibacterial properties of *Symbiodinium sp.* isolated from *S. haddoni* and four studied samples revealed that the highest inhibition was observed following penicillin treatment. Notably, the sample sourced from Hormuz showed the greatest level of inhibition, while the lowest inhibition was noted for the laboratory-cultured sample (Fig 2).

In Penicillin and methanol treatments, growth rates remained significantly high across all treatments in *Escherichia coli*. The growth values for E. *coli* in treatments of different regions (HS, ChS, and SQ) were observed to be 10.67, 12.0, and 12.33, respectively (Fig 2). This means that the E. *coli* cells were resistant or tolerant to the an-

Table 1. List of abbreviations utilized in this article

Abbreviated names	Area	season	Sample
SH	Hormuz	summer	1
CHS	Chabahar	summer	2
SQ	Qeshm	summer	3
Culture	laboratory	Pure and cultured	4
		Symbiodinium extract	5

Table 2. Physicochemical properties of the examined stations during the summer season

Physico-chemistry Analysis	SH, X±SD	SQ, X±SD	CHS, X±SD
DO mg/L	6.90±0.10	7.39±0.01	6.95±0.01
Ph	8.44±0.01	8.35±0.01	8.25±0.005
Salinity psu	40.71±0.01	39.40±0.10	39.70±0.10
Temperature °C	34.90±0.02	33.03±0.60	24.60±0.10
%O	93.00±1.00	109.30±0.10	102.60±0.10

Different letters indicate significant differences between treatments based on Duncan's test at $P< 0.05$

timicrobial actions of Penicillin under those experimental conditions and differences in treatment source. *Staphylococcus aureus*, on the other hand, had a much greater response concerning treatments. Observed inhibition of growth in S. *aureus* was relatively more marked with Penicillin treatment than with the HS, Culture, and SQ groups. Growth rates for S. *aureus* under these treatments were 3.33, 0.67, and 2.67, respectively. These results indicate a synergism of Penicillin with the extracts or additives used, with superior effects in the Culture treatment, possibly due to the nature of the *Symbiodinium* extract (Fig 2). Treatments from HS, ChS, and SQ also showed varying degrees of efficacy but generally proved more than HS as compared with the rest. These results indicate that on one hand, while *Escherichia coli* had almost no susceptibility to this combination of Penicillin and methanol, *Staphylococci* were more susceptible to the antimicrobial efficacy of this treatment, which could be boosted further with special treatments, specifically with the cultured *Symbiodinium* extract. Thus, showing the differences in efficacy of treatment on Gram-negative and Gram-positive bacteria and the viability of region-specific antimicrobial strategies. However, overall, these results were not statistically significant, and fantastically no antimicrobial activity was shown by any of the treatments. This means that, under the experimental setting, Penicillin with methanol and the various treatments were unable to demonstrate strong antimicrobial effects on inhibition of bacterial growth (Fig 2).

Figure of heat map of relationships between physico-chemical factors of antibacterial properties of *Symbiodinium* sp. extracts. In the summer season and the purified and cultured sample in laboratory conditions plotted through R, the clustering of multivariate data values based on Pearson correlation and rows are centered. A unit variance scale is applied to the rows (Figure 3). . The data were clustered using multivariate *techniques* based on Pearson correlation, and the rows were centered to emphasize their relative differences. The color scale ranges from blue (low values) to red (high values), with red indicating stronger antibacterial effects. The analysis reveals how certain physicochemical factors, such as pH, temperature, and concentration, are strongly correlated with the antibacterial properties, forming clusters that highlight their influence on inhibiting *Escherichia coli* and *Staphylococcus aureus*. The heatmap offers valuable insights into the conditions that maximize antibacterial efficacy (Figure 3).

This heatmap provides an overview of the complex relationships between physicochemical parameters and the antibacterial activity of *Symbiodinium*. However, a more detailed statistical analysis and consideration of other influencing factors are necessary to fully understand these interactions. The findings of this study can contribute to the management and conservation of marine ecosystems, as well as the development of commercial applications for bioactive compounds extracted from *Symbiodinium*. This study examined the antibacterial properties of extracts from *Symbiodinium* sp. symbiotic with *Stichodactyla haddoni* collected from Qeshm Island, Hormuz Island, Chabahar Bay, and from laboratory-cultured

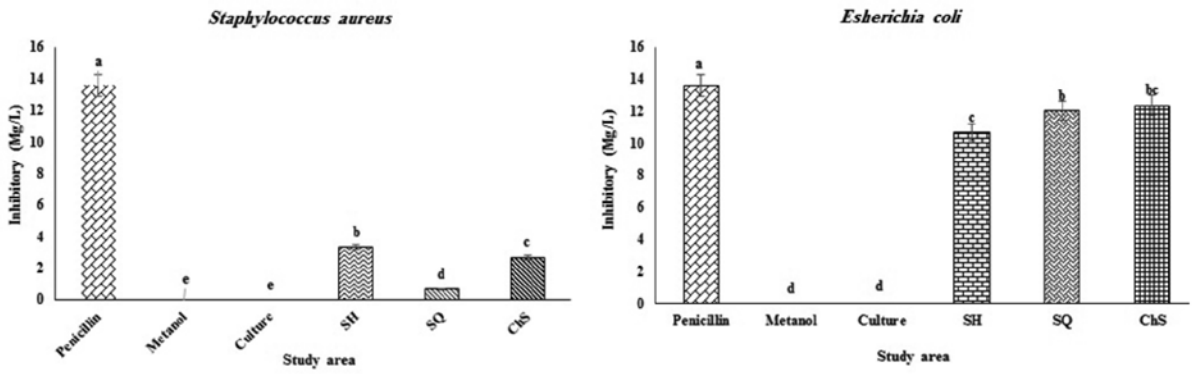

Fig 2 . The antibacterial properties of *Symbiodinium* sp. isolated from S. *haddoni* and from six other studied samples, along with laboratory-cultured pure *Symbiodinium* sp., were tested against *Staphylococcus aureus* and *Escherichia coli*.

Means sharing the same letter, according to the LSD test at the 0.05 level, were not significantly different from one another

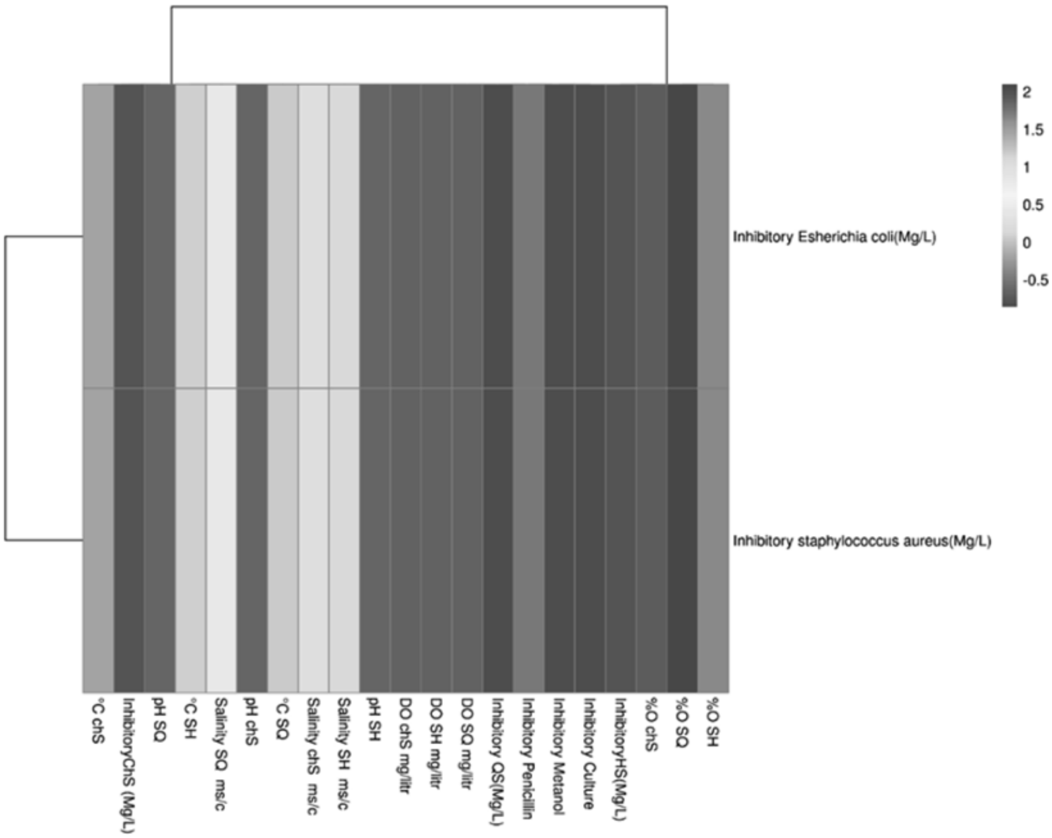

Fig. 3. The relationships between the physicochemical factors and the antibacterial properties of *Symbiodinium* sp. extracts during the summer season

Both rows and columns are clustered using correlation distance and mean linkage. 2 rows, 21 columns. 1.

Symbiodinium sp.. The extracts were tested against two human pathogenic microorganisms, *Escherichia coli* and *Staphylococcus aureus*. The results indicated that the extracts from *Symbiodinium* sp. associated with S. *haddoni* demonstrated very weak antibacterial activity. Williams et al. (2007) investigated hexane extracts from S. *haddoni* tissues against the fish pathogen *Stichodactyla haddoni*, reporting significant inhibition (24 mm diameter) compared to other pathogens. They noted that tissue extracts exhibited more promising antimicrobial activity compared to non-cellular extracts, suggesting the potential for further exploration of new antimicrobial agents from S. *haddoni*. Trick et al. (1984) identified β-diketone as an active antibiotic metabolite extracted from marine dinoflagellates, showing that environmental factors did not significantly affect its production. This metabolite may function in natural environments to reduce algae degradation by bacteria. Additionally, Leutou et al. (2020) isolated mono-galactosyl diglyceride (MGDG), mono-galactosyl monoacyl glycerol (MGMG), and methyl ester of unsaturated and polyunsaturated fatty acids (PUFAME) from *Karenia mikimotoi*, none of which showed antibacterial or antifungal activity. The findings of this study underscore that extracts of *Symbiodinium* sp. symbiotic with S. *haddoni* from the three studied locations exhibit very weak antibacterial properties. *Symbiodinium* sp., as a symbiotic organism, does not typically face direct bacterial stress that would necessitate the production of secondary antibacterial metabolites for survival. As a result, its antibacterial properties are relatively weak.

Conclusion

This study highlights the ecological significance of *Symbiodinium* sp., a pivotal symbiotic organism of sea anemones, particularly within the marine ecosystems of the Persian Gulf and Gulf of Oman. While *Symbiodinium* sp. plays a critical role in maintaining coral health and marine biodiversity, the antibacterial potential of its extracts associated with *Stichodactyla haddoni* from the studied regions was found to be exceptionally weak. These findings corroborate previous studies reporting minimal antibacterial activity in *Symbiodinium* sp. extracts. Despite the organism's importance in ecological balance, its ability to produce potent antibacterial compounds appears limited under the tested conditions. The lack of significant antimicrobial activity could be attributed to the symbiotic nature of *Symbiodinium* sp., which likely does not face substantial bacterial stress in its natural habitat, reducing the need for the production of antibacterial metabolites. Therefore, while the antimicrobial properties of *Symbiodinium* sp. may be limited, further research is warranted to explore other bioactive compounds and the complex environmental factors that might influence their production. This could provide deeper insights into their potential applications in pharmaceuticals, as well as in marine environmental management and conservation strategies.

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