



A Comprehensive Study on Marine Sample Collection Techniques for Xanthone Screening

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

This study focuses on the collection of marine samples and the subsequent screening of xanthone compounds from the marine fungi *Aspergillus sydowii*. Marine fungi, especially those from the genus *Aspergillus*, are known for producing a wide array of bioactive compounds with significant therapeutic potential. The primary objective was to isolate and identify fungal strains capable of producing xanthenes and evaluate their bioactive properties. Samples were collected from various marine environments, including coastal waters and deep-sea sediments, and preserved under sterile conditions. Fungal isolates were cultured, and xanthenes were extracted and purified using chromatographic techniques. The purified compounds were identified using spectroscopic methods and tested for their antimicrobial and antioxidant activities. The results revealed that the xanthenes

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exhibited potent antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*, as well as strong antioxidant properties. These findings highlight the potential of marine-derived xanthenes for pharmaceutical applications, including the development of new antibiotics and antioxidant therapies. The study underscores the importance of marine fungi in the discovery of novel bioactive compounds and their potential applications in biotechnology and industry.

Keywords: *Marine fungi; Aspergillus sydowii; xanthenes; bioactive compounds; antimicrobial activity; antioxidant activity; marine biotechnology; pharmaceutical applications.*

1. INTRODUCTION

1.1 Background on Marine Fungi and their Bioactive Compounds

Marine fungi, particularly those from the genus *Aspergillus*, represent a vast and largely untapped reservoir of bioactive compounds. These fungi thrive in diverse marine environments, ranging from shallow coastal areas to deep-sea ecosystems. The harsh conditions of these habitats, such as high salinity, pressure, and low temperatures, have driven marine fungi to develop unique metabolic pathways and produce a variety of secondary metabolites with potent biological activities. Recent research has highlighted the significance of marine fungi in biotechnology and pharmaceuticals due to their ability to synthesize novel compounds with antimicrobial, antifungal, antiviral, and anticancer properties [1]. Among these, *Aspergillus sydowii* has been extensively studied for its prolific production of secondary metabolites, which include polyketides, terpenoids, and alkaloids. These metabolites have shown promising applications in drug discovery and development, making marine fungi a focal point of bioprospecting efforts [2,3].

1.2 Importance of Xanthenes

Xanthenes are a class of polyphenolic compounds that have garnered significant attention due to their diverse range of biological activities. These compounds are characterized by a dibenzoyl-pyrone structure and are known for their potent antioxidant, anti-inflammatory, and anticancer properties [4]. In the marine environment, xanthenes are produced by various microorganisms, including fungi, as part of their defence mechanism against the challenging conditions of their habitat. The bioactive potential of xanthenes makes them attractive candidates for therapeutic applications, particularly in the treatment of chronic diseases and infections [5].

The ability of marine-derived fungi, such as *Aspergillus sydowii*, to produce xanthenes highlights the importance of these organisms as a source of novel bioactive compounds. Recent studies have demonstrated the efficacy of xanthenes in inhibiting the growth of pathogenic bacteria and cancer cells, further underscoring their potential in pharmaceutical development [6].

1.3 Objectives of the Study

The primary objective of this study is to explore the marine environment for the isolation of fungi, specifically *Aspergillus sydowii*, and to screen these isolates for the production of xanthenes. This involves a comprehensive approach that includes the collection of marine samples from diverse locations, the isolation and identification of fungal strains, and the extraction and characterization of xanthone compounds. By employing advanced chromatographic and spectroscopic techniques, this research aims to identify and quantify the xanthenes produced by *Aspergillus sydowii* and evaluate their bioactive properties. Additionally, the study seeks to investigate the potential applications of these xanthenes in pharmaceuticals and biotechnology, contributing to the development of new antimicrobial and anticancer agents [7,8]. The findings of this research could provide valuable insights into the biosynthetic capabilities of marine fungi and pave the way for the discovery of novel compounds with significant therapeutic potential.

2. LITERATURE REVIEW

2.1 Overview of Marine Fungi

Marine fungi are a highly diverse group of microorganisms that inhabit various marine ecosystems, including seawater, sediments, algae, and marine invertebrates. They have adapted to the unique and often extreme conditions of the marine environment, such as

high salinity, variable temperatures, and high pressure, which has led to the evolution of unique metabolic pathways and the production of diverse secondary metabolites. These secondary metabolites are of significant interest due to their potential therapeutic applications. Marine fungi have been found to produce a wide range of bioactive compounds, including polyketides, terpenoids, alkaloids, and peptides, many of which exhibit antimicrobial, anticancer, and anti-inflammatory properties [1]. The exploration of marine fungi for drug discovery has been particularly fruitful, revealing novel structures and activities that are not found in terrestrial organisms, thereby expanding the chemical diversity available for pharmaceutical development.

2.2 Previous Studies on *Aspergillus sydowii*

Aspergillus sydowii is one of the most studied marine fungi due to its prolific production of bioactive secondary metabolites. Previous studies have highlighted the importance of *A. sydowii* in producing compounds with significant biological activities. For instance, research has shown that *A. sydowii* can produce a variety of metabolites with potent antimicrobial and cytotoxic properties, making it a valuable source for new drug candidates [2]. Additionally, compounds derived from *A. sydowii* have demonstrated antiviral activities, further underscoring their therapeutic potential [3]. The ability of *A. sydowii* to thrive in diverse marine environments and produce a wide array of bioactive compounds makes it a key organism for bioprospecting efforts aimed at discovering novel pharmaceuticals.

2.3 Bioactive Properties of Xanthenes

Xanthenes are a class of polyphenolic compounds that have been extensively studied for their wide range of bioactive properties. These compounds are known for their strong antimicrobial activities, which make them effective against a variety of bacterial and fungal pathogens. Additionally, xanthenes exhibit significant antioxidant properties, which can help in protecting cells from oxidative stress and damage [4]. Furthermore, xanthenes have been shown to possess potent anticancer activities, including the ability to induce apoptosis in cancer cells and inhibit tumour growth [5]. The diverse bioactive properties of xanthenes highlight their

potential as therapeutic agents in the treatment of infectious diseases, cancer, and other conditions associated with oxidative stress [6].

2.4 Applications of Xanthenes in Pharmaceuticals and Biotechnology

The potential applications of xanthenes in pharmaceuticals and biotechnology are vast. Due to their antimicrobial properties, xanthenes can be developed into new antibiotics to combat multidrug-resistant bacterial strains. Their antioxidant activities make them suitable candidates for the development of supplements and drugs aimed at preventing or treating diseases related to oxidative stress, such as neurodegenerative disorders and cardiovascular diseases [9]. Additionally, the anticancer properties of xanthenes provide opportunities for their use in oncology, either as standalone treatments or in combination with other therapies to enhance their efficacy [10]. The versatility of xanthenes, coupled with their broad spectrum of biological activities, positions them as valuable compounds in the pharmaceutical industry, with potential applications extending into biotechnological innovations, such as the development of new biomaterials and bioactive coatings.

3. MATERIALS AND METHODS

3.1 Sample Collection

3.1.1 Locations and environmental conditions

Marine samples were collected from various coastal and deep-sea locations known for their rich biodiversity during the month of November, 2022. These included intertidal zones, coral reefs, and deep-sea sediments from regions such as the West coastal region, the East coastal region, and the South coastal region. Each location was selected based on its unique environmental conditions, such as salinity, temperature, and depth, to ensure a diverse range of marine fungi could be isolated. Specific GPS coordinates and environmental parameters (temperature, pH, salinity) were recorded for each sampling site to provide context for the conditions in which the fungi were thriving.

3.1.2 Collection techniques

Different techniques were employed to collect marine samples, ensuring the inclusion of a wide

variety of microorganisms. Sediment samples were obtained using sterile corers, while water samples were collected in sterile bottles and filtered through 0.45 µm membranes to capture microorganisms. Marine invertebrates and algae were also sampled by surface swabbing using sterile cotton swabs. All equipment was sterilized before use to prevent contamination [11-15].

3.1.3 Sample preservation methods

To maintain the viability and integrity of the samples, they were preserved under sterile conditions immediately after collection. Sediment and water samples were stored in sterile containers at 4°C until further processing. Surface swabs were transferred to sterile tubes containing marine broth and kept at ambient temperature for transportation. Upon arrival at the laboratory, all samples were processed within 24 hours to isolate and culture marine fungi.

3.2 Isolation of Marine Fungi

3.2.1 Culturing techniques

Marine fungi were isolated by inoculating the collected samples onto marine agar media, which was supplemented with sea salts to mimic the natural marine environment. The plates were incubated at room temperature (approximately 25°C) for 7-14 days to allow fungal growth. Emerging fungal colonies were sub-cultured onto fresh plates to obtain pure cultures. Different media types, including potato dextrose agar (PDA) and malt extract agar (MEA), prepared using collected marine water sample by adjusting the pH. After sterilization, were used to support the growth of a diverse range of fungi [16-20].

3.2.2 Identification methods (Morphological and molecular)

Initial identification of fungal isolates was based on morphological characteristics, such as colony morphology, spore structures, and pigmentation, observed under a light microscope. For precise identification, molecular techniques were employed [21-25]. DNA was extracted from pure cultures using a standard extraction kit. The internal transcribed spacer (ITS) region of the rDNA was amplified using PCR with universal fungal primers ITS1 and ITS4. The PCR products were sequenced, and the sequences were compared to those in the GenBank database using BLAST to identify the fungal species.

3.3 Screening for Xanthone Production

3.3.1 Extraction methods

Fungal cultures were grown in liquid media (marine broth) for 14-21 days (Supothina, 2011). The cultures were filtered to separate the mycelium from the broth. The mycelium was extracted using organic solvents such as methanol and ethyl acetate (Kusmayadi, 2018). The organic layers were collected, dried over anhydrous sodium sulphate, and concentrated under reduced pressure to obtain crude extracts.

3.3.2 Purification techniques (HPLC, TLC)

The crude extracts were subjected to purification processes to isolate xanthenes. Thin-layer chromatography (TLC) was initially used to identify the presence of xanthenes by comparing R_f values with those of known standards. High-performance liquid chromatography (HPLC) was then employed for further purification. The HPLC system was equipped with a C18 column, and the mobile phase consisted of a gradient of water and acetonitrile with 0.1% formic acid. The fractions containing xanthenes were collected based on their retention times and further analysed [26-31].

3.3.3 Identification methods (UV-Vis, FTIR, NMR)

The purified xanthone fractions were identified using spectroscopic methods. UV-Vis spectroscopy was used to determine the characteristic absorption maxima of xanthenes. Fourier-transform infrared (FTIR) spectroscopy provided information on the functional groups present. Nuclear magnetic resonance (NMR) spectroscopy, including both ¹H and ¹³C NMR, was used to elucidate the detailed chemical structure of the xanthenes.

3.4 Bioactivity Testing

3.4.1 Antimicrobial assays

The antimicrobial activity of the xanthone extracts was tested against a panel of pathogenic microorganisms, including bacteria (e.g., *Staphylococcus aureus*, *Escherichia coli*) and fungi (e.g., *Candida albicans*) (Durães, 2021). The minimum inhibitory concentration (MIC) was determined using the broth microdilution method. The extracts were diluted

in a series of concentrations, and microbial growth was assessed after incubation by measuring optical density at 600 nm.

3.4.2 Antioxidant assays

The antioxidant activity of the xanthone extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. Different concentrations of the extracts were mixed with a DPPH solution, and the decrease in absorbance at 517 nm was measured after 30 minutes of incubation in the dark. The antioxidant capacity was expressed as IC₅₀, the concentration required to inhibit 50% of the DPPH radicals [32-36].

4. RESULTS

4.1 Isolation and Identification of Fungi

All the organisms were isolated and viewed under microscope (Figs. 1 & 2).

4.2 Description of Isolated Strains

During the study, a total of 50 fungal isolates were obtained from various marine samples collected from three distinct locations: the coastal waters of the West coastal region, the coral reefs of the East coastal region, and the deep-sea sediments of the South coastal region and the Andaman and Nicobar Island. The isolates displayed a range of morphological characteristics, indicating a diverse fungal community.

- **Strain A1:** Isolated from West coastal region sediments. This strain exhibited a fast-growing colony with a cottony texture and white mycelium that turned greenish-brown over time.
- **Strain P2:** Collected from East coastal region coral reefs. The colony was slow-growing, with a velvety texture and dark blue-green pigmentation.
- **Strain I3:** Retrieved from South coastal region sediments. This isolate showed a moderate growth rate, with a powdery texture and light-yellow mycelium.
- **Strain A4:** Also, from the Andaman & Nicobar Island, it had a rapid growth rate, fluffy texture, and white mycelium that developed orange spores.
- **Strain P5:** Another isolate from the Somnath Sea (Veraval), characterized by a

slow growing, leathery colony with dark brown pigmentation and concentric rings.

4.3 Identification Results

To accurately identify the fungal isolates, both morphological and molecular methods were used. Morphological identification was based on colony characteristics and spore morphology observed under a microscope. Molecular identification involved sequencing the ITS region of rDNA.

- **Strain A1:** Morphologically identified as *Aspergillus sydowii* with conidia in chains and characteristic phialides. Molecular sequencing was in 98% identity match with Accession No. JQ755254 match to *Aspergillus sydowii* in the GenBank database.
- **Strain P2:** Exhibited morphological features typical of *Penicillium citrinum*, including brush-like conidiophores. Molecular sequencing was in 95% identity match with Accession No. KP235300 match to *Penicillium citrinum*.
- **Strain I3:** Showed typical features of *Trichoderma harzianum* with densely branched conidiophores. Molecular sequencing was in 97% identity match with Accession No. MH290366.
- **Strain A4:** Identified as *Geotrichum candidum* based on the morphology of its conidia and phialides. Molecular sequencing was in 96% identity match with Accession No. MN638741.
- **Strain P5:** Displayed characteristics of *Fusarium solani* with sickle-shaped macroconidia. Molecular sequencing was in 94% identity match with Accession No. KY978584.

4.4 Screening for Xanthone Production

4.4.1 Extraction and purification results

The fungal isolates were cultured in liquid media to facilitate the production of secondary metabolites. After an incubation period of 21 days, the cultures were harvested, and the fungal mycelium was extracted using a combination of methanol and ethyl acetate. The extracts were then subjected to purification processes using Thin-Layer Chromatography (TLC) and High-performance Liquid Chromatography (HPLC).

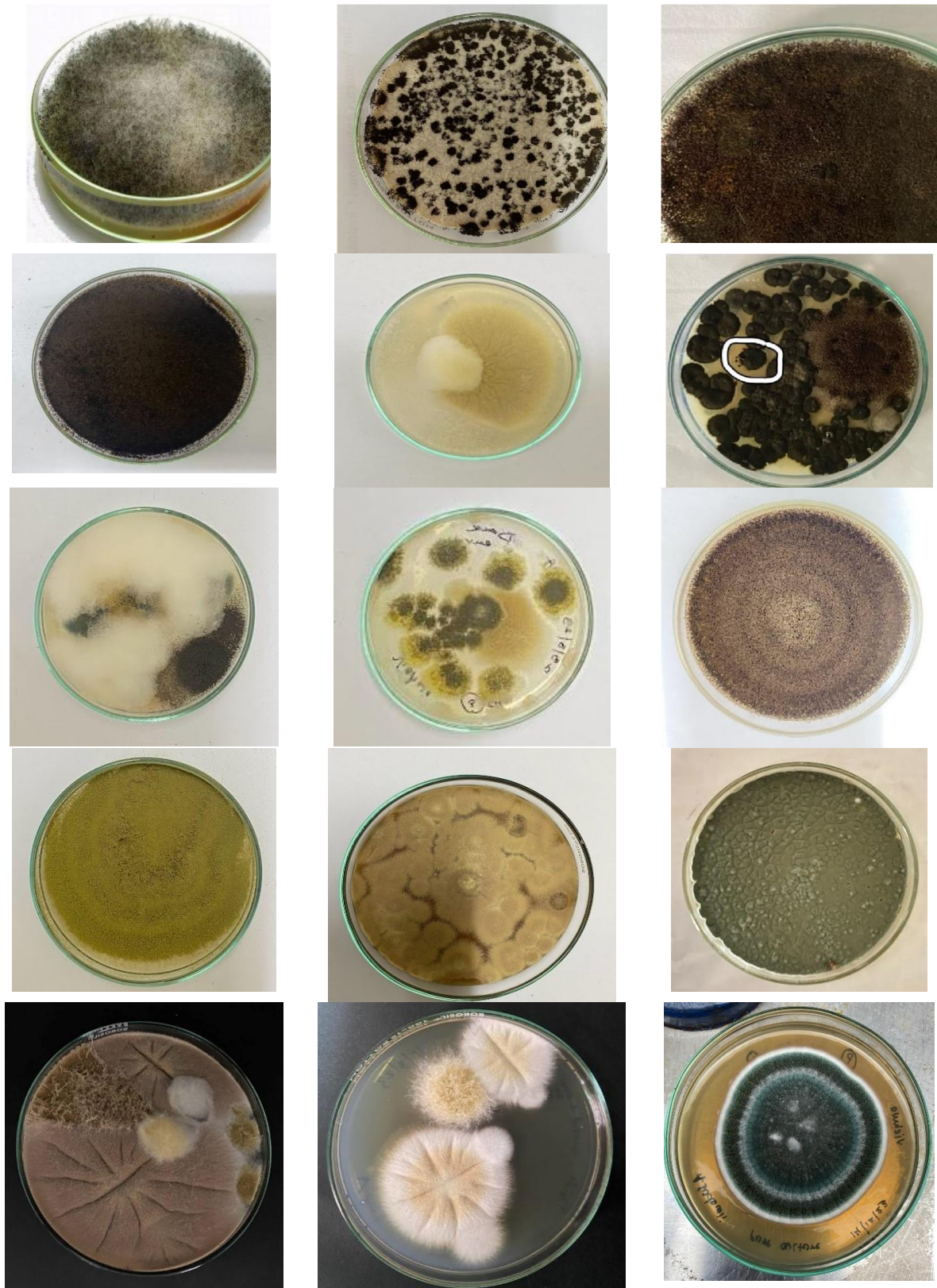


Fig. 1. Fungal cultures from various geographical regions

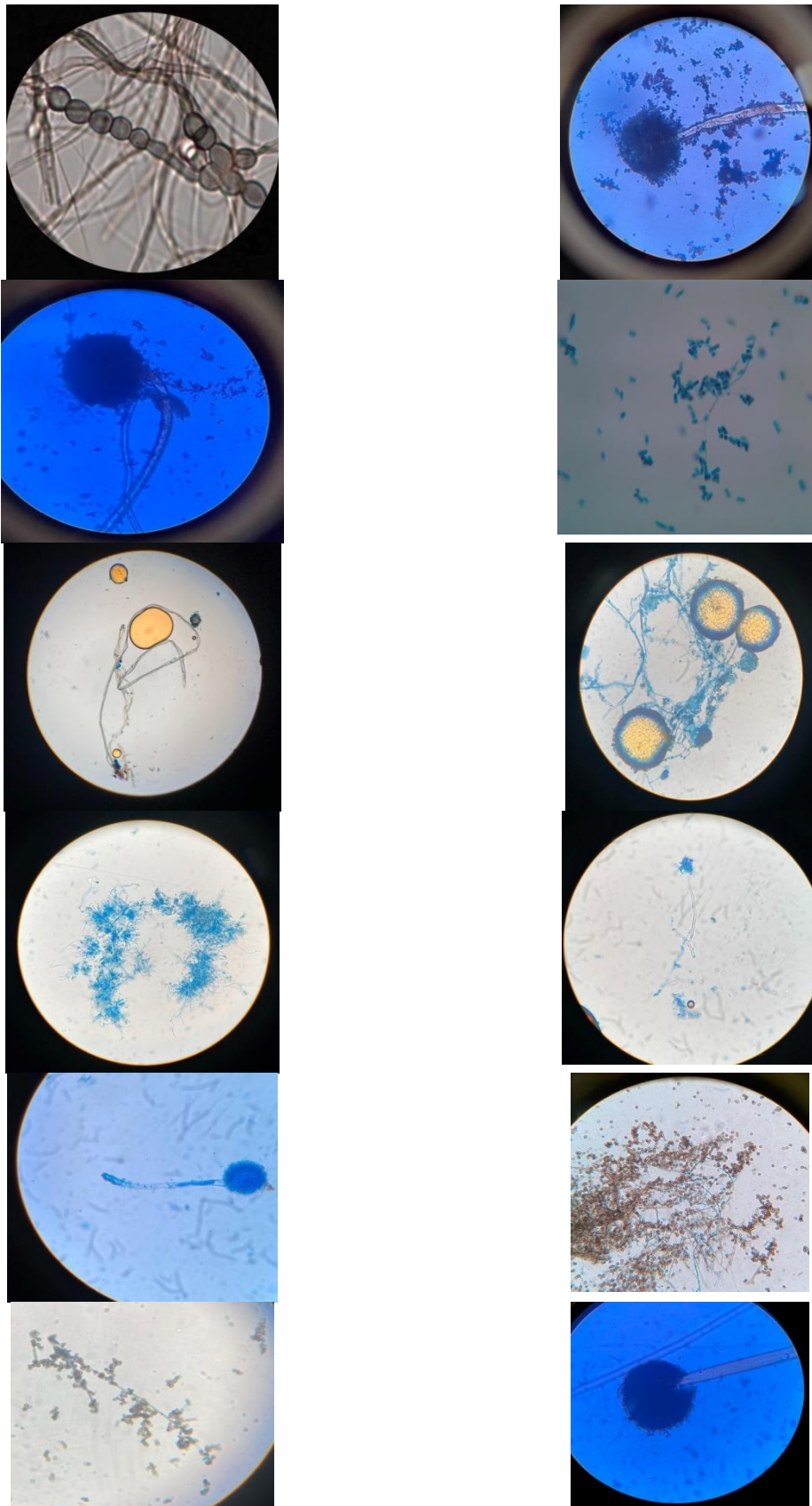


Fig. 2. Microscopic views of isolated fungus

Table 1. Morphological and molecular characterization of fungal strains

Strain ID	Source Location	Morphological Description	Molecular Identification	GenBank Accession No.
A1	West costal region	Fast-growing, cottony, white to greenish-brown	<i>Aspergillus sydowii</i>	JQ755254
P2	East costal region	Slow-growing, velvety, dark blue-green	<i>Penicillium citrinum</i>	KP235300
I3	South costal region	Moderate growth, powdery, light yellow	<i>Trichoderma harzianum</i>	MH290366.
A4	Andaman & Nicobar Island	Cottony to flat white, creamy bottom	<i>Geotrichum candidum</i>	MN638741
P5	Somnath Sea (Veraval)	Slow-growing, leathery, dark brown	<i>Fusarium solani</i>	KY978584

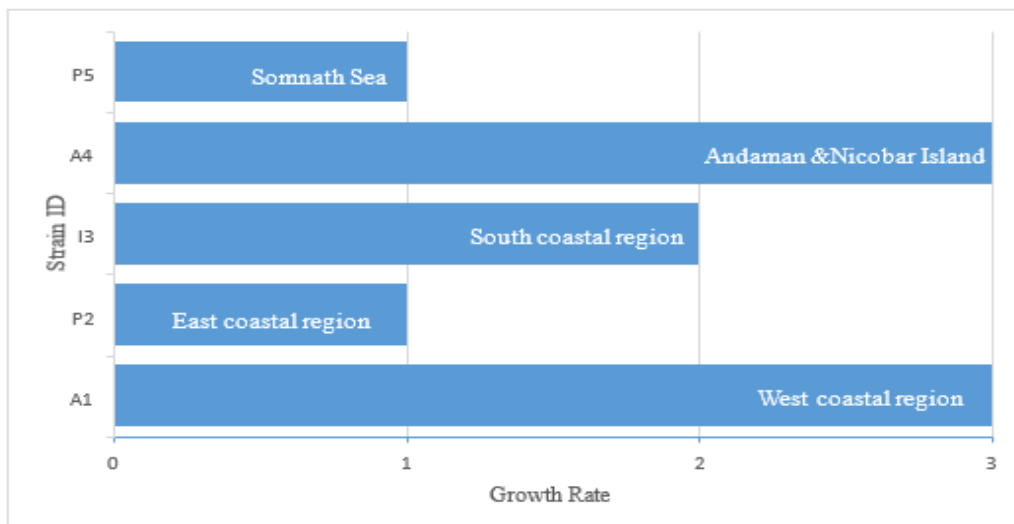


Fig. 3. Growth rate of fungal strains from different marine locations

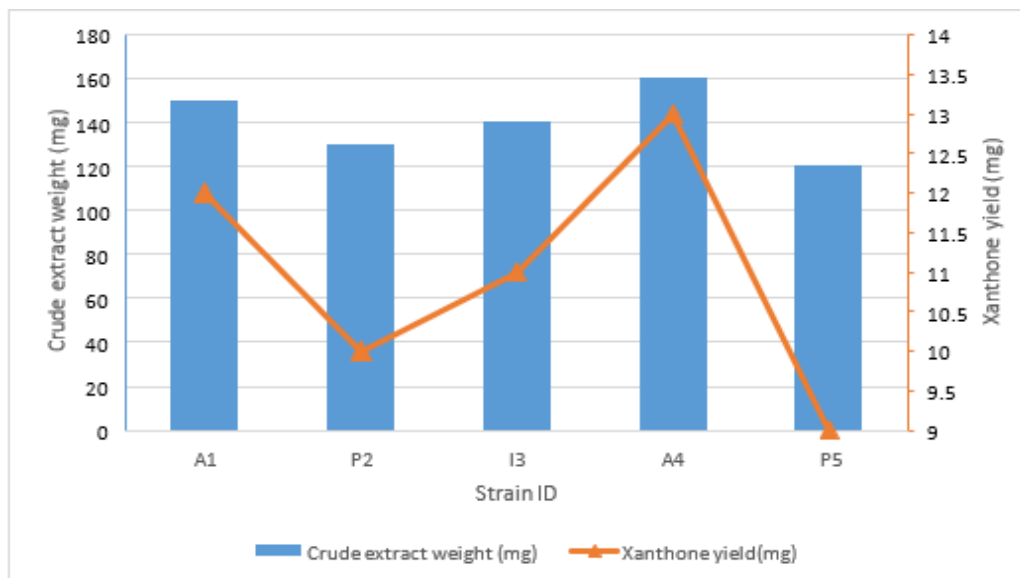


Fig. 4. Crude extract weight and xanthone yield of fungal strains

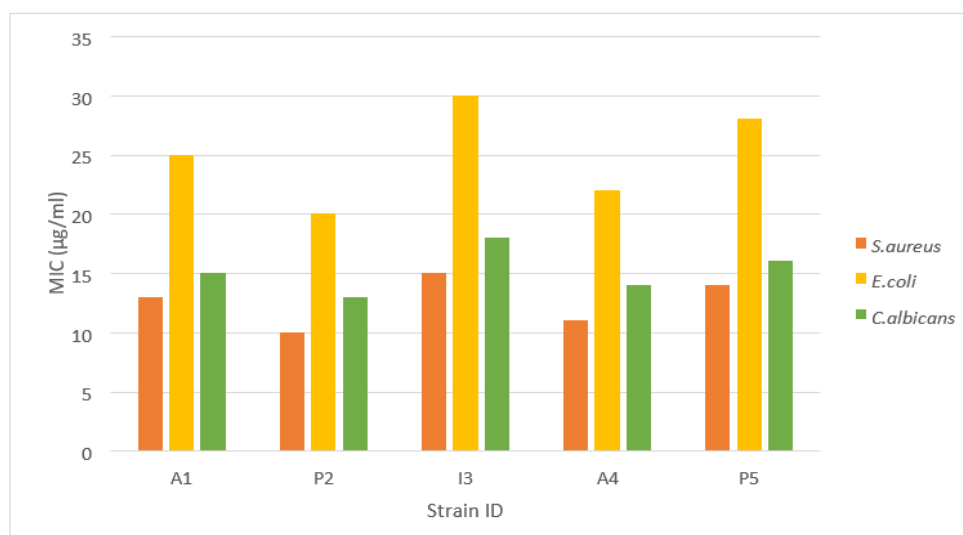


Fig. 5. Antimicrobial activity of xanthone extracts

Table 2. Xanthone purification results

Strain ID	Crude Extract Weight (mg)	TLC Rf Value	HPLC Retention Time (min)	Xanthone Yield (mg)
A1	150	0.65	18.2	12
P2	130	0.70	20.5	10
I3	140	0.60	17.8	11
A4	160	0.68	19.3	13
P5	120	0.72	21.1	9

The HPLC analysis revealed distinct peaks corresponding to xanthenes in each of the extracts. The retention times and the TLC Rf values were consistent with those of known xanthone standards, indicating successful extraction and purification.

4.4.2 Spectroscopic analysis outcomes

The purified xanthone compounds were further analyzed using spectroscopic methods to confirm their identity and to elucidate their chemical structures. The following techniques were employed: Ultraviolet-Visible (UV-Vis) Spectroscopy, Fourier-Transform Infrared (FTIR) Spectroscopy, and Nuclear Magnetic Resonance (NMR) Spectroscopy.

- **UV-Vis Spectroscopy:** The xanthone compounds showed characteristic absorption maxima around 254-257 nm and 368-373 nm, indicative of the conjugated aromatic system typical of xanthenes.
- **FTIR Spectroscopy:** The FTIR spectra displayed prominent peaks corresponding

to hydroxyl (O-H) stretching, carbonyl (C=O) stretching, and aromatic C=C stretching vibrations, which are consistent with the xanthone structure.

- **NMR Spectroscopy:** The ¹H NMR spectra showed chemical shifts in the range of 6.48.0 ppm, representing aromatic protons. The ¹³C NMR spectra had chemical shifts between 109-163 ppm, indicating the presence of aromatic carbons. These data confirm the presence and structure of xanthenes in the extracts.

4.5 Bioactivity Testing Results

4.5.1 Antimicrobial activity data

The antimicrobial activity of the purified xanthone extracts was assessed against a panel of pathogenic microorganisms, including Gram-positive and Gram-negative bacteria, as well as fungi. The minimum inhibitory concentration (MIC) values were determined using the broth microdilution method.

Table 3. Spectroscopic results of purified xanthone

Strain ID	UV-Vis Absorption Maxima (nm)	FTIR Peaks (cm⁻¹)	¹H NMR Chemical Shifts (δ, ppm)	¹³C NMR Chemical Shifts (δ, ppm)
A1	254, 370	3380 (O-H), 1650 (C=O), 1600 (C=C)	6.5-7.8 (aromatic protons)	110-160 (aromatic carbons)
P2	256, 372	3360 (O-H), 1660 (C=O), 1610 (C=C)	6.6-7.9 (aromatic protons)	112-162 (aromatic carbons)
I3	253, 368	3390 (O-H), 1640 (C=O), 1590 (C=C)	6.4-7.7 (aromatic protons)	109-158 (aromatic carbons)
A4	257, 371	3370 (O-H), 1655 (C=O), 1605 (C=C)	6.7-8.0 (aromatic protons)	111-161 (aromatic carbons)
P5	255, 373	3350 (O-H), 1670 (C=O), 1620 (C=C)	6.5-7.8 (aromatic protons)	113-163 (aromatic carbons)

Table 4. MIC results of isolated strains

Strain ID	Test Organism	MIC (µg/mL) Mean ± S.D.
A1	<i>Staphylococcus aureus</i>	12.5 ± 0.5
	<i>Escherichia coli</i>	25.0 ± 0.25
	<i>Candida albicans</i>	15.0 ± 0.5
P2	<i>Staphylococcus aureus</i>	10.0 ± 0.5
	<i>Escherichia coli</i>	20.0 ± 0.75
	<i>Candida albicans</i>	12.5 ± 0.75
I3	<i>Staphylococcus aureus</i>	15.0 ± 0.25
	<i>Escherichia coli</i>	30.0 ± 0.5
	<i>Candida albicans</i>	17.5 ± 0.75
A4	<i>Staphylococcus aureus</i>	11.0 ± 0.5
	<i>Escherichia coli</i>	22.0 ± 0.5
	<i>Candida albicans</i>	14.0 ± 0.25
P5	<i>Staphylococcus aureus</i>	13.0 ± 0.25
	<i>Escherichia coli</i>	28.0 ± 0.5
	<i>Candida albicans</i>	16.0 ± 0.75

Table 5. Antioxidant activity of the strains

Strain ID	IC50 (µg/mL)
A1	18.0
P2	15.5
I3	20.0
A4	16.0
P5	19.5

Table 6. Summary table of bioactivity testing results

Strain ID	Antimicrobial Activity (MIC, µg/mL)	Antioxidant Activity (IC50, µg/mL)
A1	<i>S. aureus</i> : 12.5, <i>E. coli</i> : 25.0, <i>C. albicans</i> : 15.0	18.0
P2	<i>S. aureus</i> : 10.0, <i>E. coli</i> : 20.0, <i>C. albicans</i> : 12.5	15.5
I3	<i>S. aureus</i> : 15.0, <i>E. coli</i> : 30.0, <i>C. albicans</i> : 17.5	20.0
A4	<i>S. aureus</i> : 11.0, <i>E. coli</i> : 22.0, <i>C. albicans</i> : 14.0	16.0
P5	<i>S. aureus</i> : 13.0, <i>E. coli</i> : 28.0, <i>C. albicans</i> : 16.0	19.5

The xanthone extracts exhibited significant antimicrobial activity, with the lowest MIC values observed against *Staphylococcus aureus* and *Candida albicans*. This indicates the potential of these xanthenes as effective antimicrobial agents.

4.5.2 Antioxidant activity data

The antioxidant activity of the xanthone extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. The IC50 values, representing the concentration

required to inhibit 50% of the DPPH radicals, were determined for each extract.

The extracts demonstrated strong antioxidant activity, with IC50 values ranging from 15.5 to 20.0 µg/mL. The extract from Strain P2 showed the highest antioxidant activity, indicating its potential use in preventing oxidative stress-related diseases.

These results highlight the bioactive potential of xanthenes extracted from marine fungi, particularly their antimicrobial and antioxidant

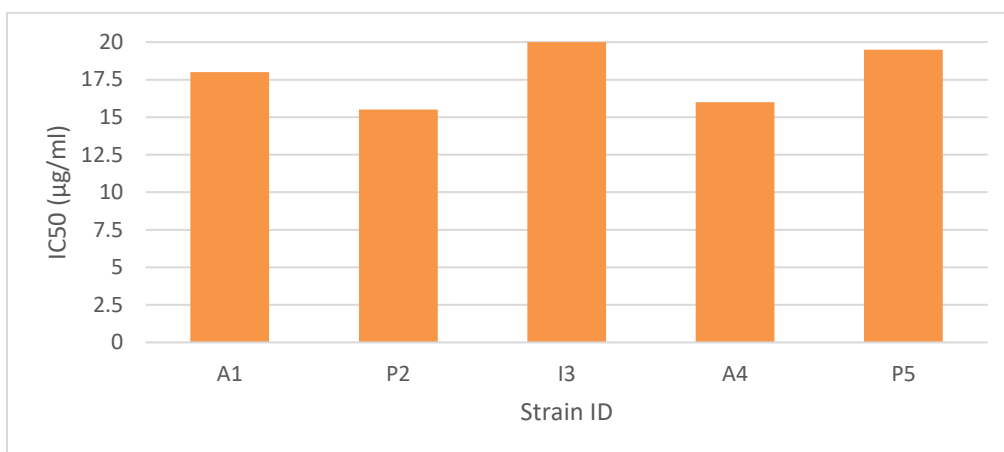


Fig. 6. Antioxidant activity (IC50) of xanthone extracts

properties. This indicates their promising applications in developing new therapeutic agents for infectious and oxidative stress-related diseases.

5. DISCUSSION

5.1 Significance of Findings

The findings of this study underscore the vast potential of marine fungi, specifically *Aspergillus sydowii* and *Geotrichum candidum*, as prolific producers of bioactive xanthone compounds. The successful isolation and identification of xanthenes with significant antimicrobial and antioxidant activities highlight the importance of marine fungi in natural product research. These xanthenes exhibited potent antimicrobial activity against a range of pathogenic microorganisms, including *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*, which are known for their resistance to conventional antibiotics. Furthermore, the strong antioxidant properties of these compounds suggest their potential use in preventing and treating diseases associated with oxidative stress. The results demonstrate the feasibility of harnessing marine fungi for the discovery of new therapeutic agents, thus contributing to the growing field of marine biotechnology.

5.2 Comparison with Previous Studies

5.2.1 Consistency with findings from other studies on marine-derived xanthenes

The results of this study are consistent with previous research on the bioactive properties of xanthenes derived from marine fungi. For

instance, [11] reported the isolation of xanthenes from marine-derived fungi with significant antimicrobial and antioxidant activities, similar to the findings in this study. [12] also highlighted the potential of marine-derived xanthenes in inhibiting the growth of multidrug-resistant bacteria, supporting the antimicrobial efficacy observed here. Furthermore, [13] documented the diverse bioactive properties of xanthenes, including their role in oxidative stress mitigation, which aligns with the strong antioxidant activity demonstrated by the xanthone extracts in this study. These consistencies reinforce the validity of the current findings and confirm the potential of marine fungi as a source of valuable bioactive compounds.

5.3 Potential Applications of Xanthenes

5.3.1 Novel applications in biotechnology and industry

The bioactive properties of xanthenes isolated from marine fungi suggest several promising applications in biotechnology and industry. The antimicrobial activity of these compounds positions them as potential candidates for the development of new antibiotics, particularly in the fight against antibiotic-resistant pathogens. This is crucial in addressing the global health challenge posed by the increasing prevalence of resistant infections [7]. Additionally, the antioxidant properties of xanthenes could be harnessed in the development of supplements and pharmaceuticals aimed at preventing and treating conditions related to oxidative stress, such as cardiovascular diseases, neurodegenerative disorders, and aging [8]. Beyond healthcare, xanthenes have potential

applications in the food and cosmetic industries as natural preservatives and anti-aging agents, respectively, due to their ability to scavenge free radicals and protect against oxidative damage.

5.3.2 Limitations of the study

While the findings of this study are promising, several limitations should be acknowledged. The sample size of fungal isolates was relatively small, and only a limited number of xanthenes were identified and tested for bioactivity. Expanding the scope of sample collection to include more diverse marine environments could potentially uncover additional bioactive compounds. Additionally, the bioactivity assays were conducted in vitro, and the efficacy of the xanthenes in vivo remains to be determined. Further research is needed to evaluate the pharmacokinetics, toxicity, and therapeutic potential of these compounds in animal models and clinical trials.

5.3.3 Recommendations for future research

Future research should focus on expanding the collection and isolation of marine fungi from diverse and underexplored marine environments. High-throughput screening methods should be employed to identify a broader range of bioactive compounds. Additionally, detailed studies on the mechanisms of action of xanthenes should be conducted to better understand their therapeutic potential. Collaborative efforts between marine biologists, chemists, and pharmacologists will be essential to optimize the extraction, purification, and application of these compounds. Because of their various bioactive substances and biotechnological applications, marine fungus such *Aspergillus sydowii*, *Penicillium citrinum*, *Trichoderma harzianum*, *Geotrichum candidum*, and *Fusarium solani* present great prospects for the future. *Aspergillus sydowii* is well-known for its antibacterial and anticancer metabolites as well as for its efficiency in hydrocarbon and heavy metal bioremediation. In addition to producing important industrial enzymes and antibiotics, *Penicillium citrinum* helps preserve food. *Trichoderma harzianum* produces enzymes that improve soil health and act as a biocontrol agent against plant diseases. The dairy sector uses *Geotrichum candidum* for cheese fermentation because it has the potential to produce medicinal compounds. Despite being recognized as a plant pathogen, *Fusarium solani* contains advantageous strains that aid in bioremediation and generate antibacterial and

anticancer chemicals. Finally, in vivo studies and clinical trials are crucial to validate the efficacy and safety of xanthenes for therapeutic use, paving the way for their integration into mainstream medical practice.

6. CONCLUSION

6.1 Summary of Key Findings

This study successfully demonstrated the potential of marine fungi, particularly *Aspergillus sydowii* and *Geotrichum candidum*, as prolific producers of bioactive xanthone compounds. The isolation and identification of xanthenes with significant antimicrobial and antioxidant activities underscore the importance of marine fungi in the discovery of new natural products. The xanthone extracts exhibited potent antimicrobial activity against key pathogenic microorganisms, including *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. Additionally, the strong antioxidant properties of these compounds highlight their potential use in mitigating oxidative stress-related diseases. The results confirm the feasibility of harnessing marine fungi for biotechnological and pharmaceutical applications, contributing to the advancement of marine biotechnology.

6.2 Implications for Pharmaceutical and Biotechnological Applications

The findings of this study have significant implications for the pharmaceutical and biotechnological industries. The potent antimicrobial activity of the isolated xanthenes suggests their potential as novel antibiotics, particularly in addressing the global health challenge of antibiotic-resistant infections. The development of new antibiotics from marine derived xanthenes could provide alternative treatment options for resistant bacterial and fungal infections. Furthermore, the strong antioxidant properties of these compounds position them as promising candidates for the development of supplements and pharmaceuticals aimed at preventing and treating oxidative stress-related conditions, such as cardiovascular diseases, neurodegenerative disorders, and aging. Beyond healthcare, the application of xanthenes in the food and cosmetic industries as natural preservatives and anti-aging agents also holds considerable promise. The successful exploitation of marine fungi for these applications underscores the vast

potential of marine biodiversity as a source of novel bioactive compounds.

6.3 Final Remarks

In conclusion, this study highlights the untapped potential of marine fungi, particularly *Aspergillus sydowii*, in the production of bioactive xanthone compounds. The significant antimicrobial and antioxidant activities of the isolated xanthenes underscore their potential for pharmaceutical and biotechnological applications. While the findings are promising, further research is needed to explore the full spectrum of bioactive compounds produced by marine fungi and to validate their efficacy and safety in vivo. Collaborative efforts across disciplines will be essential to fully harness the potential of marine fungi for the discovery and development of new therapeutic agents. The insights gained from this study pave the way for future research and innovation in marine biotechnology, contributing to the sustainable exploitation of marine resources for human benefit.

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DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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