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Marine Fungi – An Expandable Source for Production of Novel Bioactive Metabolites: A Comprehensive Review

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Abstract

The marine environment, representing the largest and most extreme biome on Earth, serves as an unparalleled reservoir of biodiversity. Within this vast ecosystem, marine fungi have emerged as a prolific source of structurally diverse and pharmacologically active secondary metabolites. Dwelling in habitats characterized by extreme high salinity, high hydrostatic pressure, and low temperatures, these microorganisms have evolved unique biochemical and metabolic pathways to synthesize novel compounds, including pyrrole–imidazole alkaloids, cyclic dipeptides, polyketides, and terpenoids. This comprehensive review evaluates the recent advancements in the discovery, isolation, and functional characterization of bioactive metabolites from marine-derived and endophytic fungi. It extensively highlights their potent antimicrobial and antibiofilm properties, particularly targeting multidrug-resistant bacterial strains where biofilms increase resistance up to 1000-fold. Furthermore, the review explores the potent cytotoxic and anticancer profiles of sponge-associated fungal metabolites, emphasizing mechanisms such as Chk2 kinase inhibition and the suppression of tumor cell migration. Additionally, the emerging field of marine dermocosmetics has been analyzed, detailing the role of fungal antioxidants, UV-photoprotective agents, and enzymatic inhibitors (targeting collagenase, elastase, and hyaluronidase) in anti-aging formulations. Through a PRISMA-based systematic evaluation, the biotechnological potential of these novel marine metabolites will be analyzed critically, providing a structured baseline for future drug discovery initiatives and clinical scale-up.

Keywords: Antibiofilm, cytotoxicity, dermocosmetics, marine fungi, secondary metabolites

INTRODUCTION

The marine environment covers over 70% of the Earth's surface and represents a largely untapped frontier for natural product discovery. Fungi constitute a substantial proportion of this microbial diversity, existing in dynamic symbiotic relationships with marine sponges, macroalgae (seaweeds), corals, and mangroves [1, 2]. To survive in extreme marine conditions, which feature severe environmental stressors, such as drastic salinity shifts, extreme hydrostatic pressures, limited nutrient availability, and intense ultraviolet (UV) radiation, marine fungi have developed specialized physiological and molecular adaptations [3]. These environmental pressures trigger distinct biosynthetic gene clusters that remain translationally silent in terrestrial fungi, making marine fungi an extraordinary and unique reservoir for novel secondary metabolites.

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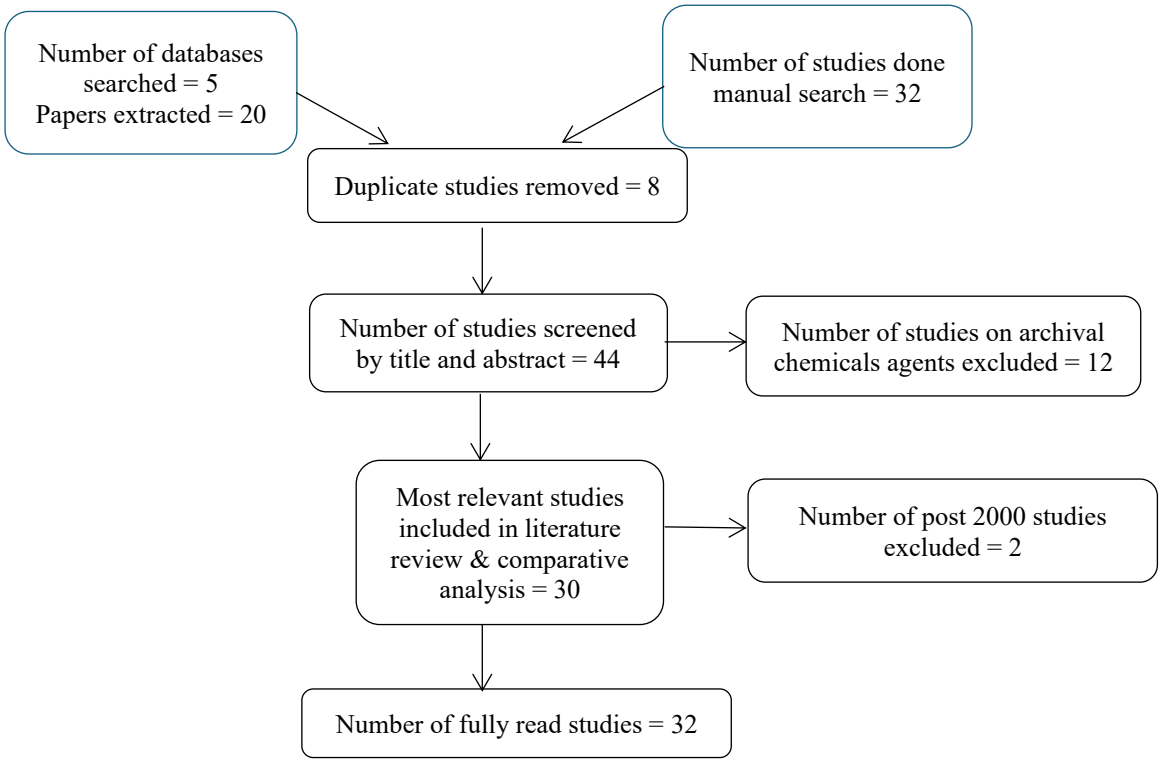
Over the past few decades, marine endophytic and sponge-associated fungi have yielded thousands of biologically active compounds [4, 5]. These secondary metabolites act as chemical defenses for the fungi and their hosts against microbial competitors, pathogens, and predators, exhibiting a broad spectrum of pharmacological activities [6, 7]. Given the escalating global health threats of multidrug-resistant bacterial infections, the need for

novel therapies, and the rising demand for natural anti-aging cosmeceuticals, the targeted exploration of the aquatic microbes offers a highly promising avenue for therapeutic discovery [2, 5, 8, 9].

PRISMA-BASED METHODOLOGY

This systematic review was conducted adhering to the core principles of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure a comprehensive and reproducible literature synthesis as follows:



- *Identification:* A systematic literature search was executed using academic databases including Academia Edu, Google Scholar, PubMed, Scribd, and Web of Science. Search strings utilized combinations of Boolean operators and terms such as "marine endophytic fungi," "seaweed endophytic fungi antibiofilm compounds," "cytotoxicity," "dermocosmetics," and "secondary metabolites".
- *Screening:* Initial screening involved evaluating the titles and abstracts of the retrieved records. Duplicate entries, non-English language articles, and studies lacking a primary focus on marine-derived fungal metabolites were excluded.
- *Eligibility:* Full-text articles were assessed for their relevance to the chemical profiling, biological activity, and pharmacological applications of marine fungal compounds. Studies were required to include explicit methodological techniques compatible with isolating marine-derived or endophytic fungi (e.g., surface sterilization using sodium hypochlorite).
- *Inclusion:* Articles providing explicit empirical data on the isolation, structural elucidation (via LC-MS/MS, NMR), and *in vitro* bioactivity assays (e.g., MTT, minimum biofilm eradication concentration) of marine fungal metabolites were included to synthesize this review and construct the comparative analyses.

KEY SECONDARY METABOLITES PRODUCED BY MARINE FUNGI

Including their chemical classifications, structural characteristics, specific marine sources, and extensively researched biological activities.

Echinulin and Neoechinulin A

- *Chemical Class:* Diketopiperazine-type indole alkaloids.
- *Marine Source:* Endophytic fungi, such as *Aspergillus chevalieri* (formerly *Eurotium chevalieri*) and *Penicillium* sect. *Exilicaulis*, are frequently isolated from the marine brown alga *Halopteris scoparia*.
- *Structural Characteristics:* These metabolites consist of three core structural moieties: An indole ring, an isoprenyl group, and a diketopiperazine ring. A critical structural feature in Neoechinulin A is the presence of a C8/C9 double bond, which is heavily correlated with its heightened antioxidant and cytoprotective properties.
- *Biological Activity and Mechanism:*
  - *Dermocosmetics:* Both compounds exhibit exceptionally high total phenolic content and extreme efficacy in scavenging DPPH free radicals [3, 10] (Figure 1). They function as robust photoprotective shields against UV-induced reactive oxygen species (ROS) production in human fibroblasts. Furthermore, they actively inhibit extracellular matrix-degrading enzymes, specifically collagenase and elastase, making them highly promising anti-aging agents [5, 11, 12].
  - *Neuroprotection and Anti-Inflammatory:* Neoechinulin A protects neuronal PC12 cells against peroxynitrite-induced cell death. It also acts as an anti-inflammatory lead molecule by inhibiting the NF-κB and p38 MAPK signaling pathways, thereby downregulating pro-inflammatory mediators like nitric oxide (NO) and TNF-α (Figure 2).

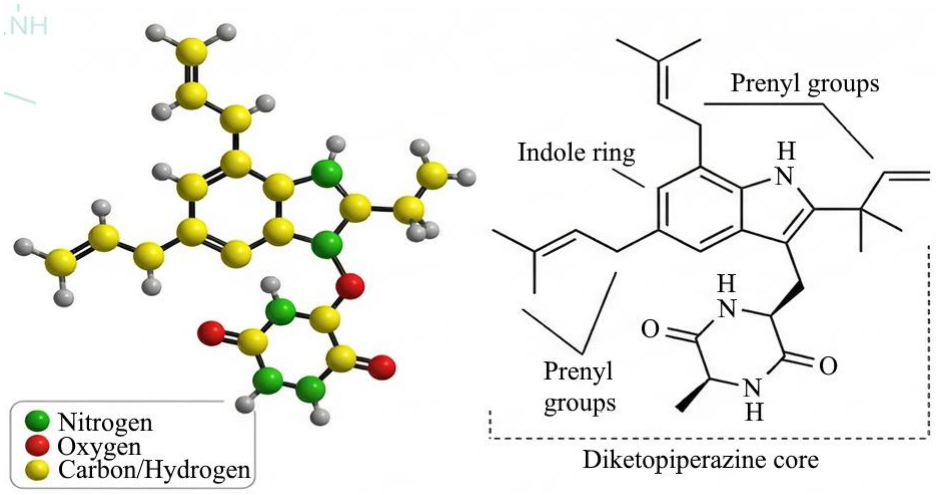


Figure 1. Echinulin.

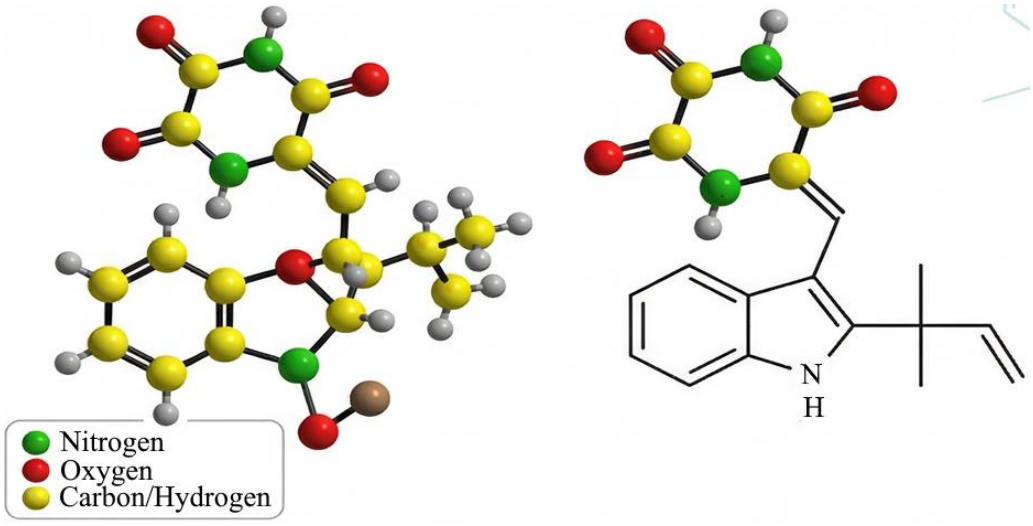


Figure 2. Neoechinulin – A.

Hymenialdisine and Spongiacidin D

- *Chemical Class:* Brominated pyrrole–imidazole alkaloids.
- *Marine Source:* The microbial flora symbiotically associated with marine sponges, most notably the Red Sea sponge *Stylissa carteri*.
- *Biological Activity and Mechanism:*
  - *Cytotoxicity:* These alkaloids display highly potent cytotoxic and anti-proliferative effects against various human cancer cell lines, including HepG2 (liver carcinoma) and HCT-116 (colon cancer). *In vitro* assays indicate that extracts containing these compounds can achieve up to 80% growth inhibition and suppress cancer cell migration (wound healing) and colony formation significantly [13].
  - *Molecular Targets:* Molecular docking and dynamics simulations reveal that hymenialdisine and spongiacidin D exhibit a rigid, high-affinity binding (e.g., –8.35 kcal/mol for hymenialdisine) to the ATP-binding pocket of the human checkpoint kinase 2 (Chk2) receptor. Chk2 is a critical regulator of the DNA damage response; its inhibition by these fungal metabolites heavily impairs the ability of cancer cells to replicate and survive [13, 14].

Epicorazines A and C

- *Chemical Class:* Thiodiketopiperazines.
- *Marine Source:* The marine-derived endophytic fungus *Epicoccum nigrum*, which has been isolated from the Antarctic seaweed *Phaeurus antarcticus* and various seagrasses.
- *Structural Characteristics:* Epicorazines are distinguished by a highly complex, rare cross-ring sulfur (disulfide) bridge within their molecular architecture, which is a known pharmacophore for extreme biological reactivity.
- *Biological Activity and Mechanism:*
  - *Antibiofilm Potential:* These metabolites demonstrate profound antibiofilm properties, successfully inhibiting and eradicating biofilms produced by multidrug-resistant bacterial strains. Against methicillin-resistant *Staphylococcus aureus* (MRSA) [15], Epicorazines A and C exhibit impressive minimum biofilm eradication concentrations (MBEC) as low as 50μg/mL and 25μg/mL, respectively. They act by disrupting the extracellular polymeric substance (EPS) matrix without exerting the intense evolutionary pressure typical of standard bactericidal antibiotics.

Fellutamides (A, B, C, and F)

- *Chemical Class:* Lipopeptides.
- *Marine Source:* Initially isolated from *Penicillium fellutanum* (derived from the gastrointestinal tract of the marine fish *Apogon endekataenia*) and subsequently found in sponge-derived *Aspergillus versicolor*.
- *Structural Characteristics:* Fellutamides are characterized by a unique C-terminal aldehyde and a β-hydroxy alkanoate tail (specifically derived from (3R)-hydroxy lauric acid in Fellutamide B). They also often incorporate the non-ribosomal amino acid β-L-threo-hydroxy-glutamine.
- *Biological Activity and Mechanism:*
  - *Proteasome Inhibition and Cytotoxicity:* Fellutamides are highly potent proteasome inhibitors. By blocking the ubiquitin–proteasome system (UPS) – a cellular recycling machinery vital for cell cycle progression and apoptosis regulation – they trigger rapid cell death in cancer cells. They display significant *in vitro* cytotoxicity against human epidermoid carcinoma (KB), leukemia, colon (HCT-15), lung (A549), and ovarian (SK-OV-3) cancer cell lines, frequently operating at nanomolar to low micromolar IC_{50} ranges [2, 13, 16, 17].

Simplicilliumtides (J-O)

- *Chemical Class:* Cyclic and linear hexapeptides.
- *Marine Source:* The deep-sea-derived fungal strain *Simplicillium obclavatum* EIODSF 020, isolated from East Indian Ocean marine sediments.
- *Biological Activity and Mechanism:*
  - *Antifungal and Antiviral:* Simplicilliumtides display potent antifungal activity, particularly against phytopathogenic fungal strains, like *Alternaria solani* and *Aspergillus versicolor*, acting

through antibiosis and cell wall disruption [18]. Furthermore, they exhibit pronounced antiviral activity against the Herpes Simplex Virus Type 1 (HSV-1), making them highly versatile molecules for both agricultural protection and human virology.

Coniosulfide E

- *Chemical Class:* Nitrogen-containing secondary metabolite.
- *Marine Source: Aspergillus unguis*, isolated from extreme deep-sea environments (often near hydrothermal vents).
- *Biological Activity and Mechanism:*
  - *Anti-Inflammatory:* While *A. unguis* is well known for producing cyclic heptapeptides (unguisins) and rare sterols (aspersterols), coniosulfide E is structurally distinct and demonstrates targeted anti-inflammatory properties [12]. It acts by downregulating the over-secretion of nitric oxide (NO) and suppressing the release of pro-inflammatory cytokines, such as Interleukin-6 (IL-6) in macrophage models, positioning it as a structural lead for the treatment of chronic inflammatory conditions.

PHARMACOLOGICAL POTENTIAL OF MARINE FUNGAL METABOLITES

Antimicrobial and Antibiofilm Compounds

The global rise of multidrug-resistant bacterial infections necessitates the discovery of novel antimicrobial agents. Bacterial biofilms are responsible for 40% to 80% of bacterial illnesses and can render bacteria up to 1000 times more resistant to conventional antibiotics than in their planktonic stages. Biofilms are structured communities embedded in extracellular polymeric substances (EPS) that hinder antibiotic penetration and facilitate quorum sensing (QS). Marine fungi are prolific producers of compounds capable of disrupting these resilient structures [7, 9, 13].

Endophytic fungi associated with marine plants and algae produce an array of antibiofilm agents. For instance, *Epicoccum nigrum* isolated from seaweed has yielded compounds, like 1-hydroxy-4,10,13-trimethyl-17-(6-methyl-5-methyleneheptan-2-yl)-3-oxo-2,3,4,7,8,9,10,11,12,13-decahydro-1H-cyclopenta[a]phenanthrene-4-carboxylic acid, which demonstrates significant minimum biofilm eradication concentration (MBEC) against methicillin-resistant *Staphylococcus aureus* (MRSA) [13]. Furthermore, polyketides, such as aspulvinones extracted from *Aspergillus flavipes* and tenellic acid C from *Neosartorya spinosa*, have shown profound biofilm inhibitory effects. By targeting quorum sensing rather than strictly acting as bactericidal agents, these fungal metabolites cause less evolutionary pressure, thereby reducing the likelihood of resistance development [19, 20].

Cytotoxic and Anticancer Agents

Marine fungi, particularly those existing in symbiotic relationships with marine sponges, are actively sought for their antineoplastic and cytotoxic potential. Red Sea sponges, such as *Stylissa carteri*, *Hemimycale arabica*, and *Negombata magnifica*, possess distinct microbial ecosystems that biosynthesize potent secondary metabolites. Extensive *in vitro* analyses using HepG2 human liver carcinoma cells have demonstrated that the methanolic and dichloromethane extracts of *S. carteri* exhibit extraordinary cytotoxicity, achieving 80% growth inhibition and a highly significant suppression of cancer cell colony formation and migration.

The chemical profiling of these cytotoxic extracts via UPLC/ESI-MS reveals an enrichment of unique brominated pyrrole–imidazole alkaloids. Key metabolites isolated include hymenialdisine, oroidin, and spongiacidin D. Molecular docking and dynamics simulations reveal that hymenialdisine exhibits strong binding affinity (–8.35 kcal/mol) to the ATP-binding site of the human checkpoint kinase 2 (Chk2) receptor. Chk2 is a crucial regulator of the DNA damage response; its inhibition heavily impairs cancer cell proliferation and survival [3, 8, 21, 22]. The stable complex formed between hymenialdisine and Chk2 is characterized by rigid structural thermodynamics and critical hydrogen bonding with key residues (Glu76 and Met78), underscoring the potential of marine fungal alkaloids as targeted oncological therapies [23, 24].

Marine Peptides and Dermocosmetic Applications

Beyond systemic therapies, marine fungal metabolites are being increasingly explored for their highly lucrative dermocosmetic applications [25, 26]. Skin aging is a complex physiological process accelerated by extrinsic factors, predominantly UV-induced oxidative stress. This leads to the generation of reactive oxygen species (ROS) and the subsequent enzymatic degradation of the extracellular matrix by collagenase, elastase, and hyaluronidase [9, 13, 16].

Marine endophytic fungi, associated with the Portuguese coast seaweed *Halopteris scoparia*, exhibit remarkable anti-aging properties. Fungal isolates belonging to the taxa *Aspergillus chevalieri* and *Penicillium* sect. *Exilicaulis* have been identified as potent producers of dermocosmetically relevant compounds. Crude extracts from *A. chevalieri* demonstrated exceptionally high total phenolic content and extreme efficacy in scavenging DPPH free radicals, acting as a robust photoprotective shield against UV-induced ROS production in human fibroblasts [8, 25, 27, 28]. High-resolution tandem mass spectrometry (HRMS/MS) of these extracts identified the indole alkaloids echinulin and neoechinulin A as the primary bioactive drivers [29]. Furthermore, these fungal metabolites successfully inhibited the activity of collagenase and elastase, enzymes responsible for skin wrinkling, while also exhibiting antimicrobial activity against *Cutibacterium acnes*, highlighting their dual utility in both anti-aging and anti-acne cosmetic formulations.

COMPARISON OF BIOACTIVE METABOLITES

The following table synthesizes key marine fungi, their isolated metabolites, and their corresponding biological activities.

Fungal species	Marine source/host	Major identified metabolite(s)	Primary biological activity
Aspergillus chevalieri	Seaweed (*Halopteris scoparia*)	Echinulin, Neoechinulin A	High antioxidant capacity, strong collagenase inhibition, UV-photoprotection, and inhibition of *C. acnes*.
Stylissa carteri (associated mycobiome)	Red Sea Sponge	Hymenialdisine, Spongiacidin D, Oroidin	Potent cytotoxicity against HepG2 liver cancer cells, inhibition of cell migration, and Chk2 kinase receptor binding.
Epicoccum nigrum	Seaweed (*Phaeurus antarcticus*)	Epicorazines A and C	Significant antibiofilm properties and minimum biofilm eradication concentration (MBEC) against MRSA.
Penicillium sect. *Exilicaulis*	Seaweed (*Halopteris scoparia*)	Unspecified phenolic compounds	Strong inhibition of hyaluronidase and collagenase activities, mild photoprotective properties.
Diaporthe sp.	Seaweed (*Halopteris scoparia*)	Unspecified complex extracts	Strong inhibition of tyrosinase activity (skin-whitening potential) and significant anti-inflammatory capacity.

OPPORTUNITIES AND CHALLENGES

Despite the immense pharmaceutical potential of the marine mycobiome, substantial biochemical and ecological challenges remain. A primary bottleneck is that many secondary metabolite biosynthetic gene clusters (BGCs) remain silent or cryptic under standard laboratory cultivation [30]. To overcome this, researchers are employing advanced strategies, such as the OSMAC (One Strain Many Compounds) approach, co-cultivation with competing bacteria, and chemical epigenetic modifiers (e.g., DNA methyltransferase inhibitors), to induce the expression of these silent pathways. Furthermore, integrating high-throughput omics technologies – such as LC-MS/MS networking paired with multivariate metabolomics – is accelerating the dereplication process, ensuring that novel compounds, like anti-migratory alkaloids and anti-aging peptides, are identified rapidly and accurately.

CONCLUSION

Marine-derived and endophytic fungi represent an unparalleled, yet vastly underexplored, treasure trove of bioactive secondary metabolites. Their evolutionary adaptations to harsh oceanic conditions have endowed them with the biochemical machinery to synthesize complex molecular scaffolds rarely

found in terrestrial organisms. From potent cytotoxic alkaloids, like hymenialdisine that target specific cancer kinase pathways, to robust antibiofilm agents that disarm multidrug-resistant bacteria, these compounds offer innovative solutions to modern pharmacological crises. Furthermore, the discovery of photoprotective and enzyme-inhibitory metabolites, like echinulin, opens highly lucrative avenues for the dermocosmetic industry. Continued interdisciplinary research, leveraging advanced metabolomics and synthetic biology, is essential to fully harness and sustainably scale the therapeutic and commercial potential of the marine mycobiome.

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