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Article

Antimicrobial and Antibiofilm Activity of Marine *Streptomyces* sp. NBUD24-Derived Anthraquinones Against MRSA

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Abstract

Antimicrobial resistance (AMR) has emerged as a global health crisis, with methicillin-resistant *Staphylococcus aureus* (MRSA) representing one of the most clinically significant multidrug-resistant pathogens. In this study, three structurally unique anthracycline derivatives—keto-ester (**1**), 4-deoxy-ε-pyrromycinone (**2**), and misamycin (**3**)—were first isolated and characterized from the fermentation broth of the marine-derived *Streptomyces tauricus* NBUD24. These compounds exhibited notable antibacterial efficacy against MRSA, with minimum inhibitory concentrations (MICs) ranging from 16 to 32 μg/mL. Cytotoxicity assays confirmed their safety profile at therapeutic concentrations. The biofilm formation assay demonstrated that 4-deoxy-ε-pyrromycinone inhibited biofilm formation of MRSA ATCC43300, with an inhibition rate of 64.4%. Investigations of antibacterial mechanisms revealed that these compounds exert antibacterial effects primarily through disruption of bacterial cell wall integrity and destruction of DNA structure. These findings underscore the potential of marine-derived microbial metabolites as promising scaffolds for developing next-generation antimicrobial candidates to combat drug-resistant infections.

Keywords: marine natural products; *Streptomyces tauricus* NBUD24; MRSA; antibacterial; antibiofilm

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1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA), the primary pathogen causing soft tissue and skin infections, bacteremia, and joint infections [1], is listed as one of the six multidrug-resistant pathogens requiring urgent attention by the WHO [2]. The Antibiotic Resistance Threats Report released by the US Centers for Disease Control and Prevention (CDC) shows that the number of cases of serious invasive diseases and deaths caused by MRSA is estimated to reach 72,000 and 10,000, respectively, in the US each year [3,4]. The treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) poses significant challenges due to the development of antimicrobial tolerance and persistence among clinically used agents [5]. Vancomycin and linezolid, regarded as last-line therapeutics for MRSA infections [6], have seen their efficacy compromised by the emergence of corresponding resistant strains following increased clinical utilization [7]. Therefore, there is an urgent need to develop new drugs with novel antibacterial mechanisms to overcome antibiotic resistance problems.

The vast ocean covers nearly 70% of the Earth's surface [8]. Its extreme conditions, such as high salinity, high pressure, and low oxygen [9,10], have driven marine organisms

to evolve diverse secondary metabolites with remarkable bioactivities, demonstrating significant potential for antibiotic development [11]. In our previous studies, three compounds, Quinosumycin [12], Xiamycin, and Chloroxiamycin [13], were isolated from *Streptomyces diastaticus* NBU2966 and *Streptomyces* sp. NBU3429 and characterized. These compounds demonstrated potent antibacterial activity against MRSA, with minimum inhibitory concentrations (MICs) ranging from 8 to 32 μg/mL. Quinosumycin represents the first reported heterodimeric scaffold featuring a thioether-bridged quinolinone–quinazolinone structure, demonstrating selective anti-MRSA activity [12]. Two indole sesquiterpene compounds, Xiamycin and its chlorinated metabolite, Chloroxiamycin, which were first isolated from the fermentation broth of marine *Streptomyces* sp. NBU3429, exhibited antibacterial/antibiofilm activity against MRSA [13]. These reports indicate that marine *Streptomyces* are an important source of potential antibacterial compounds, offering broad prospects for developing novel therapeutics against resistant pathogens.

As part of our ongoing research on bioactive *actinomycetes*, we isolated *Streptomyces tauricus* NBUD24 from the tissue of the mesophotic zone sponge (*Dasychalina* sp.). From this strain, three anthracycline derivatives—keto-ester (**1**), 4-deoxy-ε-pyrromycinone (**2**), and misamycin (**3**)—were obtained and structurally characterized. Herein, we report the isolation, structural elucidation, and evaluation of the antibacterial and antibiofilm activities of compounds **1**–**3**, along with a preliminary investigation of their mechanism of action.

2. Results

2.1. Structures of Compounds

As shown in Figure 1, the structures of compounds **1**–**3** were identified as keto-ester, 4-deoxy-ε-pyrromycinone, and misamycin. The ^{1}H NMR spectrum, ^{13}C NMR spectrum, UV spectrum, and HRESIMS spectrum of these compounds are shown in Supplementary Figures S1–S3. In addition, the ^{1}H and ^{13}C NMR data of compounds **1**–**3** in $CDCl_3$ are shown in Supplementary Table S1.

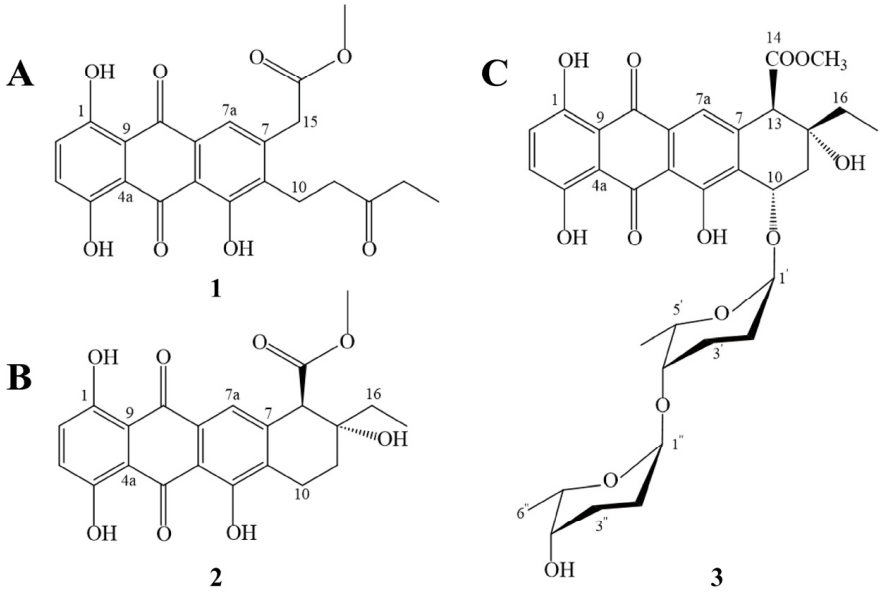

Figure 1. Structure of keto-ester (**A**), 4-deoxy-ε-pyrromycinone (**B**), and misamycin (**C**).

The molecular formula of **1** (Figure 1A) was determined as $C_{22}H_{21}O_8$ (*m*/*z* 413.1239 $[M + H]^+$) by HRESIMS (Figure S1). The ^{1}H NMR spectrum (600 MHz, $CDCl_3$; Table 1) of compound **1** exhibited two methyl singlets at δ_H 1.28 (H_3-14) and 1.45 (H_3-17), three

olefinic methine protons at δ_H 6.32 (H-2), 6.15 (H-3), and 5.87 (H-7a), and four methylene groups at δ_H 2.65 (H_2-10), 2.82 (H_2-11), 3.12 (H_2-13), and 2.35 (H_2-15). Concurrently, the ^{13}C NMR spectrum (150 MHz, $CDCl_3$; Table 1) revealed 22 distinct carbon signals, comprising 2 methyl carbons (δ_C 8.0, 52.6), 3 olefinic methine carbons (δ_C 122.0, 129.6, 130.1), and 13 quaternary carbons, including an ester carbonyl (δ_C 170.8) and a ketone carbonyl (δ_C 186.3). Comparative analysis with published NMR data for anthracycline derivatives conclusively identified compound **1** as a keto-ester [14].

Table 1. The MICs of compounds against MRSA ATCC43300 and *E. coli* ATCC25922.

Drugs	MICs			
	ATCC43300		ATCC25922	
	(μg/mL)	(μM)	(μg/mL)	(μM)
Keto-ester	16	38.70	>128	>309.61
4-Deoxy-ε-pyrromycinone	16	38.70	>128	>309.61
Misamycin	32	48.73	>128	>194.92
Vancomycin	1	0.69	/	/
Polymyxin B	/	/	2	1.54

The molecular formula of **2** (Figure 1B) was determined as $C_{22}H_{21}O_8$ (*m*/*z* 413.1239 $[M + H]^+$) by HRESIMS (Figure S2). Comparative analysis with published NMR data for anthracycline analogues conclusively identified compound **2** as 4-deoxy-ε-pyrromycinone [15].

The molecular formula of **3** (Figure 1C) was determined as $C_{34}H_{40}O_{13}$ (*m*/*z* 657.2586 $[M + H]^+$) by HRESIMS (Figure S3). Comparative analysis with the literature NMR data for structurally related anthracyclines conclusively established compound **3** as misamycin [16].

2.2. MICs

The antibacterial activities of keto-ester, 4-deoxy-ε-pyrromycinone, and misamycin were tested by determining MIC values using the broth microdilution method. The MIC values of these compounds were measured against MRSA ATCC43300 and *Escherichia coli* (*E. coli*) ATCC25922, with vancomycin and polymyxin B as positive controls. As shown in Table 1, these compounds showed varying degrees of inhibition against MRSA ATCC43300 (MIC 16–32 μg/mL). However, the MIC values for *E. coli* ATCC25922 exceeded the maximum test range.

2.3. Cytotoxicity

The toxicity of compounds to macrophagocyte RAW 264.7 cells was determined. As illustrated in Figure 2, all compounds exhibited cytotoxicity to varying degrees at the highest test concentration. Under the treatment of keto-ester, 4-deoxy-ε-pyrromycinone, and misamycin at the concentrations of 128 μg/mL, the cell survival rates were 73.5%, 84.7%, and 85.6%, respectively. Within the test concentration range (1–128 μg/mL) of keto-ester, the cell survival rate was 73.5–88.8%. Except at the highest test concentration, the cell survival rates of 4-deoxy-ε-pyrromycinone and misamycin were both higher than 95%. 4-Deoxy-ε-pyrromycinone and misamycin can be used safely within the normal antibacterial range.

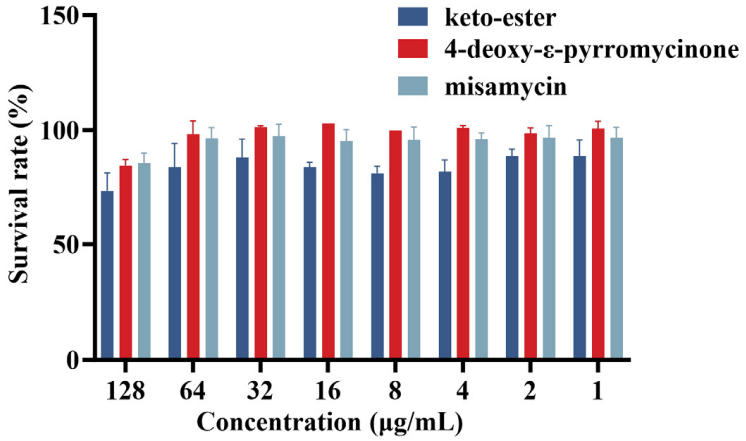

Figure 2. The toxicity of compounds against the RAW 264.7 cells.

2.4. Scanning Electron Microscopy (SEM) Observation of Bacterial Morphology

To investigate the impacts of compounds on bacterial cell walls, the cell morphology of MRSA ATCC43300 was visualized by SEM. As shown in Figure 3A–D, the MRSA ATCC43300 of the control group (CK) exhibited normal morphology and a smooth surface. After 2 h of exposure to 4× MIC compounds (keto-ester and 4-deoxy-ε-pyrromycinone: 64 μg/mL; misamycin: 128 μg/mL), shrinkage, bulging of bullae, and filamentous adherent material appeared on the bacterial surface. These results indicated that these compounds could damage the cell wall of MRSA ATCC43300 and exert antibacterial activity through wall damage.

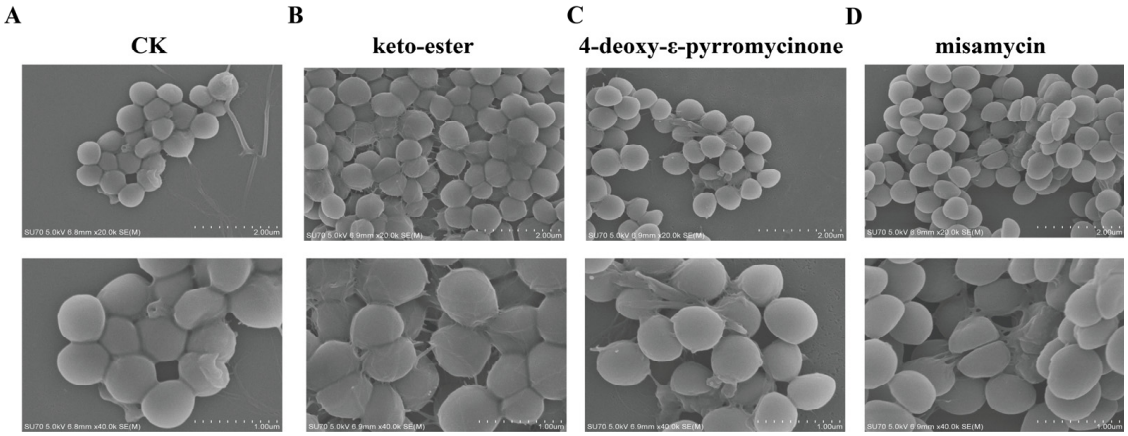

Figure 3. Effects of compounds on cell wall and membrane. (**A**–**D**) Scanning electron microscopy (SEM) analysis of MRSA ATCC43300 treated with 4× MIC of compounds. CK: treated with an equal volume of DMSO.

2.5. Effects on Bacterial Genomic DNA

DNA migration was assessed by electrophoresis on a 1% agarose gel. As can be seen in Figure 4A, the bacterial genome was not subjected to a blocking effect, and thus, the compounds had a relatively minor impact on bacterial DNA-binding ability. Then we speculated whether the compounds could damage the secondary structure of DNA. Cyclization of the hexacyclic scaffold exhibited negligible effects on MIC values, and we aimed to investigate whether the presence of glycosyl moieties would interfere with the secondary structural integrity of DNA. Therefore, we used 4-deoxy-ε-pyrromycinone and misamycin for a CD spectrometer assay. As displayed in Figure 4B, the positive and negative peaks of normal bacterial DNA occurred at about 275 nm and 245 nm. After treatment with compounds, although the overall shape of the DNA chromatogram was

similar to the control group, the peak value at 245 nm decreased for both drug-treated groups, indicating that the compounds had an impact on the structure of DNA.

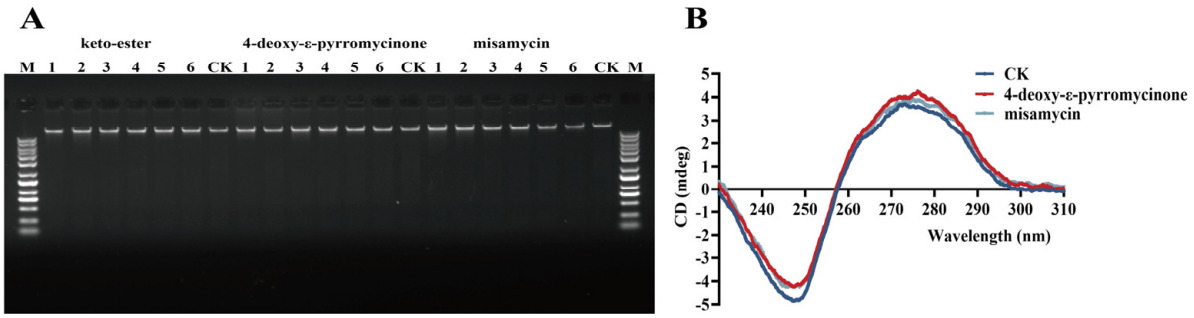

Figure 4. Interaction of compounds with MRSA ATCC43300 genomic DNA. (**A**) Interactions of compounds with MRSA ATCC43300 genomic DNA, which were tested by a gel migration assay. M: DNA marker; 1–6: the concentrations of compounds were 64, 32, 16, 8, 4, and 2 μg/mL, respectively; CK: treated with an equal volume of DMSO. (**B**) CD spectra of genomic DNA from MRSA ATCC43300 in the presence of keto-ester, 4-deoxy-ε-pyrromycinone, and misamycin. The concentrations of compounds and DNA were 64 and 150 μg/mL, respectively.

2.6. Transcriptome Analysis of MRSA ATCC43300 Treated with Compounds

Transcriptomic analysis provides crucial molecular insights into elucidating the antimicrobial mechanisms of many antimicrobial agents [17–19]. To further explore the potential antibacterial mechanisms by which the compounds act on *S. aureus*, transcriptome analysis was performed on *S. aureus* treated with compounds (1× MIC) for 4 h. As shown in Figure 5A–F, 4-deoxy-ε-pyrromycinone and misamycin treatment identified 142 (79 upregulated and 63 downregulated) and 193 (85 upregulated and 106 downregulated) significantly differentially expressed genes (DEGs, $|\log2(\text{fold change})| > 1$, FDR < 0.05), respectively. All of the compound treatment groups downregulated genes involved in cell wall synthesis, including *ezrA*, *mraZ*, *cwrA*, etc. [20–22]. It was indicated that the compounds could inhibit the formation of the bacterial cell wall, which is consistent with the results of SEM. In addition, many genes were significantly upregulated after being treated with compounds; for example, *recA*, *lexA*, and *uvrB* for DNA replication and repair systems were increased to protect cells from the corresponding stress response [23,24]. KEGG pathway analysis identified 20 metabolic pathways affected by the treatments. Notably, both compounds significantly downregulated genes associated with microbial metabolism in diverse environments and glycolysis/gluconeogenesis pathways. We speculate that the addition of the compound triggers the bacterial stress mechanism, which then regulates metabolism and DNA damage repair to adapt to the presence of the compound, ultimately ensuring the survival and proliferation of the bacteria in an unfavorable environment. These pathways are critically involved in amino acid metabolism and energy production—essential biological processes for bacterial survival. The marked suppression of these metabolic pathways suggests that the compounds likely exert their antibacterial effects by disrupting bacterial amino acid metabolism synthesis and energy homeostasis.

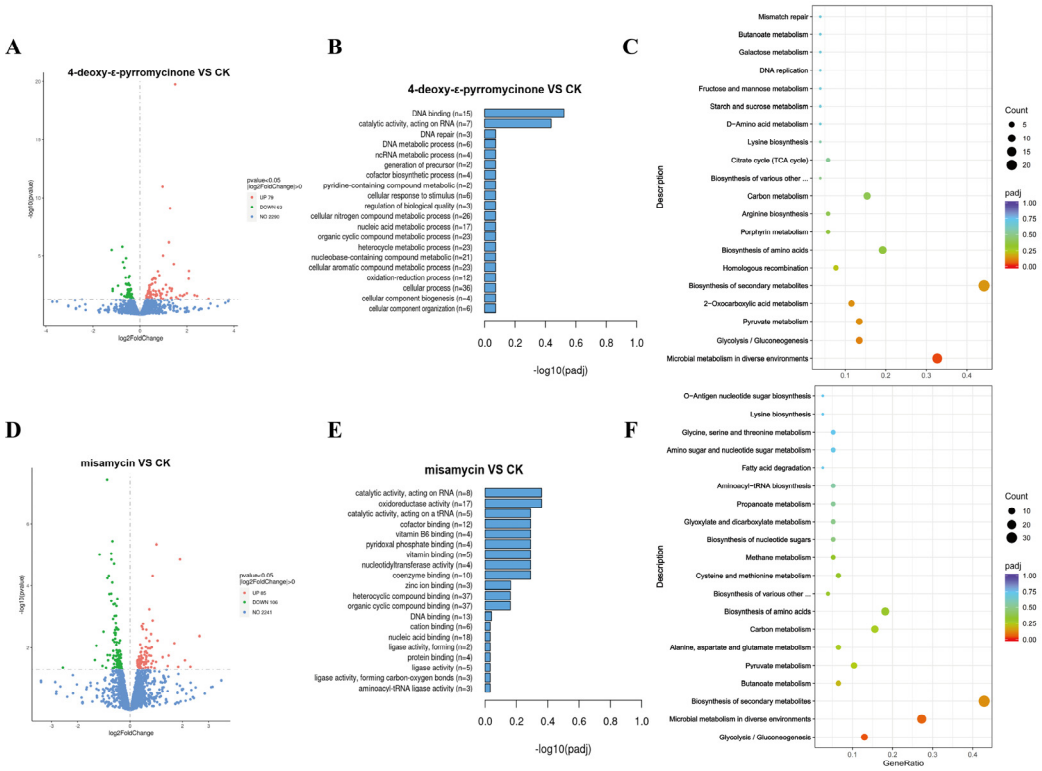

Figure 5. Transcriptome analysis of *S. aureus* treated with compounds (1× MIC). (**A**) Volcano plot of the significantly differentially expressed mRNA genes in *S. aureus* following treatment with 4-deoxy-ε-pyrromycinone. (**B**) GO function analysis chart of significantly differential genes in *S. aureus* following treatment with 4-deoxy-ε-pyrromycinone. (**C**) Bubble chart for the KEGG pathway enrichment of significantly upregulated and downregulated genes in *S. aureus* following treatment with 4-deoxy-ε-pyrromycinone. (**D**) Volcano plot of the significantly differentially expressed mRNA genes in *S. aureus* following treatment with misamycin. (**E**) GO function analysis chart of significantly differential genes in *S. aureus* following treatment with misamycin. (**F**) Bubble chart for the KEGG pathway enrichment of significantly upregulated and downregulated genes in *S. aureus* following treatment with misamycin.

2.7. Biofilm Formation Assay

As illustrated in Figure 6, at the highest tested concentration, 4-deoxy-ε-pyrromycinone demonstrated an inhibitory effect on biofilm formation with an inhibition rate of 64.4%. In comparison, the inhibition rates of the other two compounds were 25.6% and 14.4%, respectively. At lower tested concentrations (ranging from 1 to 64 μg/mL), all compounds exhibited relatively low inhibition rates against biofilm formation.

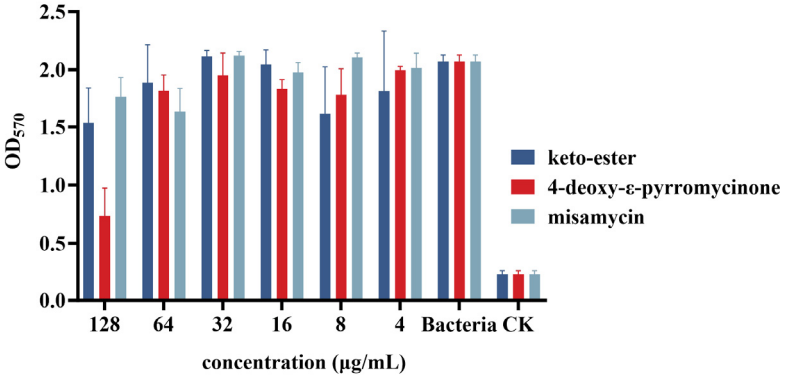

Figure 6. The inhibition abilities of compounds against MRSA biofilms.

3. Discussion

The MICs of the compounds against MRSA ranged from 16 to 32 μg/mL, and there was no significant difference in antibacterial activity between the compounds. Based on the MIC results, all of our compounds have a parent structure—1,4,5-trihydroxy-9,10-anthraquinone. Therefore, we speculate that this structure is the key point for the compounds' antimicrobial effect. Our result is consistent with the previous research conclusions that molecules with similar structures usually have similar drug effects [25]. Among these compounds, keto-esters show a certain structural similarity to 4-deoxy-ε-pyrromycinone, as both contain methyl and ester bond structures. Their main difference lies in whether the six-membered ring forms a closed loop, but it does not change the MIC values. The side chain of misamycin contains a unique disaccharide structure — α-narbosine B, which may increase its MIC to 32 μg/mL. None of the compounds exhibit antibacterial activity against *E. coli* ATCC25922 at a concentration of 128 μg/mL. This might be due to the presence of the outer membrane of Gram-negative bacteria, which prevents the drugs from exerting their effects.

Given the cytotoxicity associated with anthraquinones in clinical oncology practice, we conducted a comprehensive safety assessment of these compounds. The result showed that, although the compounds exhibit dose-dependent cytotoxicity, the cell survival could reach 73.3–85.6% at the highest tested concentration (128 μg/mL). When antimicrobial treatment is performed using 1× or 2× MIC concentrations, cell survival was consistently maintained above 80%. This indicates an acceptable safety profile in antimicrobial applications. In particular, 4-deoxy-ε-pyrromycinone and misamycin exhibit improved safety margins relative to keto-esters and doxorubicin, a widely used antitumor agent [26].

Subsequently, we explored the compounds' antibacterial mechanism. The cell wall of Gram-positive bacteria is mainly a reticular scaffolding structure formed by peptidoglycan. In this study, we observe that compounds containing anthraquinone structures can disrupt the integrity of bacterial cell walls, which is consistent with the findings of Tang et al. [27]. Moreover, transcriptomic profiling demonstrated significant downregulation of *ezrA*, *mraZ*, and *cwrA* genes in compound-treated strains. The downregulation of *ezrA* and *mraZ* disrupts the FtsZ protein assembly and Z-ring formation, leading to defective cell wall synthesis during division [20,21]. Concurrently, the reduced expression of *cwrA* promotes peptidoglycan hydrolysis, interfering with the structural integrity of the cell wall [22]. Following cellular internalization, the compounds can further damage the secondary structure of genomic DNA, which extends the previous conclusions that anthraquinones are considered to be inhibitors of bacterial topoisomerases I and II [28]. Therefore, we speculate that our compounds can target the bacterial DNA topoisomerase, thereby inhibiting the replication and transcription of DNA and then blocking the expression of proteins. Hence, bacteria need to upregulate DNA-damage-repair-related genes (*recA*, *lexA*, and *uvrB*) to activate damage repair pathways and sustain vital cellular processes under stress conditions.

MRSA can form biofilms, which increase bacterial resistance to antibiotics [29]. Once these bacteria establish biofilms, it is tough to eliminate them, which is one of the main reasons for persistent and chronic infections [30]. There is an urgent clinical need to develop novel therapeutic agents effective against MRSA and its biofilm-associated infections. In our study, we find that 4-deoxy-ε-pyrromycinone shows a better antibiofilm activity. Compared with keto-esters and misamycin, 4-deoxy-ε-pyrromycinone effectively suppressed biofilm growth, achieving an inhibition rate of 64.4% at 8× MIC (128 μg/mL). We hypothesize that 4-deoxy-ε-pyrromycinone inhibits biofilm formation by decreasing mRNA levels of cwrA and *ezrA*. The downregulation of *cwrA* induced the activation of the *icaR* operon, thereby decreasing PIA production and reducing biofilm adhesion capacity [31]. The transcription inhibition of *ezrA* leads to reduced extracellular polysaccharide levels and architectural changes, resulting in impaired biofilm formation [32].

4. Materials and Methods

4.1. General Experimental Procedures

The ^{13}C NMR and ^{1}H chemical shifts were obtained relative to the solvent signal $CDCl_3$ (δ_H 7.26/δ_C 77.16) on a Bruker AVANCE NEO 600 MHz instrument (Bruker Bio spin AG, Fällanden, Switzerland). Tetramethylsilane (TMS) was used as an internal standard. Medium-pressure liquid chromatography (MPLC) separations were carried out on a Bonna-Agela FLEXA purification system. C18 reversed-phase silica gel (50 μm, YMC Co., Ltd., Tokyo, Japan) and silica gel (200–300 mesh, Qingdao, China) were used for column chromatography. HRESIMS data were acquired using a Waters G2-XS Q-TOF mass spectrometer (Milford, MA, USA) coupled with liquid chromatography (LC/MS). Preparative HPLC was conducted on an Agilent 1260 system (Agilent Technologies, Lexington, MA, USA) equipped with a DAD and a YMC C18 column (10 × 250 mm, 5 μm, YMC).

4.2. Antimicrobial Agents

Streptomyces tauricus NBUD24 (*actinobacterial* strain) was derived from the South China Sea, China. It was obtained from tissue samples of the mesophotic zone sponge *Dasychalina* sp. The strain was defined by 16S rDNA sequence amplification (GenBank accession number PRJNA1187314) and deposited at Ningbo University, Ningbo, China.

4.3. Fermentation, Extraction, and Isolation

A fresh *actinomycete* colony was obtained by culturing on ISP2 plates at 25 °C for 3 days. The *strain* was subsequently inoculated in 300 mL ISP-2 medium (4.0 g/L yeast extract, 4.0 g/L glucose, 30 g/L sea salt, 10 g/L malt extract, pH 7.2). The culture flasks were incubated for 3d (28 °C, 220 rpm). The seed culture (6.0 L) was transferred to a rotary 1000 mL Erlenmeyer flask, with 300 mL of a liquid medium (9 g artificial sea salt, 1.5 g yeast extract, 3 g glucose, 1.5 g malt extract, 0.15 g $CaCO_3$, 6.0 g starch). 12 days later, all fermentation broths were extracted three times with ethyl acetate (EtOAc) to generate crude extract (22.6 g).

The crude extract was subjected to silica gel column chromatography (200–300 mesh) using a stepwise gradient of ethyl acetate in petroleum ether (PE) (100:0, 95:5, 90:10, 85:15, 80:20, 70:30, 50:50, 0:100), yielding 3 major fractions (Fr. A-C). Fr. B was further fractionated on an ODS column (H_2O/MeOH, 2:3, 0:1), yielding six subfractions (Fr.B.1-6). Subsequent purification of Fr.B.2, Fr.B.4 and Fr.B.5 by semi-preparative HPLC (34%, 53%, and 56% MeCN respectively; flow rate 2 mL/min) afforded compounds **1** (tR = 45 min, 4.8 mg), **2 (tR = 29 min**, 3.5 mg) and **3** (tR = 37 min, 5.6 mg).

4.4. Structure Elucidation

Compound **1** was isolated as a yellow amorphous powder. Then the structure of compound **1** was identified as a keto-ester by comparing the NMR data. The ^{1}H and ^{13}C NMR spectroscopic data is as follows: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.75 (s, H-7a), 7.30 (d, J = 9.4 Hz, H-2, H-3), 3.72 (s, H-17), 3.49 (s, H-15), 3.03 (t, J = 7.5 Hz, H-10), 2.80 (t, J = 7.5 Hz, H-11), 2.44 (q, J = 7.3 Hz, H-13), 1.07 (t, J = 7.3 Hz, H-14); ^{13}C NMR (150 MHz, $CDCl_3$) δ 210.6 (C-12), 191.1 (C-5), 186.3 (C-8a), 170.8 (C-16), 161.5 (C-6), 158.4 (C-4), 157.9 (C-1), 142.5 (C-7), 137.4 (C-6a), 131.1 (C-8), 130.1 (C-2), 129.6 (C-3), 122.0 (C-7a), 114.6 (C-5a), 112.8 (C-4a), 112.7 (C-9), 52.6 (C-17), 40.5 (C-11), 39.5 (C-15), 36.0 (C-13), 21.4 (C-10), 8.0 (C-14).

4.5. Bacterial Strains and Cells

The strains (MRSA ATCC43300 and *E. coli* ATCC25922) were purchased from the American Type Culture Collection (Manassas, VA, USA). The RAW 264.7 cell line was donated by Dr. Guo Hua (Ningbo University).

4.6. MICs

MICs of the compounds were determined using the micro broth dilution method, as previously described [33]. In the assay, Mueller–Hinton (MH) broth and Luria–Bertani (LB) broth were used to culture MRSA ATCC43300 and *Escherichia coli* ATCC25922, respectively. The mid-logarithmic phase of bacteria was diluted to 1×10^5 CFU/mL. The compounds were diluted with DMSO using two-fold serial dilution, with a concentration gradient from 1 to 128 μg/mL. After 2 μL of the compound and 98 μL of the bacterial suspension were added to a 96-well plate, the plate was incubated at 37 °C for 16–24 h. The MIC value is defined as the lowest concentration at which no bacterial growth is observed.

4.7. Cytotoxicity

The effect of compounds on the viability of murine peritoneal RAW 264.7 macrophage cells was determined by the CCK-8 method [34]. Cells were added to a 96-well plate at a density of 2.5×10^4 cells/well and cultured at 37 °C for 24 h in a 5% CO_2 air environment. Cells were exposed to serial concentrations of test compounds (1–128 μg/mL) for 24 h, with DMSO-treated cells serving as a control. Each well was supplemented with 10 μL of WST-8 solution. The plate was protected from light and cultured at 37 °C for 4 h. The absorbance at 460 nm was measured. The following formula was used to calculate cell viability: cell viability (%) = OD 460 nm of treated sample/OD 460 nm of control × 100%.

4.8. Scanning Electron Microscopy Observation of Bacterial Morphology

The mid-logarithmic phase of MRSA ATCC43300 (1×10^8 CFU/mL) was cultured with 4× MIC compounds for 2 h at 37 °C. After three washes with PBS, the bacteria were fixed overnight at 4 °C with 2.5% glutaraldehyde. A graded series of ethanol (20–100%) was used to dehydrate bacteria, with each concentration applied for 5 min, followed by drying with CO_2. The surface of the samples was sprayed with gold–palladium, and the bacteria were observed using an S4800 scanning electron microscope (Hitachi, Tokyo, Japan) [35].

4.9. Effects on Bacterial Genomic DNA

4.9.1. DNA Gel Migration Assay

Genomic DNA was obtained by a bacterial genome extraction kit (Aidlab Biotechnologies Co., Ltd., Beijing, China). Compounds (2–64 μg/mL) were incubated with equal volumes of bacterial genomic DNA solution and then incubated at room temperature for 10 min. The DNA-binding effects of compounds **1**–**3** were evaluated using 0.8% agarose gel electrophoresis [36].

4.9.2. Circular Dichroism Spectroscopy

Circular dichroism (CD) spectroscopy was used to further assess the DNA-binding affinity of compounds **1**–**3**. Each compound (64 μg/mL) was added to a genomic DNA solution (150 μg/mL) and then incubated at room temperature for 10 min. Measurements were conducted using a 1.0 mm path length quartz cuvette on a J-1700 CD spectrometer (JASCO, Tokyo, Japan), with spectra recorded from 220 to 320 nm (scan rate of 10 nm/min) [36].

4.10. Transcriptome Analysis of MRSA ATCC43300 Treated with Compounds

The mid-logarithmic phase of MRSA ATCC43300 (OD value = 0.5) was incubated with 1× MIC compounds for 4 h at 37 °C [32,33]. The bacterial suspension was centrifuged at 4 °C and 4000 rpm for 10 min, followed by two washes with PBS. The bacteria were immediately placed in liquid nitrogen and frozen for 15 min. Three biological replicates were collected for each condition. The total RNA extracted from the bacterial samples was characterized and quantified at Novogene (Beijing, China).

4.11. Biofilm Formation Assay

The crystalline violet method was used to study the biofilm formation inhibition of the compounds. The mid-logarithmic phase cultures of *Staphylococcus aureus* ATCC43300 were diluted to a concentration of 1×10^8 CFU/mL by tryptic soy broth (TSB) medium, with test compounds at final concentrations spanning 1–128 μg/mL. The compound–bacterium mixtures were in a 96-well plate at 37 °C for 24 h [27,28]. Next, the biofilm was washed twice with PBS and fixed for 15 min with 2.5% glutaraldehyde. The biofilm was stained for 0.5 h with 0.1% crystal violet and dried overnight. After drying, the sample was dissolved in 95% ethanol for 30 min and then measured at the absorbance of 570 nm.

5. Conclusions

In this study, we identified three anthracycline derivatives—keto-ester (**1**), 4-deoxy-ε-pyrromycinone (**2**), and misamycin (**3**)—that exhibited antibacterial and antibiofilm activity against MRSA. Cytotoxicity assays confirmed their favorable safety profiles at therapeutically relevant concentrations. Mechanistic investigations revealed that these compounds disrupt the bacterial cell wall, induce DNA damage, and impair metabolic synthesis, collectively leading to bacterial cell death. Our findings highlight the potential of these anthracyclines as novel dual-functional agents capable of simultaneously targeting bacterial viability and biofilm formation, offering a promising way for combating MRSA infections.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/md23080298/s1: Figure S1: The ^{1}H NMR spectrum, ^{13}C NMR spectrum, UV spectrum, and HRESIMS spectrum of keto-ester.; Figure S2: The ^{1}H NMR spectrum, ^{13}C NMR spectrum, UV spectrum, and HRESIMS spectrum of 4-deoxy-ε-pyrromycinone. Figure S3: The ^{1}H NMR spectrum, ^{13}C NMR spectrum, UV spectrum, and HRESIMS spectrum of misamycin. Table S1: The ^{1}H and ^{13}C NMR data of compounds **1-3** in $CDCl_3$ (δ in ppm).

Author Contributions: X.W., L.D. and R.M. conceived and designed experiments. Y.Y., Z.Z., G.H. and S.Y. carried out all the experiments. Y.Y., X.W., L.D. and R.M. contributed to writing. X.W. and L.D. contributed to funding acquisition. All authors have read and agreed to the published version of the manuscript.

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ARTICLES FOR FACULTY MEMBERS

ELUCIDATION OF ANTIBIOFILM FORMATION MECHANISMS USING MARINE EXTRACTS AGAINST INFECTION AND ANTIMICROBIAL RESISTANCE (AMR)

Antimicrobial, antibiofilm, cytotoxicity, and anti-DNA topoisomerase activity of Streptomyces sp. 22SH with ADME and in silico study / Hassan, M. G., Abdel-Monem, M. O., Sleem, A. S. M. A., El Awady, M. E., & Hamed, A. A.

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Antimicrobial, antibiofilm, cytotoxicity, and anti-DNA topoisomerase activity of *Streptomyces* sp. 22SH with ADME and in silico study

Mervat G. Hassan[1*], Mohamed O. Abdel-Monem[1], Al Shaimaa M. A. Sleem[1], Mohamed E. El Awady[2] and Ahmed A. Hamed[3*]

Abstract

The genus *Streptomyces* has been recently proven to be a valuable and rich source of producing several bioactive compounds with substantial biological activity and applications in many fields such as medicine, environmental science, food industries, and agronomy. This study highlights the importance of Streptomyces as an antimicrobial, antibiofilm, and anticancer. Out of the 75 actinobacteria isolated from both marine and soil habitats, one isolate, HG2, was selected based on its potent antimicrobial activity. The isolate has been identified morphologically by studying colony and spore chain morphology using TEM and genetically by sequencing their 16 sr RNA gene as Streptomyces sp. 22SH with Accession number OK326829.1. Bioassay-guided fractionation of the Streptomyces sp. 22SH crude extract led to the isolation and purification of Cis-9-Octadecenoic. Biological evaluation including antimicrobial and antibiofilm activity of the crude and purified compound was performed on four clinical microbes (S. aureus ATCC6538-P, B. subtilis ATCC6633, and P. aeruginosa ATCC27853). The compound showed the ability to eradicate the biofilm formation by the tested pathogens. Additionally, the antitumor activity was assessed, and the compound showed a cytotoxic effect against liver carcinoma and breast cancer cells, with IC50 values of 17.48 ± 0.94 and 88.73 ± 4.78 µg/ml, respectively. While it displayed anti-topoisomerase activity with an IC_{50} of 0.65 ± 0.023 µg/ml. Furthermore, the compound's ADME-related physicochemical features and docking analysis were investigated.

Keywords *Streptomyces* sp., Bioactive metabolites, Antibacterial, Antibiofilm, anti-DNA topoisomerase

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Introduction

Antimicrobial resistance (AMR) is the main cause of the public health disaster caused by antibiotic misuse in recent decades [1]. According to the World Health Organization, pathogenic diseases have become more difficult to cure, and death rates have risen due to antibiotic resistance (AR). New sources of antibiotics are urgently needed to combat these superbugs lying within the WHO priority list [2]. The financial impact of antimicrobial resistance (AMR) on national economies and health systems is significant because it makes patients or those who care for them less productive due to extended hospital admissions and the need for more expensive and intense treatment [3].

The number of patients whose treatments fail or who pass away from infections will rise in the absence of efficient instruments for the avoidance and effective management of drug-resistant illnesses and enhanced access to current and novel dependable antimicrobials. Surgery, such as hip replacements or cesarean sections, chemotherapy for cancer, and organ transplants, will all become more dangerous medical procedures (WHO, 2022) [4].

Coastal habitats are among the most productive and valuable habitat types in ecosystems around the globe [5]. Since they are home to creatures unique to the marine environment, marine ecosystems have proven to be a good source of innovative and distinctive natural products (NPs). Marine NPs are a valuable source for drug discovery since certain characteristics of the chemical diversity reported from marine organisms align with those of recognized medications [6]. Today, more marine medications are being used. Another 23 marine NPs are currently undergoing clinical studies, while 14 marine NPs or their derivatives are already approved as medications [7].

Actinomycetes, gram-positive filamentous bacteria, function as saprophytes and break down intricate biopolymers [8]. Actinobacteria, particularly those from the genus *Streptomyces*, are among the most significant producers of antibiotics. For example, *Streptomyces* species have been responsible for the discovery of clinically important antibiotics like streptomycin, tetracycline, and erythromycin, which are widely used to treat bacterial infections [9]. Given the increasing problem of germs that are resistant to several drugs, the search for novel antibiotics derived from actinobacteria is very critical. Moreover, actinobacteria have produced a number of anticancer chemicals, such as anthracyclines, which are used in cancer treatment because they may stop DNA production and cause cancer cells to die. Actinobacteria have the unusual capacity to synthesis a wide range of bioactive compounds, which emphasizes its potential to improve cancer and infectious disease therapies [10].

Additionally, they can create a variety of secondary metabolites, many of which have antibacterial or antifungal characteristics. The current study aims to screen and examine the bioactive secondary metabolites with antimicrobial and anticancer activity of marine and terrestrial actinomycetes obtained from different sources in the Egyptian region.

Materials and methods

Sample collection

Samples were taken from two habitats in Egypt: marine habitats (Ain Sokhna sediment (29.6725° N, 32.3370° E), Ras Sedr sediment (29.5933° N, 32.7178° E), and Hurghada sea water (27.2579° N, 33.8116° E) and soil habitats (Mansoura, Dakahlia Governorate). Samples were collected, coded, and transferred to the Lab and kept in a fridge at the Microbial chemistry department till further processing [11].

Isolation of marine actinomycetes

Serial dilution techniques were utilised in order to successfully isolate actinomycetes from samples taken from both marine and soil environments. Utilizing starch casein media for marine samples and starch nitrate agar medium for soil samples was the method of choice. Isolation of actinomycetes was accomplished by utilizing their distinctive morphological traits, which included deep-sitting colonies, sporulation, and a colour that was easily identifiable.

Biological screening of isolated actinomycetes small-scale extracts

To prepare the small-scale crude extract of the isolated actinomycetes, 75 actinomycetes isolates were cultured on rice media for 10 days at 30 °C. After incubation, each separate culture medium was mixed with ethyl acetate. The isolates' crude extracts were then assessed for their antimicrobial potential toward a range of test pathogens using the agar disc diffusion method using nutrient agar media and potato dextrose agar with incubation period 24 h for bacteria and 48 h for fungi toward a panel of test microbes including gram-positive (*Staphylococcus aureus* ATCC6538-P subsp. *aureus*), a gram-negative bacterium (*Escherichia* coli ATCC 14169), and fungi (*Aspergillus niger* NRRL A-326). All test microbes were collected from Egypt's National Research Center, Egypt [12].

Identification of the most potent isolate (HG2)

Phenotypic identification and genotypic identification

The most potent isolate, HG2, showed a pronounced antimicrobial activity and has been identified by studying its morphological, physiological, and biochemical characteristics HG2's colony formation on various growth mediums, color, textures, shapes, and sizes, and

microscopic investigation of cellular features such as hyphal arrangements and spore generation revealed its morphology [13–16]. Identity confirmation of the selected isolate was carried out via sequencing of the 16 S rRNA gene. Genomic DNA was extracted and amplified using the following primers: Reverse 27 F (5′-AGAGTT TGATCCTGGCTCAG-3′) and forward 1492R (5′-TAC-GGYTACCTTGTTACGACTT-3′). The sequencing process made use of the Big Dye terminator cycle sequencing kit (Applied BioSystems, USA). The sequencing results were then evaluated using the Applied BioSystems model 3730XL, an automated DNA sequencing machine (Applied BioSystems, USA). The obtained 16 S rRNA sequence was then analyzed by aligning it using the BLAST online tool with other 16 S rRNA sequences at the GenBank, National Center for Biotechnology Information (NCBI) database. Bacterial strains with the highest similarity to our isolate's 16 S rRNA gene were chosen, aligned, and used to build a phylogenetic tree.

Large scale fermentation

To prepare the large-scale crude extract of the *Streptomyces* sp. 22SH, the spore suspension of the isolate was prepared by inoculation of the isolate into 100 mL of the ISP2 medium and then cultivated at 30 °C for three days. The cultivated seed culture was then used to inoculate 10 × 1 L Erlenmeyer flasks with 100 g of commercial rice and 100 mL of 50% seawater pH 6.5. After that, the inoculated cultures were incubated for 14 days at 28 °C [17]. The rice culture was then extracted with ethyl acetate (ratio 1:3) before being filtered, and concentrated in vacuo. The crude extract was then dried down to yield 4.5 gram.

Bioassay guided fractionation and identification of isolated compound

Four grams of the obtained crude extract were placed on a normal-phase silica column with a diameter of seven centimeters. The adsorbent (silica gel) to solute (crude extract) ratio is 20:1. A total of 100 fractions of 5 mL each were gathered and subjected to a thin-layer chromatography (TLC) analysis. TLC was performed on Silica Gel 60 F254 (layer thickness 0.2 mm, E. Merck, Darmstadt, Germany) precoated TLC plates with Dichloromethane (DCM): methanol (90:10, v/v) as the solvent system. Based on the TLC results, the obtained similar fractions were recombined after confirmation by Ultra Violet and anisaldehyde/sulfuric acid reagent [18]. Biological evaluation of the obtained fractions was carried out using a variety of test microorganisms, including gram-positive bacteria *Staphylococcus aureus ATCC6538-P subsp. aureus* and *Bacillus subtilis* ATCC6633, and a gram-negative bacteria *Pseudomonas areuginosa* ATCC 27,853 and *Escherichia coli* ATCC25955, and fungi *Candida albicans* ATCC10231.

Structural elucidation

LCMS uses liquid chromatography to separate chemicals and mass spectrometry to identify and quantify them by mass-to-charge ratio. Analyzing metabolites, small compounds, and peptides in complicated combinations is routine. NMR analyzes atomic nuclei's interaction with a magnetic field and radiofrequency pulses to identify chemical structures. Its chemical shift and coupling data identify functional groups, molecular dynamics, and 3D structures, making it crucial for structural elucidation and conformational analysis. The most potent fraction was then submitted to Sephadex LH-20 column and the obtained fractions were biologically evaluated. The purified molecule was identified by measuring its molecular weight using Liquid Chromatography Mass Spectroscopy and Nuclear Magnetic Resonance (NMR).

Biological evaluation of purified compound

Antibiofilm activity

To evaluate the antibiofilm activity of the obtained purified compound, an MTP assay was completed utilizing four clinical microorganisms (*P. aeruginosa* ATCC 27853, *S. aureus* ATCC6538-P, *E. coli* ATCC 25955, *and B. subtilis* ATCC6633) [19]. The study included inoculating sterile 96-well plates with overnight bacterial suspensions in nutrient-rich broth and adding the chemical at conc of 10 µg/mL. We rinsed wells with PBS to eliminate planktonic cells after 24 h of biofilm development at 37 °C, then stained with 0.1% crystal violet solution for 15 min. Removed excess stain, washed, and air-dried wells before solubilizing dye with ethanol. We assessed biofilm inhibition using 570 nm optical density (OD).

Cytotoxic effect

The antitumor activity was assessed at the Regional Center for Microbiology and Biotechnology, Al-Azhar University, Cairo, Egypt. The cell lines (HepG2 malignancy cells from the liver and the MCF7 cell line for breast cancer) were purchased from the American Type Culture Collection (ATCC, Rockville, MD). The Cells were cultured in appropriate media and incubated under standard conditions (37 °C, 5% CO_2). Serial dilutions of test compounds were prepared, and cells were exposed to varying concentrations for 24–72 h. Staurosporine was a positive control, while untreated cells acted as negative controls. Cell viability was assessed using the MTT assay with 5 mg/mL MTT reagent. After incubation, formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm with a reference wavelength of 630 nm. The half-maximal inhibitory concentration (IC_{50}) was calculated using dose-response curves, representing the

concentration required to inhibit 50% cell viability. Staurosporine was used as a drug control agent [20].

DNA topoisomerase Inhibition activity

To measure the relaxation of supercoiled pBR322 plasmid DNA The extracted compound was used in a slightly altered version of a previously described procedure [21]. Etoposide was used as a drug control. Briefly, 0.5 µg of supercoiled pBR322 plasmid DNA was combined with a reaction buffer containing Tris-HCl, KCl, $MgCl_2$, and DTT. Topoisomerase I enzyme was added with varying concentrations of the test compound (1, 5, and 10 µM) or etoposide (10 µM) for the control. Reactions were incubated at 37 °C for 30 min, then stopped by adding loading dye. Samples were resolved on a 1% agarose gel stained with ethidium bromide and visualized under UV light.

In silico forecasts prediction of toxicity and physicochemical characteristics associated with ADME

SwissADME web tools (https://www.swissadme.ch/) accessed (June 2024) were used to plan the obtained physicochemical properties of a compound's ADME characteristics [22]. Using the ProTox ii web server, compounds' in-silico toxicity predictions were carried out as previously published [23].

The molecular docking

Outer membrane proteins (OMPs) of gram-negative bacteria are crucial in mediating bacterial virulence and antibiotic resistance, which in turn affect how harmful the bacteria are. Proteins in the outer layer and the OMPX (1QJ8) crystal structure were obtained from the Protein Data Bank (PDB) database and refined by removing water molecules and heteroatoms, followed by hydrogen atom addition to ensure stability. Energy minimization was conducted using MOE to optimize the receptor's conformation. The *cis*-9-Octadecenoic acid structure was retrieved from PubChem in SDF format, converted to PDB via the https://cactus.nci.nih.gov. and subjected to energy minimization and geometric optimization before docking analysis. The prepared PDB files of both the receptor and ligand were uploaded to the PatchDock server, with receptor-ligand interaction mode set to default parameters. A root mean square deviation (RMSD) cutoff of < 4.0 Å was applied to refine the docking results. The highest-scoring docked complex was selected based on the PatchDock algorithm, and further interaction analysis was conducted in MOE. Residues within 4 Å of the ligand-binding site were examined to identify key molecular interactions, including hydrogen bonding, hydrophobic contacts, and electrostatic interactions.

Results

Isolation of streptomycetes and pre-screening

Seventy-five streptomycetes isolates were isolated from various marine and soil habitats, including Hurghada marine sea, Ras Sedr sediment, and Ain Sokhna sediment. While the soil sample was collected from Mansoura. *Streptomyces* sp. is isolated based on distinctive colony morphology, typically forming circular, convex-shaped colonies. Then, the isolated streptomycetes were cultivated on a rice medium for small-scale fermentation to obtain the bioactive compounds. Therefore, the antimicrobial activity of ethyl acetate extracts for isolated *Streptomyces* spp. was tested using the agar disc diffusion method against *E. coli* ATCC 14,169, *S. aureus* ATCC6538-P and *A. niger* NRRLA-326. Ciprofloxacin and Amphotericin B were used as controls. Among all tested actinomycetes, 20 isolates exhibited antimicrobial activity, including HG2, HG3, HG5, HG9, HG12, RS8, RS9, RS10, RS11, RS12, RS15, AS8, AS9, AS23, AS24, M1-2, M1-4, M1-7, M1-9, and M2-13. Out of the 20 isolates, one strain, HG2, exhibited potent antimicrobial activity (supplementary tables S1–S5).

Phenotypic identification of most potent streptomycetes isolate HG2

To formally describe a novel taxon, actinobacteria are currently defined using a polyphasic approach that includes a combination of phenotypic, chemotaxonomic, and genotypic data. The primary components that need to be collected and examined in prokaryote characterization studies were described [24]. Therefore, the strain (HG2) was identified primarily by visualizing its spore morphology using TEM, and the obtained result showed that the strain has rectiflexible spore chains with a smooth spore surface, according to spore chain morphology Figure 1. Tyrosine agar does not produce melanin pigments in most cases. The spore mass is whitish and produces yellow diffusible pigments. The isolate HG2 demonstrates unique pigmentation patterns upon several media. In the starch nitrate medium, the aerial mycelium manifested as reddish grey, accompanied by greyish yellow substrate mycelium, but other media, including starch-ammonium sulfate and glycerol-asparagine, displayed grey aerial mycelium and yellowish grey substrate, indicating diversity in pigment synthesis (Table 1). Physiological and chemo-taxonomical studies are presented in Table 2. The obtained results showed that the isolated HG2 was able to produce melanin pigment only on tyrosine agar, while no melanin pigment was observed on peptone iron agar. The enzymatic capabilities of the isolate were also looked into, and the findings indicated that the isolate HG2 has proteolysis and lecithinase activity. The utilization of different carbon sources was also assessed, and results showed that the selected isolate

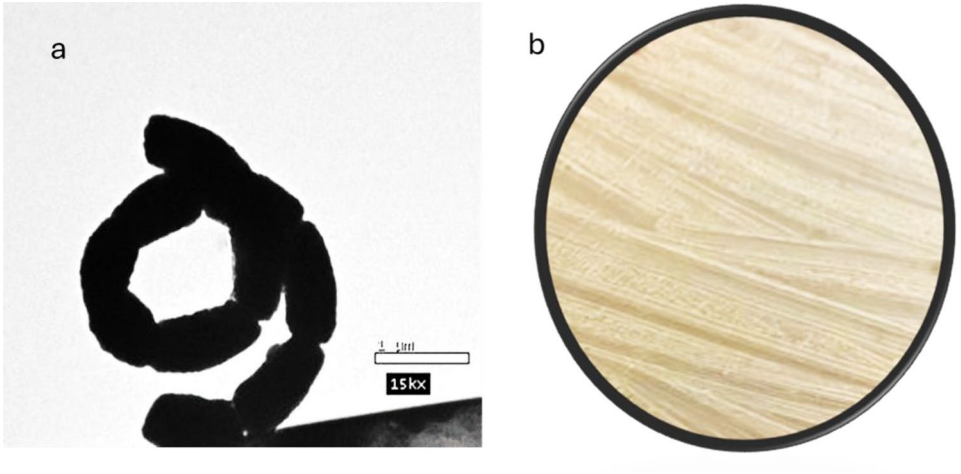


Fig. 1 **a** TEM photomicrograph showing smooth spore surface (isolate HG2 × 15000). **b** colony morphology

Table 1 Cultural properties of isolate no. HG2 grown on different culture media

Medium	Color of		
	Aerial mycelium	Diffusible pigments	Substrate mycelium
1- Starch nitrate medium	Reddish gray	+ve	Grayish yellow
2- Starch-ammonium sulphate medium	Gray	-ve	Yellowish gray
3- Glycerol-asparagine medium	Gray	-ve	Yellowish gray
4- Oat-meal medium	Pale gray	-ve	Yellowish gray
5- yeast/malt extract agar medium	Whitish Gray	-ve	Pale gray
6- Czapeks medium	Gray	-ve	Pale gray

HG2 can utilize D glucose, L Arabinose, and D fructose, while it can't consume Rhamnose, Sucrose, D-xylose, D-mannitol, I-inositol, Galactose, and Raffinose.

Genotypic identification of most potent streptomycetes isolate HG2

Analysis of the 16 S rDNA and sequencing of the most potent isolate *Streptomyces* isolate (HG2), started by isolating genomic DNA, and amplification. Sequencing and analysis of the Genomic DNA of the most potent isolate showed a high similarity score of isolate HG2, with *Streptomyces* sp., reaching 100% similarity. The *Streptomyces* sequence was submitted to the Gene Bank as *Streptomyces* sp. 22SH (Accession number: OK326829.1). The phylogenetic tree was constructed using MEGAX and presented in Figure 2.

Large-scale production and purification of bioactive substances from *Streptomyces* sp. 22SH

The strain *Streptomyces* sp. 22SH was grown in a rice medium with ethyl acetate used for the extraction. After evaporation, the ethyl acetate was evaporated, and the obtained extract was 4.5 gram. The obtained extract was principally separated into 100 fractions by using flash column chromatography. All fractions were chemically screened using TLC. Based on the TLC screening, the 100 fractions were recombined into 14 fractions. The 14 obtained fractions were biologically screened by evaluation of their antimicrobial activity (Table 3). According to the antimicrobial results, the greatest active proportion (F2) was purified through a Sephadex LH-20 column with a DCM: Methanol gradient mobile phase. The TLC and antimicrobial evaluation of the obtained Sephadex fractions showed that only six fractions exhibited

Table 2 Physiological and chemo-taxonomical properties of the isolate HG2

Isolate no.		HG2
Melanin pigment production	Pepton iron agar	-
	Tyrosine agar	+
Enzyme activities	Proteolysis	+
	Lipolysis	-
	Lecithinase	+
Utilization of different carbon source	No suger (-)	+
	D-Glucose (+)	+
	D-Fructose	+
	Sucrose	-
	Rhamnose	-
	D-Mannitol	-
	D-Xylose	-
	Raffinose	-
	I-inositol	-
	Galactose	-
	L-Arabinose	+
Nitrate reduction		+
H2S production		-
Starch hydrolysis		+
Cellulose decomposition		-
Gelatin liquification		+

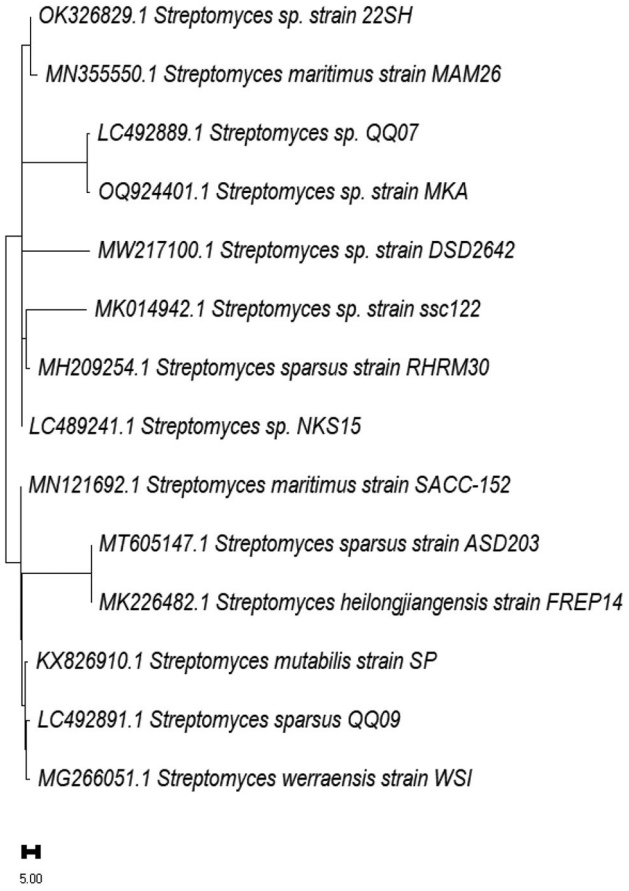


Fig. 2 Phylogenetic tree of the *Streptomyces* sp. 22SH strain (Accession no. OK326829.1)

antimicrobial activity (Table 4). Among them, the subfraction (SF15) was the most potent, as it showed broad-spectrum activity toward all tested microbes. The most potent Sephadex subfraction (SF15) was further purified to remove any impurities, and the obtained semi pure compound was structurally identified using LC-MS and NMR.

The Molecular weight (282.47) and Molecular formula ($C_{18}H_{34}O_2$) of the isolated pure compound were measured using LC-MS. The H-NMR spectrum of the compound demonstrated signs at δ 0.83 as a pair of triplets overlapped, suggesting two methyl groups at the terminals at δ 1.29 as a broad singlet for a long chain of methylene protons, at δ 1.94 for methylene groups α to C = C group, and at δ 2.24 for methylene groups α to carbonyl group. The multiple signals were visible at δ 4.13, which may be attributed to the oxymethylene group, and at δ 5.38 for an unsaturated proton. Consequently, the compound could be identified as cis-9-Octadecenoic (C1) depending on its chromatographic characteristics, proton (Supplementary S6) [25].

Table 3 Antimicrobial activity of *Streptomyces* sp. 22SH flash column fractions

Extracts	Antibacterial activity (mm)				Antifungal activity (mm)
	Gram−ve		Gram + ve		
	E. coli ATCC25955	*P. aeruginosa* ATCC27853	*B. subtilis* ATCC6633	*S. aureus* ATCC6538-*P*	*C. albicans* ATCC10231
Crude	NA	13.00 ± 0.12	14.50 ± 0.21	10.00 ± 0.13	13.5 ± 0.12
F1	NA	11.00 ± 0.14	12.50 ± 0.12	9.9.00 ± 0.15	NA
F2	12 ± 0.02	23.00 ± 0.35	25.00 ± 0.15	22.00 ± 0.09	18.00 ± 0.13
F3	NA	15.00 ± 0.21	NA	NA	10.00 ± 0.09
F4	9 ± 0.12	NA	12.00 ± 0.07	NA	9.90 ± 0.06
F5	NA	NA	8.25 ± 0.06	NA	13 ± 0.12
F6	NA	NA	NA	NA	NA
F7	NA	NA	NA	NA	NA
F8	NA	NA	14.00 ± 0.12	NA	NA
F9	NA	NA	11.00 ± 0.23	NA	NA
F10	NA	NA	NA	NA	NA
F11	NA	14.00 ± 0.08	NA	NA	NA
F12	NA	NA	NA	NA	NA
F13	NA	NA	NA	NA	NA
F14	NA	NA	NA	NA	NA
Strep.	17.2	24. 6	25.3	23.8	-
Amp	-	-	-	-	22.9

NA: Not active Strep.: Streptomycin Amp: Amphotericin B

Table 4 Antimicrobial activity of *Streptomyces* sp. 22SH Sephadex fractions

Extracts	Antibacterial activity (mm)				Antifungal activity (mm)
	Gram–ve		Gram + ve		
	***E. coli* ATCC25955**	***P. aeruginosa* ATCC27853**	***B. subtilis* ATCC6633**	***S. aureus* ATCC6538-P**	***C. albicans* ATCC10231**
Crude	NA	14.00 ± 0.23	13.50 ± 0.03	11.00 ± 0.12	15.00 ± 0.11
SF1	NA	10.00 ± 0.06	NA	7.00 ± 0.03	NA
SF2	7.00 ± 0.12	10.00 ± 0.12	NA	NA	NA
SF3	12.00 ± 0.20	13.00 ± 0.12	12.00 ± 0.01	10.00 ± 0.14	8.50 ± 0.12
SF4	11.00 ± 0.03	NA	11.00 ± 0.03	NA	9.90 ± 0.13
SF5	NA	NA	12.00 ± 0.09	NA	13.00 ± 0.23
SF6	NA	NA	10.00 ± 0.06	NA	NA
Strep.	17.2	24. 6	25.3	23.8	-
Amp	-	-	-	-	**22.9**

NA: Not active Strep.: Streptomycin Amp: Amphotericin B

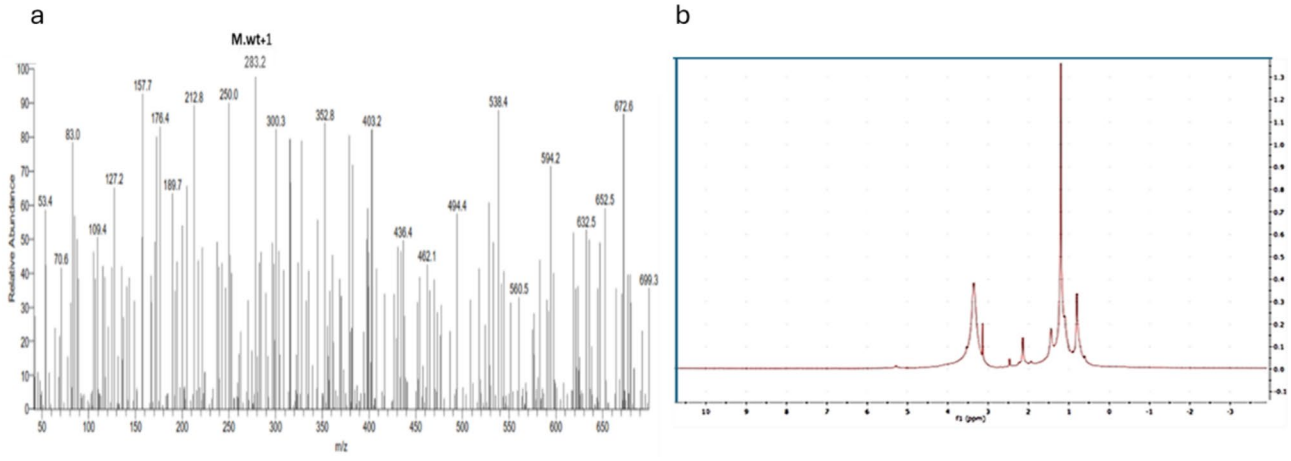
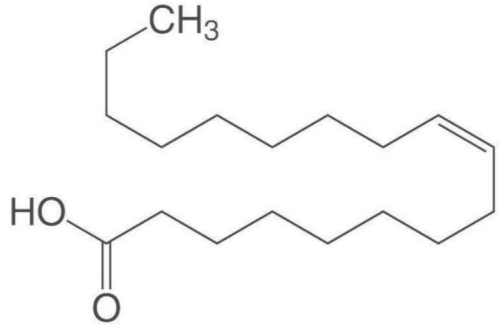


Fig. 3 **(a)** Mass spectrum of cis-9-Octadecenoic (**b**) NMR proton diagram of semi pure fraction contain cis-9-Octadecenoic



C1. Cis-9-Octadecenoic acid

Biological evaluation of identified compound C1

Antibiofilm activity

The anti-biofilm activity of C1, MTP assay was carried out using four clinical microbes (Fig. 3). It showed antibiofilm activity against *S. aureus* ATCC6538-P, *B. subitilis* ATCC6633 and *P. aeruginosa* ATCC27853 with inhibition ratios of 30.50, 50.00, and 45.26%, respectively. While it showed no antibiofilm activity against *E. coli* ATCC25955.

Cytotoxic activity

The anticancer activity of the identified compound was assessed and results showed that C1 has a pronounced anticancer effect against liver carcinoma with an IC_{50} value of 17.48 ± 0.94 µg/mL while it showed a moderate anticancer effect against breast cancer cells with an IC_{50} value of 88.73 ± 4.78 µg/ml (Fig. 4). Compared to the standard, the semi-pure compound significantly reduced MCF7 and HepG2 viability at 100 µM (log 2) to 44.26% and 35.67%, respectively, demonstrating robust anticancer activities. Cell viability rose with decreasing concentrations, peaking at 82.93% and 85.24% at the lowest dose (log 0.409), reaching 79.33% (MCF7) and 74.67% (HepG2) at 1.56 µM (log 0.1931). The results show dose-dependent cytotoxicity, with more potent effects at higher concentrations (Supplementary S7).

DNA topoisomerase Inhibition activity

DNA Topoisomerase inhibition assay was performed for cis-9-Octadecenoic, which showed anti-topoisomerase activity with an IC_{50} 0.65 ± 0.023 ug/ml, while Etoposide gave 1.87 ± 0.07 µg/ml (Fig. 5).

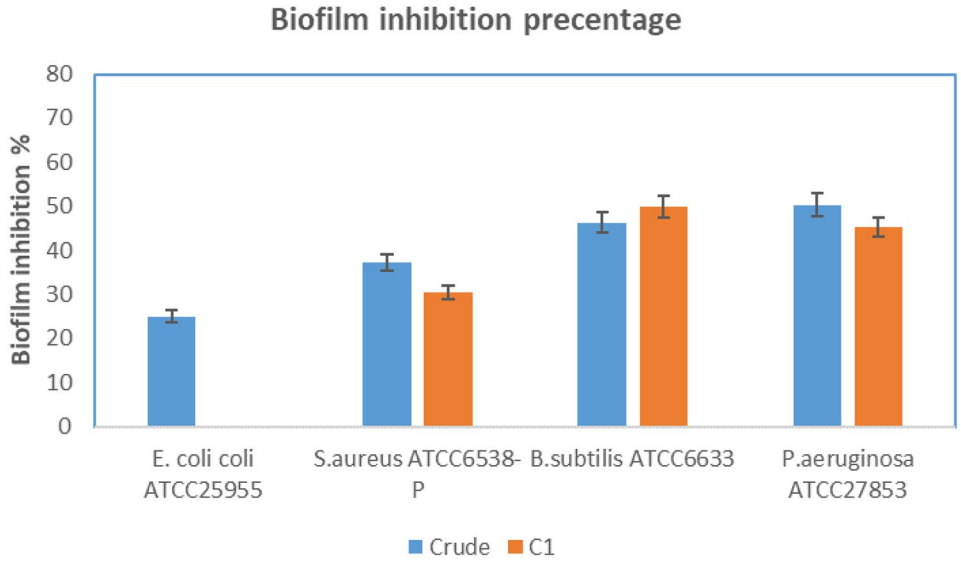


Fig. 4 Biofilm inhibition of obtained compound C1

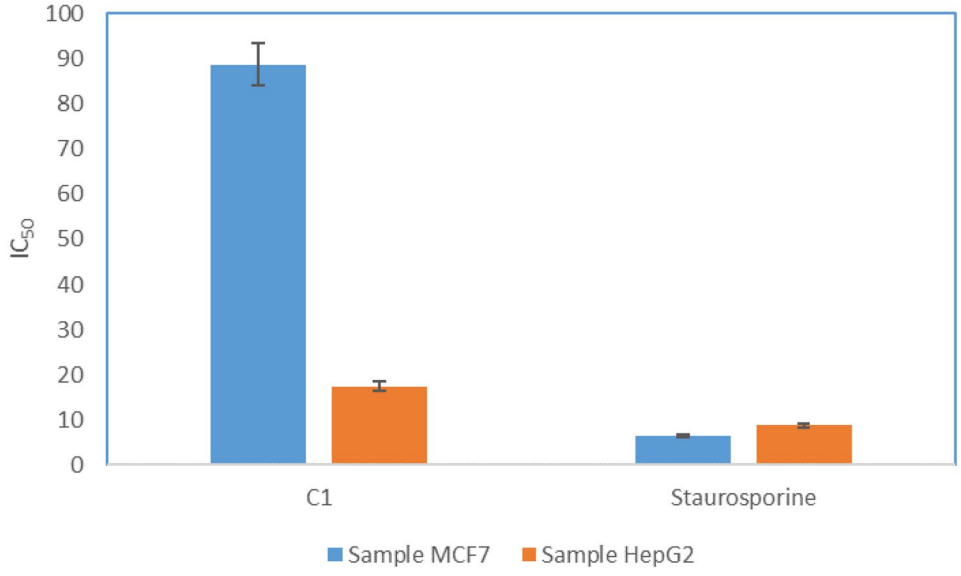


Fig. 5 Anticancer activity of obtained compound C1

The ADME-related physicochemical properties

The physicochemical characteristics of the ADME of the cis-9-Octadecenoic acid were measured, and the obtained data showed that the compound exceeded the Lipinski requirements with 1 violation (MLOGP > 4.15) but did not pass the Veber rules with 1 violation (1 violation: Rotors > 10), while one violation (WLOGP > 5.6) has been discovered using the Ghose rule. The substances have a 0.85% oral bioavailability and may be taken as a pill (Supplementary S8). Additionally, by plotting bioavailability, a quick evaluation of drug similarity was carried out. The radar image of the cis-9-Octadecenoic acid according to the six physicochemical traits size, lipophilicity, polarity, solubility, saturation, flexibility, and the pink region shows the parameter's ideal value range. Using the obtained diagram as a guide, the compound shows the optimum (pink area) for all the parameters except the flexibility parameter (Fig. 6a). The cis-9-Octadecenoic acid showed Log $P_{o/w}$ values above 5 (5.71), suggesting weak permeability and absorption throughout the cell membrane. Based on the ESOL topological model, the tested compound is moderately soluble. Regarding defining medicinal chemistry and Lead likeness, the compound property failed to meet the requirement of three (RO3), as it has two violations for this rule (Rotors > 7, XLOGP3 > 3.5). The compound had moderate synthetic accessibility, with values of 3.07 for the synthetic accessibility score (SAscore), which calculates accessibility based

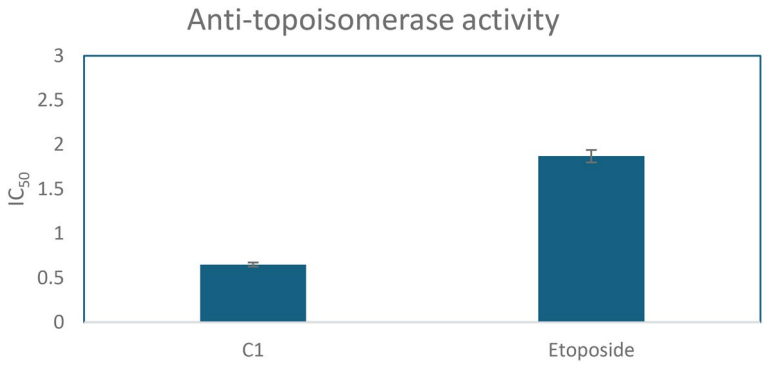


Fig. 6 DNA Topoisomerase inhibition activity

on fragment similarity and complexity penalties. Employing the vector machine algorithm (SVM) model, the pharmacokinetic characteristics of cis-9-octadecenoic acid were determined [22]. The Compound demonstrated a specific inhibition effect on CYP1A2 and CYP2C9 isoenzymes, while no selective activity against CYP2C19, CYP2D6, and CYP2C9 isoenzymes has been detected (Supplementary S8). Figures 6b indicates the boiled-egg model (Brain or Intestinal Estimate D permeation method, WLOGP vs. TPSA), which was modified from [22], The cis-9-Octadecenoic acid showed significant gastric (GI) absorption in humans. The cis-9-Octadecenoic acid are non-P-gp substrates (PGP-, red dots), while their blood-brain barrier (BBB) is permeant (TPSA < 75 Å^2), indicating the possibility of central nervous system (CNS) effects [26]. The skin permeability prediction coefficient (Kp) of cis-9-Octadecenoic was completed as outlined by Potts and Guy [27]. The cis-9-Octadecenoic showed a log (Kp) (-2.60 cm/scm/s), while the compounds with a high negative log Kp had less skin penetration. The white region (GI) indicates a very high probability of HIA (GI) absorption, while the yellow zone (yolk) indicates a very possible BBB permeability. The gray area outside represents substances that have no brain penetration and a poor absorption rate. Additionally, the points are labeled red if P-GP non-substrate (PGP) is expected and blue if P-gp substrate (PGP+) is anticipated. While forecasting the toxicity of the obtained cis-9-Octadecenoic acid was performed using the ProTox ii webserver, Results in Supplementary (S8) showed that the obtained compound acts on certain targets as expected by ProTox ii. While Supplementary (S9) shows the toxicity radar chart, which is meant to quickly demonstrate the certainty of positive toxicity data in comparison to the class average.

Molecular Docking determines the cis-9-Octadecenoic acid binding capacity to *E. coli*

A computational study and molecular docking of the possible interaction and antimicrobial activity of cis-9-Octadecenoic acid was carried out using MOE software and Patch Dock server for cis-9-Octadecenoic acid and the three-dimensional structures of *E. coli* proteins in the outer membrane, the OMPX (1QJ8) crystal structure. The interactions between the amino acid residues and cis-9-Octadecenoic and the kinds of interactions between Cis-9-Octadecenoic, and OMPX (1QJ8) reacted via Phe90, and Lys48 could result in disruption of the *E. coli* outer membrane (Fig. 7a, b and c). The binding energy between *cis*-9-octadecenoic acid and OmpX was calculated to be -5.2 kcal/mol. Several reports study the OmpX inhibition by streptomyces compounds. In the study conducted by Basharat et al. [28], Eleven compounds were analyzed, with seven satisfying Lipinski's Rule of Five, indicating favorable drug-like properties. The highest binding energies identified were in the range of -10 kcal/mol. These inhibitors interact with OmpX by forming hydrogen bonds, hydrophobic interactions, and Van der Waals forces, primarily engaging key residues such as ASP97 and ARG171. Structural changes in the active pocket upon ligand binding highlight the inhibitors' stability and effectiveness. The findings suggest that these seven compounds hold promise as potential therapeutic agents targeting OmpX-related diseases. The findings highlight how cis-9-octadecenoic acid could be a promising molecule for destabilizing bacterial membranes through specific residue targeting, advancing our understanding of its antimicrobial mechanism.

Discussion

Streptomyces species can produce many different biologically active secondary metabolites, such as antibiotics, pesticides, antiparasitic, herbicides, and enzyme inhibitors, which are very important therapeutically and commercially. About one-third of known antibiotics are extracted from *Streptomyces sp.*. Researchers are very interested in the potential of *Streptomyces* species to create secondary metabolites [29]. This capability is typically a result of the clusters of *Streptomyces* strains, which are responsible for encoding enzymes for numerous secondary metabolic processes [30]. Nevertheless, dietary

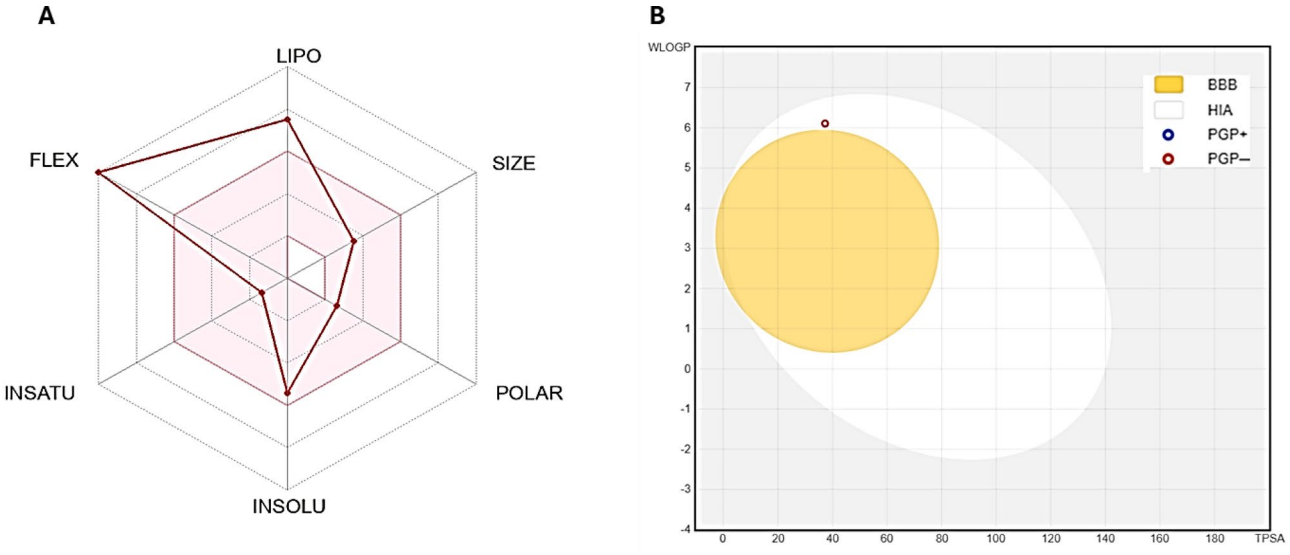


Fig. 7 **A** Bioavailability radar char and **B** boiled-egg plot

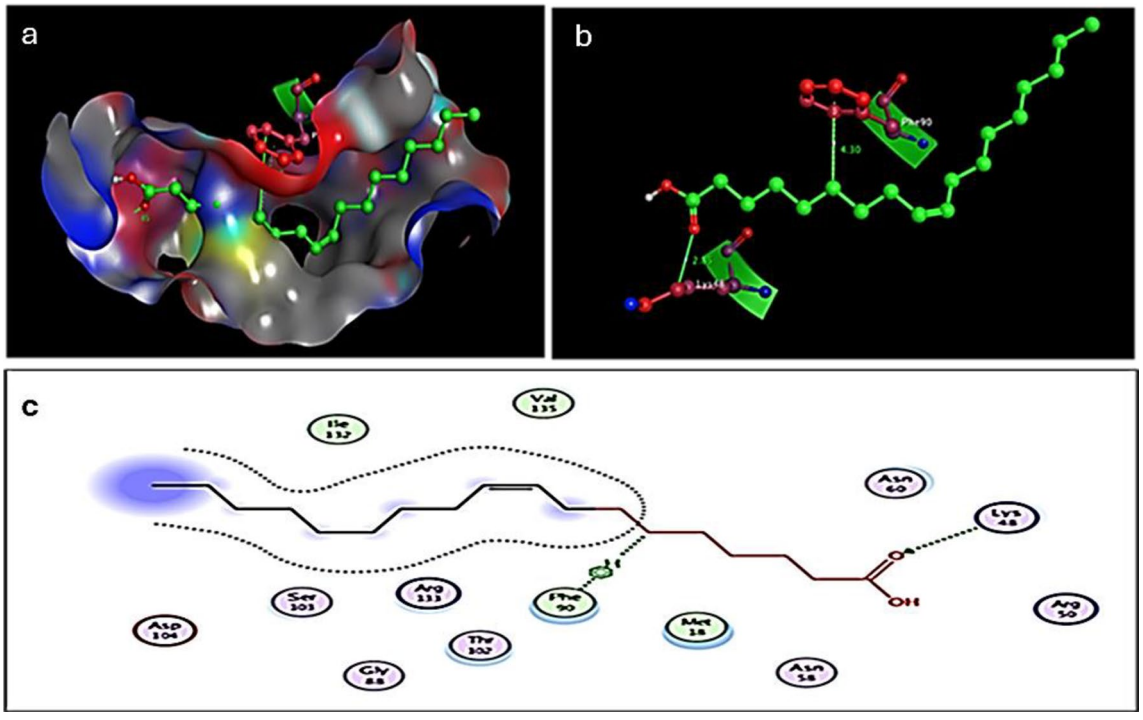


Fig. 8 Molecular docking determines cis-9-Octadecenoic binding capacity to *E. coli* outer membrane protein. (**a** and **b**) The docking model shows an interaction of the cis-9-Octadecenoic with 1QJ8 through Phe90, and Lys48, (**c**) 2D Interaction

variables and growth circumstances are also important regulators of secondary metabolite formation in microorganisms [31]. Out of 75 actinomycetes isolates purified from marine and soil habitats one actinomycetes coded as HG2, was selected as it exhibited potent antimicrobial activity. Several studies conducted by researchers showed that actinomycetes have exhibited obvious antimicrobial activities against almost all tested pathogenic strains. Chen et al. [32] isolated *Streptomyces* sp. K15 and discovered that a few of its secondary metabolites have strong antibacterial properties. Marine *Streptomyces aminophilus* exhibited activity against almost all test organisms and high activity against *Aspergillus niger*. The literature reported that this strain can produce many bioactive compounds with variable activities, such as chorismic acid, a macrolide antibiotic, and a potent immunosuppressant [33–35]. Additionally, the marine *Streptomyces baarnensis* exhibited moderate activity

against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, and *Saccharomyces cerevisiae*, high activity against *Aspergillus niger*, and no activity against *Bacillus subtilis*. This strain produced many antibiotics, such as enaminomycin A, B, and C [36]. Two methods including morphological and genetic—identified the isolate. Polyphasic morphological, chemotaxonomic, and genotypic data are used to define actinobacteria as a novel taxon [38]. In prokaryote characterization research, major components must be gathered and evaluated. Thus, the strain (HG2) was identified by examining its spore shape using TEM, which displayed rectiflexible spore chains with a smooth spore surface. Most tyrosine agar does not create melanin. Spore mass is pale and produces yellow diffusible colors. Table 2 lists physiological and chemo-taxonomical investigations. The isolated HG2 produced melanin pigment only on tyrosine agar, not peptone iron. HG2 exhibits proteolysis and lecithinase activity, according to enzymatic analysis. The isolate HG2 can use D glucose, L Arabinose, and D fructose, but not Rhamnose, Sucrose, D-xylose, D-mannitol, I-inositol, Galactose, or Raffinose. 16 S rDNA analysis and sequencing of the most potent Streptomyces isolate (HG2) began with genomic DNA isolation and amplification. Genomic DNA sequencing and analysis of the most potent isolation indicated 100% similarity between isolate HG2 and *Streptomyces* sp. The Gene Bank received *Streptomyces* sp. 22SH (Accession number: OK326829.1). To obtain the isolate crude extract, the strain was cultivated in rice media, incubated and extracted with ethyl acetate. The obtained crude extract was then partially purified using flash column chromatography and reassembled after TLC analysis into 14 fractions. Out of the assembled fractions only six fractions exhibited antibacterial activity. The most potent subfraction, SF15, demonstrated wide-ranging effectiveness against all bacteria. Additional purification removed contaminants from the most potent Sephadex subfraction (SF15). The NMR and LC-MS structural characterization of the molecule revealed its identity as cis-9-Octadecenoic (a mono-unsaturated omega-9 fatty acid) that can be found in a variety of sources, both plant and animal. Triglyceride esters of Cis-9-Octadecenoic comprise the majority of olive oil. In pharmaceuticals and aerosol goods, Cis-9-Octadecenoic acid is utilized as an emulsifying or solubilizing ingredient. It may slow the growth of the fatal condition of adrenoleukodystrophy, which damages the brain and adrenal glands, and it may improve memory. Cis-9-Octadecenoic acid may also be responsible for the Olive oil's hypotensive (or blood pressure-lowering) properties [37]. Antimicrobial resistance, which is currently recognized as a significant health issue, is closely associated with pathogenic bacterial biofilm. This biofilm serves as a crucial element in bacterial pathogenicity and is a fundamental factor in the development of persistent illnesses caused by various bacteria. Scientists have discovered the presence of an extracellular polymeric substance (EPS) that envelops biofilms, which are intricate formations composed of polysaccharides, proteins, and DNA. Biofilm formation, characterized by an acidic pH and reduced oxygen levels, significantly contributes to antibiotic resistance due to decreased drug diffusion and penetration [39]. The antibiofilm activity of the identified compound was evaluated against four clinical microbes and results showed antibiofilm activity appeared toward S. *aureus* ATCC6538-P, *B. subitilis* ATCC6633 and *P. aeruginosa* ATCC27853 with inhibition ratios of 30.50, 50.00, and 45.26%, respectively. While it showed no antibiofilm activity against *E. Coli* ATCC25955. Additionally, the anticancer activity of the identified compound showed that a pronounced anticancer activity is present against liver carcinoma with an IC_{50} value of 17.48 ± 0.94 µg/mL while it showed a moderate anticancer effect against breast cancer cells with an IC_{50} value of 88.73 ± 4.78 µg/ml. Several studies revealed an induced reduction in cell proliferation by cis-9-Octadecenoic acid among many tumor cell lines. Herein, the well-known oncogene HER2 (erbB-2), which is important for the genesis, invasive growth, and metastasis of several human malignancies, could be suppressed by cis-9-Octadecenoic acid. Cis-9-Octadecenoic acid might be involved in proliferation-related intracellular calcium signaling pathways. Regarding cell death, cis-9-Octadecenoic acid has been demonstrated to cause cell death in cancer cells. The processes via which apoptosis is triggered by cis-9-Octadecenoic acid could be related to an increase in intracellular ROS generation or caspase-3 activity. According to reports, several unsaturated fatty acids trigger apoptosis by releasing calcium from intracellular reserves. However, there is insufficient data to support such a role for cis-9-Octadecenoic acid [39]. Topoisomerase II is an enzyme necessary for chromosomal condensation, DNA replication, and chromosome segregation. Inhibitors of Topoisomerase II are crucial treatments used to treat a variety of neoplasms, such as lymphomas, sarcomas, breast cancer, lung cancer, and testicular cancer [40]. Recent molecular studies showed that inhibition of Topoisomerase II is a successful cancer chemotherapy plan. The molecular tools that have allowed an understanding of the biological functions of Topoisomerase II are also being used to learn the specifics of how drugs work. According to this research, topoisomerase II can be precisely targeted as a potent anticancer method [41]. A DNA Topoisomerase inhibition experiment was conducted for cis-9-Octadecenoic, revealing anti-topoisomerase activity with an IC50 of 0.65 ± 0.023 ug/ml. In comparison, Etoposide exhibited a concentration of 1.87 ± 0.07 µg/ml. In order to comprehend and forecast

the behavior of a molecule throughout the body, encompassing its absorption, distribution, metabolism, and excretion, an analysis was conducted on the ADME physicochemical and pharmacokinetic properties of cis-9-Octadecenoic acid. In addition, the ProTox ii website was utilized to assess the toxicity profile of cis-9-octadecenoic acid. In addition, a computational analysis was conducted to investigate the potential interaction and antimicrobial effects of cis-9-Octadecenoic. This analysis utilized the MOE software and Patch Dock server to examine the three-dimensional structures of E. coli proteins found in the outer membrane, specifically the crystal structure of OMPX (1QJ8). The interaction between Cis-9-Octadecenoic and OMPX (1QJ8) through Phe90 and Lys48 may lead to the breakdown of the outer membrane of *E. coli*. Several studies have shown the biological and agricultural potential of marine-derived actinomycetes, notably Streptomyces. Streptomyces violaceusniger KS20 has many biomedical uses, according to Chakraborty et al. [42], while Math et al. found that 7-hydroxyflavone from *Amycolatopsis* sp. HSN-02 can control Cercospora leaf spot disease in tomatoes [43]. *Streptomyces* filamentous strain KS17, from underexplored maritime environments, inhibited human infections with its broad-spectrum antibacterial action [44]. *Streptomyces* levis strain KS46's secondary metabolite profile showed potential in marine settings [45]. Thes researches demonstrate the role actinomycetes' role in producing novel antimicrobials and sustainable biocontrol methods.

Conclusion

In conclusion, our study emphasizes the importance of investigating marine microbial diversity as a significant resource bioactive compound. The bioactive metabolites obtained from streptomyces sp. exhibited potent antibacterial and antibiofilm properties against *S. aureus*, *B. subtilis*, and *P. aeruginosa*, effectively disrupting biofilm formation. Furthermore, it demonstrated significant cytotoxicity against liver and breast cancer cells, with IC_{50} values of 17.48 ± 0.94 and 88.73 ± 4.78 µg/ml, respectively, alongside vigorous anti-topoisomerase activity (IC_{50} = 0.65 ± 0.023 µg/ml). Computational analysis, including ADME profiling and molecular docking, provided insights into its pharmacokinetic potential. These findings underscore *cis*-9-Octadecenoic acid as a promising candidate for antimicrobial and anticancer applications. However, our study has limitations. The compound's efficacy and safety must be confirmed in vivo after in vitro testing. Computational research revealed pharmacokinetics, but experimental studies are needed to validate. Future study should enhance fermentation conditions and how the molecule is manufactured to increase yield and effectiveness.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-03912-w.

Supplementary Material 1

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Author contributions
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ELUCIDATION OF ANTIBIOFILM FORMATION MECHANISMS USING MARINE EXTRACTS AGAINST INFECTION AND ANTIMICROBIAL RESISTANCE (AMR)

Anti-biofilm activity of marine algae-derived bioactive compounds / Behzadnia, A., Moosavi-Nasab, M., & Oliyaei, N.

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Anti-biofilm activity of marine algae-derived bioactive compounds

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A large number of microbial species tend to communicate and produce biofilm which causes numerous microbial infections, antibiotic resistance, and economic problems across different industries. Therefore, advanced anti-biofilms are required with novel attributes and targets, such as quorum sensing communication system. Meanwhile, quorum sensing inhibitors as promising anti-biofilm molecules result in the inhibition of particular phenotype expression blocking of cell-to-cell communication, which would be more acceptable than conventional strategies. Many natural products are identified as anti-biofilm agents from different plants, microorganisms, and marine extracts. Marine algae are promising sources of broadly novel compounds with anti-biofilm activity. Algae extracts and their metabolites such as sulfated polysaccharides (fucoidan), carotenoids (zeaxanthin and lutein), lipid and fatty acids (γ-linolenic acid and linoleic acid), and phlorotannins can inhibit the cell attachment, reduce the cell growth, interfere in quorum sensing pathway by blocking related enzymes, and disrupt extracellular polymeric substances. In this review, the mechanisms of biofilm formation, quorum sensing pathway, and recently identified marine algae natural products as anti-biofilm agents will be discussed.

KEYWORDS

anti-biofilm activity, algae extracts, biofilm, marine natural products, quorum sensing, quorum quenching, quorum sensing inhibition

1 Introduction

Microbial sessile communities attached to a substratum or interphases or to each other, known as biofilms embedded in self-secreted extracellular matrices containing protein, polysaccharide, nucleic acid, and lipid substances, display an altered phenotype in comparison with planktonic microbial cells (Muras et al., 2021; Wang et al., 2022). Extracellular polymeric substances (EPS) is greatly involved in the stability of the structure and the function of biofilm (Ashrafudoulla et al., 2020). Over 80% of microbial infections are associated with biofilm formation, while it has been characterized as one of the crucial medical hurdles over the century (Brackman and Coenye, 2015; Wang et al., 2022). Moreover, bacteria cells within biofilms have shown 1,000 times more resistance to various stresses rather than the planktonic form (Ashrafudoulla et al., 2019). In addition to their impact on human health, biocorrosion and biofouling based on biofilm formation of microorganisms cause enormous financial problems in the shipping and medical industries (Muras et al., 2021; Wang et al., 2022). Therefore, due to the importance of this issue, some researchers present multiple challenges of biofilm formation and their impact on food safety, particularly poultry and seafood products

(Ashrafudoulla et al., 2023; Chowdhury et al., 2023), and fully discuss the new insights to combat biofilms in the food industry (Mevo et al., 2021; Rahman et al., 2023; Uddin Mahamud et al., 2024). In recent years, numerous natural products have been identified as antibacterial and anti-biofilm agents (Song et al., 2018; Lu et al., 2019; Mulat et al., 2022) which can inhibit cellular adhesion, abolish the quorum sensing signaling, prevent biofilm formation, disrupt extracellular matrix (ECM) structure, and decrease the production of quorum sensing-regulated virulence factors in pathogenic bacteria (Lu et al., 2019). Therefore, several research studies have been focused on natural anti-biofilm agents obtained from plants, microorganisms, and marine extracts or metabolites to control human infections (Mishra et al., 2020; Danquah et al., 2022; Ashrafudoulla et al., 2023). Among various natural products, it is well known that marine and algal bioactive compounds are utilized for prevention and treatment of bacterial biofilm (Melander et al., 2020).

Algae are known as renewable sources of bioactive compounds, in particular, carotenoids and polysaccharides, with a wide range of biological activities, such as antioxidant, antimicrobial, anti-inflammatory, anti-obesity, antidiabetic, anticancer, and anti-Alzheimer activities (Oliyaei et al., 2023). Macroalgae or seaweeds are classified into Chlorophyceae (green algae), Phaeophyceae (brown algae), and Rhodophyceae (red algae) (Oliyaei et al., 2022). Some research studies revealed that their extracts or metabolites have the potential to inhibit biofilm formation (Zammuto et al., 2022). Similarly, microalgae, microscopic and photosynthetic organisms, are rich in various metabolites (polyphenols, lipids, and carotenoids) with various bioactivities. Microalgal derivatives are promising candidates in novel, biocontrol agents against pathogens (Sampathkumar et al., 2019).

Based on the importance of biofilm, and concern about its serious problems, natural substances became attractive. Moreover, considering the potential of the use of algae as a novel source of biological agents, determining the antibiofilm activities of their derivatives is crucial. To the best of our knowledge, there is no review article about the anti-biofilm inhibitory potential of different algal-based bioactive compounds. Therefore, this review summarizes an overview of biofilm formation, quorum sensing pathway, and new identified marine algae natural products, as anti-biofilm agents will be discussed. These natural substances are interesting candidates that suggest novel strategies for controlling biofilm-related infections.

2 Biofilm formation and structural and physiological attributes

Biofilm is a sessile complex of microorganisms produced on food surfaces. Biofilms create serious concerns in the healthcare and food industries. The structure complexity of biofilms causes intrinsic resistance against antibiotics and also promotes the colonization of microorganisms by providing nutrients which protect microbial cells from antimicrobial agents (Ashrafudoulla et al., 2021). Biofilm occurrence forms in multiple steps as shown in Figure 1: (i) conditioning the surfaces with organic and inorganic molecules influencing initial adherence of the microbial cells and (ii) producing extracellular matrices causing attachment of cells to the substratum more firmly. This allows the cells to grow and mature from microcolonies to three-dimension communities. The physiological responses of the microbial cells in the biofilm community will change based on the existing conditions in the biofilm, (iii) detaching the cells from the biofilm, disperse and form a biofilm on other surfaces or release as a planktonic state (Brackman and Coenye, 2015; López and Soto, 2019).

A biofilm association is evaluated as one of the most impressive sites of genome expression of bacterial cells, in which the microbial cells are well preserved and metabolically act more efficiently. However, a biofilm arrangement with attributes, such as survival, adaptive, and protective values, behaves differently from the planktonic cells that are not very efficient and are only used for propagating and colonizing new surfaces. The population of microbial cells embedded in the biofilm matrix is between 10^4 and 10^8 cells/cm^2, by which the differences between invisibility and visibility of biofilms are being considered (Costerton et al., 1995; Lazar, 2011; Lamin et al., 2022).

Moreover, a biofilm community resists microbial cells inside the matrices to antibiotics by numerous mechanisms. The small dissemination of antibiotics across the extracellular matrices of biofilm communities, altered physiological traits of microbial cells by the low growth rate in response to restrict oxygen and nutrient quantity or environmental stress, changed phenotypic properties of cells, quorum sensing. Then, it results in exchanging the resistance of genes between microbial cells inside the biofilm and resisting the cells exposed to antimicrobial agents due to the remaining cells and cell debris. In addition, the structure of the biofilm would be effective in its mechanism of antimicrobial resistance which avoids the immune system function and phagocytosis (López and Soto, 2019). A large number of microbial species tend to communicate with other cells, make an interaction with their surrounding environment, and display multicellularity, resulting in biofilm formation (Kalia et al., 2015; Muras et al., 2021; Majdura et al., 2023). It is mostly related to the ability of microbial cells to communicate with themselves at high and uncommonly low cell densities through the signaling phenomenon called quorum sensing. Quorum sensing system was utilized to synchronize gene expression to biofilm formation, indicating cell density and facilitating collective behaviors. Cell-to-cell communication known as quorum sensing has been found to be crucial in biofilm formation of both gram-negative and gram-positive with the surrounding extracellular matrix. By detecting and quantifying the accumulation of specific self-produced signal molecules released by the community, quorum sensing allows bacteria to determine the population density. The quorum sensing system mediates changes in the production of biofilm matrix components, such as exopolysaccharides, lipids, nucleic acids, and proteins. In addition, this system is also coordinated with other environmental factors such as pH, salinity, temperature, oxidative stress, and availability of nutrients progressing the cellular adaptation to the environment and enhancing their survival likelihood. However, interfering with the quorum sensing system is known as a promising approach to avoid bacterial biofilm proliferation (Machado et al., 2020; Muras et al., 2021).

3 Quorum sensing signaling network

As mentioned above, there is a cell-to-cell communication among the microbial cells involved in the biofilm matrix. Quorum sensing is

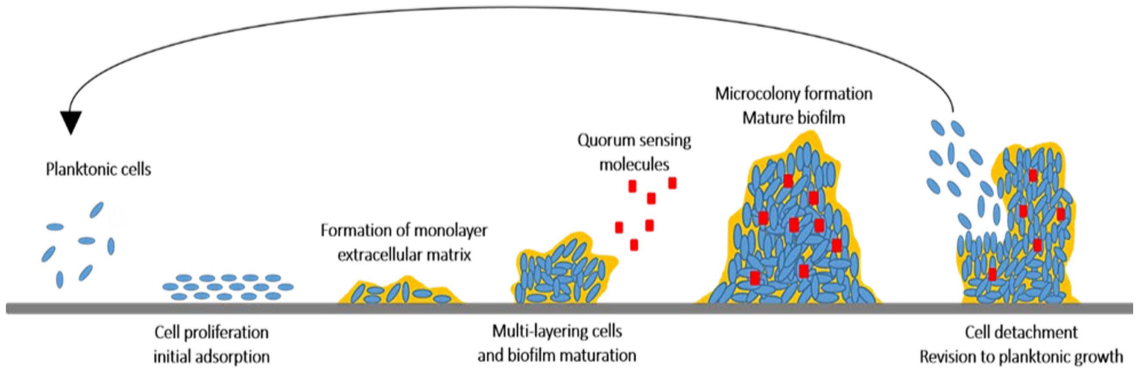

FIGURE 1
The cycle of biofilm evolution from planktonic cells to detachment of planktonic cells.

mediated by the production and secretion of low molecular weight chemicals, also known as auto-inducers (AIs) (Menaa et al., 2021), using bacterial cells depending on their cell density (Lazar, 2011; Machado et al., 2020). These diffusible signaling molecules are responsible to regulate gene expression depending on cell density and to adjust some prokaryotic phenotypes such as bioluminescence, production of secondary metabolites (toxins and antibiotics), secretion of intercellular enzymes, production of virulence factor, and biofilm formation (Borges and Simões, 2019; Lamin et al., 2022). Over the last years, the function of the quorum sensing system has been investigated in several important biological processes. It has been shown that quorum sensing mechanism regulates food spoilage. In addition to regulating gene expression, it has also an impressive effect on the survival and growth of food pathogens (Bai and Vittal, 2014; Machado et al., 2020). Studies have demonstrated the crucial role of the quorum sensing process in different stages of biofilm formation (Machado et al., 2020). The most identified microorganisms involved in biofilm formation mediated by quorum sensing signaling network include *Pseudomonas aeruginosa*, *P. fluorescens*, *Chromobacterium violaceum*, *Campylobacter jejuni*, *Aliivibro ficheri*, *Vibrio harveyi*, *V. parahaemolyticus, Escherichia coli*, *Acinetobacter* spp., *Salmonella enterica*, and *Salmonella* Thompson. They are known as human opportunistic pathogens causing infections such as pneumonia, chronic otitis, chronic wounds, and catheter infection (Ashrafudoulla et al., 2019, 2023; Ćirić et al., 2019).

However, quorum sensing signaling systems can be used in expanding quorum sensing inhibitors to control the biofilm propagation, specifically unwanted bacterial embedding in the biofilm matrix (Machado et al., 2020). The auto-inducers (AIs) are synthesized steadily and diffused into the environment with the cell density increment gaining a threshold value called quorum level of signaling molecules (Borges and Simões, 2019; Machado et al., 2020). Different types of quorum sensing signaling systems (Figure 2) distinguished between gram-negative bacteria cytoplasm and gram-positive bacteria cell membrane, exploiting the gene regulation-mediated quorum sensing (Borges and Simões, 2019). Hormone-like AIs diffusible from gram-negative bacteria, such as acyl-homoserine-lactones (AHL), non-diffusible autoinducing peptides (AIP) at gram-positive bacteria, and autoinducer-2 (AI-2) in both gram-positive and gram-negative bacteria are major quorum sensing systems which are recognized as precursor peptides releasing outside by protein transport machinery (Lazar, 2011; Brackman and Coenye, 2015; Borges and Simões, 2019). Moreover, the other typical signaling molecules such as *Pseudomonas* quinolone signal (2-heptyl-3-hydroxy-4-quinolone), fatty acids (cis-2-dodecenoic acid), and autoinducer-3 (from *E. coli* O157:H7) have been identified, while the LuxI/R-type system, the Agr system, and the LuxS/AI-2 system are known as the main quorum sensing systems (Bai and Vittal, 2014; Borges and Simões, 2019). The LuxI/R-type system consists of the proteins called *LuxI* (AHL synthase) and *LuxR* (AHL receptor), utilized by gram-negative bacteria. The regulation of the luminescence production in gram-negative marine bacterium *V. fischeri*, which is known as the cell density-dependent mechanism, is mediated by the AHL quorum sensing system (Papenfort and Bassler, 2016; Borges and Simões, 2019). The Agr system consists of RNA II and RNA III regulating signals. It is a signaling peptide presenting in gram-positive bacteria such as *Staphylococcus aureus* as activating/inhibiting peptides that contain a membrane-bound sensor and an intracellular response regulator (Brackman and Coenye, 2015; Vadakkan et al., 2018; Borges and Simões, 2019). The third signaling system is the LuxS/AI-2 system which is associated with the AI-2 production mediating the interaction between gram-negative and gram-positive bacteria (Brackman and Coenye, 2015). The LuxS protein is key a metallo-enzyme with two tetrahedral metal-binding sites being in some bacterial species, i.e., *Streptococcus* spp., *Lactococcus lactis*, *Clostridium perfringens*, *Neisseria meningitidis*, *E. coli*, and *Haemophilus influenza* (Brackman and Coenye, 2015; Wang et al., 2018).

However, it has been demonstrated that biofilm spoilage related to the quorum sensing network is highly responsible for contaminating various food products such as milk and dairy, meat, fisheries, and vegetables products (Machado et al., 2020; Lamin et al., 2022). For instance, *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes*, *E. coli*, *S. aureus,* and *Bacillus cereus* as foodborne pathogens are capable to connect different surfaces in various industries causing biofilm distribution, which would be a severe health and hygiene problem and an economic problem (Giaouris et al., 2015). Therefore, since there are still more to learn about the key role of quorum sensing in biofilm formation, antagonist molecules, known as quorum sensing inhibitors/quorum quenching agents by binding to different sites on the receptors (LuxR-type receptor), block the signal activations. The blocking agents compete or interfere with AHL signal molecules, target production of cell signaling molecules, deteriorate signal

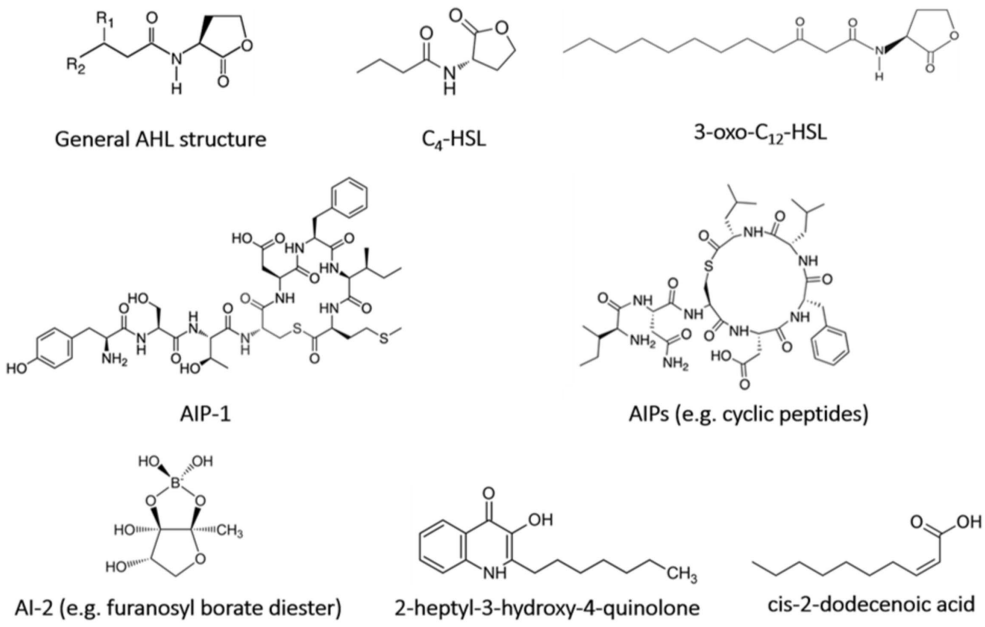

FIGURE 2
Chemical structure of quorum sensing signal molecules.

systems, and inhibit the attachment of signals to the receptors or signal transportation to prevent the biofilm formation (Bai and Vittal, 2014; Brackman and Coenye, 2015). However, quorum sensing inhibition as an alternative strategy might result in the inhibition of particular phenotype expression blocking cell-to-cell communication that would be more acceptable rather than conventional sanitizers (Muras et al., 2021).

4 Quorum sensing inhibition

Several strategies have been expanded to eradicate quorum sensing network and biofilm-related infections. Applying liposomes, bacterial interference, bacteriophages, hydrogels, iontophoresis, and nanoparticles are some approaches combatting biofilm propagation, although new approaches are also required (López and Soto, 2019). Therefore, the inhibition of the quorum sensing network using new materials and strategies is a promising tool for interfering bacterial biofilms, while they resist to a variety of regular antimicrobial agents (Lazar, 2011; Kalia et al., 2015). The polymeric matrix on the surface of the substratum, which is mostly made up of exopolysaccharides, extracellular proteins, nucleic acids, and other components, facilitates the irreversible adhesion of bacteria. Cell adhesive properties also depend heavily on extracellular elements, such as surface-exposed proteins, extracellular glucan-binding proteins, and glycosyltransferases. Hence, the anti-biofilm function of natural compounds is related to the blockage of polymer matrix synthesis, elimination of cell-to-cell and cell-to-surface attachment, extracellular matrix disruption, lowering of the formation of virulence factors, and, subsequently, hampering quorum sensing system and biofilm formation (Lu et al., 2019). Quorum sensing inhibition or quorum quenching mechanism is referred to the enzymatical inactivation of quorum sensing signals. It describes the disruption of microbial cell communication by quorum sensing inhibitory chemicals using targeted to AI signaling molecules, receptors, and downstream signaling cascades (Figure 3) (Lazar, 2011; Kalia et al., 2015; Muras et al., 2021).

It has been reported that blocking the microbial quorum sensing signals as a prevalent strategy has already been applied by a variety of organisms to protect themselves from pathogenicity and virulence (Lazar, 2011; Sampathkumar et al., 2019). This strategy inhibits intercellular signaling by (i) hindering diffusion of homoserine-lactone (HSL) signal and lowering the concentration of extracellular HSL. Signal molecules are degraded by enzymes such as AHL-lactonases and AHL-acylases, which hydrolyze the lactone ring and release free HSL and fatty acid, respectively; (ii) inhibiting the signal receptors by competitive synthetic or naturally synthesized molecules using some bacteria strains including *V. fischeri*, *Agrobacterium tumefaciens*, *Chromobacterium violaceum*, and *Aeromonas salmonicida*, producing inhibitor proteins which express new functions to cells or by some uncompetitive molecules cause disruption of HSL binding to proteins; (iii) interfering intercellular signaling systems using quorum sensing inhibitors produced by algae, microorganisms, herbs, fruits, spices, and animals (Lazar, 2011; Bai and Vittal, 2014; Machado et al., 2020). Quorum sensing inhibitors as intrinsic ammunition are considered to preserve from treatment to surviving the host cells or hindering the ecological balance (Sampathkumar et al., 2019). Generally, quorum sensing inhibitors are divided into three main classes, namely, AHL analogs, 2 (5H)-furanones, and compositions that are not structurally related to AHLs (Lazar, 2011). However, there are four enzymatic ways to degrade AHL, including AHL acylases (amidohydrolysis) and AHL lactonases (lactone hydrolysis), which hydrolyze the amide bonds of the AHL molecules irreversibly and the HSL rings reversibly; AHL oxidases and AHL reductases (oxidoreduction) alter the activity of AHL but are not able to degrade the AHL molecules (Dong et al.,

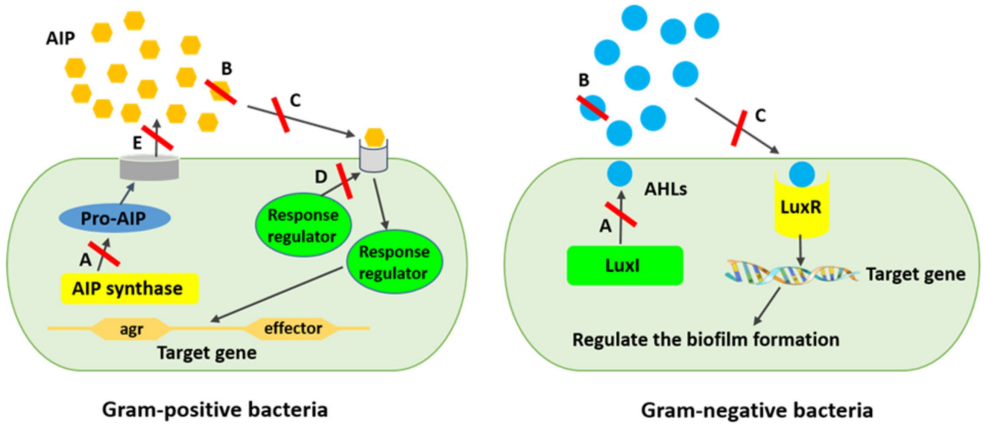

FIGURE 3
Mechanism of quorum sensing inhibition against biofilm formation. **(A)** AIs synthesis inhibition, **(B)** Degrading AIs by AHL-lactonases, **(C)** Interference of the signal receptors by AI antagonists, **(D)** Interference of the response regulators resulting in signaling cascade disturbing, **(E)** Reduction of the extracellular AIs resulting in inhibited cell-to-cell signaling. *AIPs are produced inside the bacterial cell as pro-AIP which will be processed and modified inside or outside the cell hinge upon the organism.

2000; Uroz et al., 2009; Borges and Simões, 2019). It has repeatedly been shown in several studies that AHL analogs modified the acyl side or lactone ring, or amide moiety by which the lactone moiety is replaced by cyclopentyl or cyclohexanone rings or triazolyldihydrofuranone moiety, interfering biofilm development and indicating biofilm inhibitory action against *Serratia marcescens*, *P. aeruginosa,* and *Burkholderia* spp. (Ishida et al., 2007; Morohoshi et al., 2007; Brackman et al., 2012). Nevertheless, exploring new and natural sources for inhibiting biofilm-related infections would be crucial to be used in clinical and industrial practices. Meanwhile, biological molecules from algal sources are promising agents due to their vast species diversity, habitat they live in, and great biological defense activity compared with their counterparts from other resources (López and Soto, 2019).

5 Marine anti-biofilm agents

Recently, some investigations have been focused on natural products with anti-biofilm attributes. Therefore, various bioactive compounds have been identified from marine sponges, including the diterpene alkaloid (−)-ageloxime D 121, derived from *Agelas nakamurai* with prevention of *S. epidermidis* biofilm formation (Hertiani et al., 2010), diterpene, darwinolide 122 from *Dendrilla membranosa* with inhibition of the attachment and biofilm formation of MRSA (von Salm et al., 2016), tryptamine derivative bufotenine 123, from the Mediterranean gorgonian *Paramuricea clavate* with anti-adhesion activity against *Pseudoalteromonas* sp. D41 (Pénez et al., 2011).Sometimes,thesenaturalcompounds,suchasbufotenine(5)and1,3,7-trimethylisoguanine (10), two bromoindole derivatives, are more effective than commercial compounds and have more strong anti-adhesion properties against bacteria (Pénez et al., 2011). The marine-based natural anti-biofilm agents are well reviewed by Melander, Basak (Melander et al., 2020). However, some algae species producing inhibitors such as red marine algae, *Delisea pulchra*, synthesizes halogenated furanones as the first quorum sensing inhibitor group which competitively inhibits AHL binding to intracellular receptors and then bacterial colonization and biofilm development. It has happened by preventing the key quorum sensing pathway through bacterial cells as the AHSL regulatory system in Gram-negative bacteria and also AI-2 signaling network in both Gram-negative and Gram-positive species (Manefield et al., 1999; Dong et al., 2000; Borges and Simões, 2019). Moreover, it has been observed that extracts from red seaweeds *Sarcodiotheca gaudichaudii* and *Chondrus crispus* interfere with biofilm formation by *S. enteritidis* (Kulshreshtha et al., 2016). The great anti-bacterial and anti-biofilm activities of methanolic extract of *Padina pavonica* were obtained against *S. aureus*, *Enterococcus faecalis*, *P. aeruginosa*, *Klebsiella pneumonia*, and *B. subtilis* (Makhlof et al., 2023). Attenuation of AHL function of several marine actinomycetes extracts has been discovered against biofilm formation of *Vibrio* spp., hindering quorum sensing signaling by blocking the AHL receptor site of the *LuxR* homologs (You et al., 2007).

5.1 Algae

Algae are a very diverse group of predominantly aquatic photosynthetic microorganisms that account for almost 50% of photosynthesis on this planet. Marine algae are classified into two major groups based on their size; seaweeds (macroalgae) and microalgae (Menaa et al., 2021). Seaweeds can be classified into Chlorophyceae (green algae), Phaeophyceae (brown algae), and Rhodophyceae (red algae). Marine algae have been recognized as an alternative resource of natural substances which possess a wide range of biological attributes and health benefits such as antioxidant, antibacterial, antifungal, antiviral, anti-inflammatory, and antidiabetic attributes (Pradhan et al., 2020; Oliyaei et al., 2022). Therefore, algal metabolites have gained considerable attention in the development of functional foods and nutraceutical industries and health products (Oliyaei et al., 2022). In recent years, the anti-biofilm activity of algae extracts and their derivatives has gained much attractive attention which will be reviewed (Table 1).

TABLE 1 Attributes of algal-base anti-biofilm agents.

Inhibitor	Algae	Organism	Effect	Reference
Algae extracts	*W. prolifica*	Gram-positive bacteria (*S. aureus, B. subtilis*, and *Streptococcus* spp.), gram-negative bacteria (*Shigella* spp., *Proteus* spp., and *P. aeruginosa*) and fungi (*A. niger* and *C. albicans*).	Inhibition of biofilm formation and prevention of bacterial adhesion. Antibacterial activities of Phycobiliprotein pigments, such as phycocyanin	Al-TmimiG et al. (2018)
	A. platensis	*V. parahaemolyticus* *Ch. Violaceum*, *V. alginolyticus* *A. hydrophila* *P. aeruginosa* *E. coli* *S. aureus*	Reduction of the cell surface hydrophobicity and inhibition of EPS	LewisOscar et al. (2017)
	Chlamydomonas spp.	*P. aeruginosa* (PA14 and ATCC 10145)	Reduction of cell surface hydrophobicity and extracellular polymeric substances and biofilm secretion, Acyl homoserine lactone (AHL)-like substances affect quorum sensing-controlled expression, reduction of biofilm morphology and thickness of biofilm by furanones, and the anti-biofilm activity and pathogenicity of *P. aeruginosa* by phycocyanin	Nithya et al. (2014)
	Synechococcus spp.	*V. harveyi* and *V. vulnificus*	Inhibition of the initial attachment and interfering in the biofilm structure and formation. Interfere in quorum sensing pathway by blocking the protease and gelatinase	Santhakumari et al. (2016)
	U. reticulate, S. wightii, H. macroloba, sea grasses: *H. pinifolia, C. serullata*	*Pseudomonas* spp., *Flavobacterium* spp., *Bacillus* spp., and *Cytophaga* spp.	Inhibition of biofilm formation mainly related to the functional groups such as hydroxyl and carbonyl groups, fatty acids, and amides I & II.	Prabhakaran et al. (2012)
	225 cyanobacteria microalgae species (*Cercozoa, Charophyta, Chlorophyta, Cryptophyta, Cyanobacteria, Euglenophyta, Glaucophyta, Haptophyta, Miozoa, Ochrophyta, Rhodophyta*, and 2 unknown species)	*C. albicans* and *C. cloacae*	Biofilm inhibition mechanism because of high amount of lipids and polyunsaturated fatty acids (PUFAs) such as docosahexaenoic acid	Cepas et al. (2019)
	Chlamydomonas reinhardtii	*E. cloacae, C. albicans,* and *S. aureus*	Inhibition of bacteria growth, quorum quenching, and disruption of biofilm because of active metabolites such as hydrocarbons, phenols, alcohols, and esters and two bioactive compounds;1Heptacosanol and Octadecyl chloride	Ghaidaa et al. (2020)
	H. siliquosa	*S. aureus* MRSA ATCC 33593 and *S. aureus* MRSA NCTC 10442	The strong antimicrobial properties were related to QSI	Busetti et al. (2015)
	Halimeda spp.		Inhibition of bacteria growth and EPS production	Gadhi et al. (2018)
	S. wightii, and *H. gracillis*	Gram-negative bacteria (*E. coli, P. aeruginosa*, and *V. parahaemolyticus*).	Antimicrobial activity against the biofilm formation by gram-negative bacteria, insecticidal activity	Suganya et al. (2019)

(Continued)

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TABLE 1 (Continued)

Inhibitor	Algae	Organism	Effect	Reference
	C. spongiosus, L. papillosa, and *C. arabicum*	*Candida* species (*C. krusei, C. glabrata, C. parapsilosi,* and *C. albicans*).	Inhibition of *C. krusei* biofilm formation, reduction in the viability of preformed biofilms	El Zawawy et al. (2020)
	Sargassum spp.	*S. epidermidis*	Inhibition of biofilm formation by *Staphylococcus* spp.	Alreshidi et al. (2023)
	U. lactuca *S. scoparium*, and *P. capillacea*	*S. aureus*	Reduction in the surface hydrophobicity of *S. aureus* and inhibition of initial adhesion and proliferation of *S. aureus* and the biofilm development	Rima et al. (2022)
Bioactive compounds	Furanones derived from *D. pulchra*,		Interfering in gene expression of the AHL and act as quorum sensing inhibitor	Manefield et al. (1999)
	Alginate oligosaccharides derived from *L. hyperborean*	*P. aeruginosa*	Weakening the EPS integrity and decomposition of its structure	Powell et al. (2018)
	Fucoidan F85	*S. mutans* and *S. sobrinus*	Inhibition of the planktonic cell growth	Vishwakarma and Vavilala (2019)
	Fucoidan F85 derived from *F. vesiculosus*	*S. mutans* and *S. sobrinus*	Inhibition of biofilm production and planktonic cell growth	Jun et al. (2018)
	Zeaxanthin	*P. aeruginosa*	Interfering in the gene expression of quorum sensing and prevention of *LasIR* and *RhlIR* quorum sensing system	Gökalsın et al. (2017)
	Lutein derived from *C. pyrenoidosa*	*P. aeruginosa*.	Inhibition of biofilm formation and degeneration of CSH and EPS. Interact with *LasI*, *LasR*, *RhlI,* and *RhlR* taking part in quorum sensing process	Sampathkumar et al. (2019)
	Glycolipid derived from *Shewanella algae*	*B. cereus*, *S. pneumoniae*, *P. aeruginosa*, *E. coli*, *K. pneumoniae*, and *Acinetobacter* spp.	Growth inhibition of clinical bacterial pathogens and disruption of the preformed biofilms	Gharaei et al. (2022)
	Lipid from *S. platensis* extract	*C. albicans*	Anti-biofilm growth activity and anti-biofilm activity	Boutin et al. (2019)
	Lipids from *S. brasiliensis*, *E. acutiformis* and cyanobacteria (*Sphaerospermopsis* spp.)	*K. pneumoniae*, *E. coli, P. aeruginosa*, *E. cloacae, S. aureus*, Coagulase Negative *Streptococcus*, *S. epidermidis*, *C. parapsilosis*, and *C. albicans*	Effective inhibition of biofilm and antivirulence potency	Prasath et al. (2020)
	Palmitic acid derived from *Oscillatoria subuliformis*	*P. aeruginosa*	Reduction of biofilm formation by downregulation of *abaR* gene, reducing N-acyl-homoserine lactone production and interference in quorum sensing system	LewisOscar et al. (2018)
	Phlorotannins from *Ascophyllum nodosum*	*E. coli*	Inhibition of cell proliferation and synthesis of exopolysaccharides	Bumunang et al. (2019)
	Phlorotannins from *Hizikia fusiforme*	*P. aeruginosa*	Reduction of pyocyanin production, disruption of QS, reduction of the motility of bacterial, synthesis of protease, and hemolysin, and suppression of the biofilm formation	Tang et al. (2020)
	Polyphenols from *Sargassum muticum*	*E. coli* and *P. aeruginosa*	Anti-biofilm activity by suppressing the biofilm formation	Puspita et al. (2017)

(Continued)

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TABLE 1 (Continued)

Inhibitor	Algae	Organism	Effect	Reference
Green silver nanoparticles	Silver nanoparticles in combination with seaweeds extracts (*U. fasciata*, *Grateloupia* spp., *P. capillacea* and *C. mediterranea*)	*E. coli*, *S. aureus*, *S. faecalis*, *P. aureogenosa* and *V. damsela*.	Anti-biofilm activity	Negm et al. (2018)
	Silver nanoparticle with *Oscillatoria* spp. extract	*S. aureus*, *E. coli*, *P. aeruginosa*, *Citrobacter* spp., *S. typhi*, and *B. cereus*.	Antibacterial activity against pathogen bacteria, strong antibiofilm activity	Adebayo-Tayo et al. (2019)
	Silver nanoparticle using red algae *G. corneum* extract	*C. albicans* and *E. coli*	Antibiofilm efficacy in two stages (prebiofilm and postbiofilm)	Öztürk et al. (2020)
	Silver nanoparticles with *G. corticata*	*K. pneumonia*	Influenced the protein responsible for EPS production and disrupting the protective layer in biofilm	Rajivgandhi et al. (2020)
	Silver nanoparticles with *Sargassum myriocystum*	*P. aeruginosa,* and *S. epidermidis*	Biofilm inhibition at MIC due to the inhibitory effect on gene expressions related to motility and biofilm formation	Balaraman et al. (2020)
	Silver nanoparticles using *Spirogyra* extract	*S. aureus* and *Acinetobacter baumannii*.	Lethal to biofilm-forming bacteria and inhibiting biofilm development	Danaei et al. (2021)
	Iron oxide nanoparticles prepared using *S. vulgare*, *U. fasciata* and *J. rubens* extract	Gram-positive and Gram-negative bacteria	Antibiofilm efficiency on steel and wood surfaces	Salem et al. (2020)
	Silver nanoparticles with *S. angustifolium*		Increasing lethality with increasing concentration	Bita et al. (2015)
	Silver nanoparticles with *Urospora* spp.		Inhibitory activity against pathogen bacteria	Suriya et al. (2012)
	Silver nanocomposite capped with k-carrageenan	*S. aureus* and *P.aeruginosa*	Reduction in the growth of biofilm forming bacteria	Goel et al. (2019)
	Silver nanoparticles capped with k-carrageenan	*C. albicans* and *C. glabrata*	Cell membrane penetration and interaction with extracellular matrix of biofilms	Gupta et al. (2021)
Dental resin	Dental acrylic resin containing seaweeds *U. pinnatifida*	*S. mutans*	Reduction in the colony counts of microorganisms and antibiofilm activity	Pourhajibagher et al. (2019)
	Silver nanoparticles with *U. lactuca*	*S. mutans*	Reduction of virulence genes expression and biofilm formation ability	Pourhajibagher et al. (2021)
	Nanocomposite with *S. muticum* extract containing Zn and CuO nanoparticles	*P. mirabilis*, *P. aeruginosa*, *S. aureus*.	Reduction of biofilm production and inhibition of adhesion	Sadek et al. (2019)

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5.1.1 Algae extract

Some researchers have investigated the anti-biofilm activity of algae extracts. The blue-green algae *Westiellopsis prolifica* extract showed anti-biofilm activity potential against gram-positive bacteria (*S. aureus*, *B. subtilis*, and *Streptococcus* spp.), gram-negative bacteria (*Shigella* sp., *Proteus* sp., and *P. aeruginosa*), and fungi (*Aspergills niger* and *Candida albicans*). It was reported that the *W. prolifica* extracts were more effective on gram-negative bacteria, and the crude acetone extract showed the highest biofilm inhibition against *Shigella* sp. According to the Congo red agar method, the *W. prolifica* acetone extract exhibited significant anti-biofilm activity at 50 µL, while hexane extract was less active. The *W. prolifica* extract can inhibit bacterial biofilm formation and prevent bacterial adhesion, which depends on its antibacterial components such as phycobiliprotein pigments such as phycocyanin, phycoerythrin, and allophycocyanin (Al-TmimiG et al., 2018). The methanolic *Arthrospira platensis* extract at a concentration of 100 ng mL^{-1} also prevented the biofilm formation of *V. parahaemolyticus* (90%), *Chromobacterium violaceum* (89%), and *V. alginolyticus* (88%). Moreover, spirulina extract lowered the cell surface hydrophobicity (CSH) of *A. hydrophila*, *E. coli,* and *S. aureus* impressively and also exhibited extracellular polymeric substances (EPS) inhibition of approximately 50–88% for *P. aeruginosa*, *E. coli*, *S. aureus,* and *A. hydrophila* (LewisOscar et al., 2017). Accompanying quorum sensing, CSH and EPS play a significant role in attachment and biofilm forming ability of bacteria. The structural integrity and stability of biofilm is influenced by EPS. Surface adhesion of bacteria and enhancement of biofilm mass are also strongly affected by CSH. Therefore, CSH diminish can intercept initial attachment and reduce early stage biofilms, thereby reducing the bacterial communities in biofilm and biofilm-related disorders (LewisOscar et al., 2017). Similarly, *Chlamydomonas* sp. extract can decrease CSH and biofilm secretion of *P. aeruginosa*. *Chlamydomonas* extract has some Acyl homoserine lactone (AHL)-like substances that affect quorum sensing-controlled expression. Moreover, reduction of the biofilm morphology, thickness by furanones, and anti-biofilm activity and pathogenicity of *P. aeruginosa* by phycocyanin are reported (Nithya et al., 2014).

In addition, the crude extract of *Synechococcus* sp. possesses anti-biofilm and anti-quorum sensing activity against aquatic bacterial pathogens, such as *V. harveyi* and *V. vulnificus*, and significantly decreased the biofilm formation (approximately 71 and 84%) and EPS secretion (approximately 66 and 68%). It has been reported that this extract successfully inhibited the initial attachment, interrupted the biofilm structure, and therefore prevented biofilm formation. Moreover, the *Synechococcus* extract can interfere in the quorum sensing pathway by blocking the protease and gelatinase, which were positively regulated by the quorum sensing. The anti-quorum sensing activity of the *Synechococcus* extract was related to the presence of hexadecanoic acid in the extract, and molecular docking revealed its potential to antagonistic binding to the AHL receptor protein (Santhakumari et al., 2016).

Several researchers reported that polar and non-polar extracts from brown algae, including *Sargassum vulgare*, *Padina* sp., *S. furcatum*, and *Dictyota* sp., and red algae, such as *Asparagopsis taxiformis*, *Pterocladiella capillacea*, *Spyridia aculeate*, and *Peyssonnelia capensis,* exhibited anti-QS activity (Dahms and Dobretsov, 2017). It has also been reported that different extracts from seaweeds such as *U. reticulate*, *Sargassum wightii*, *Halimeda macroloba*, and sea grasses, such as *Halodule pinifolia* and *Cymodocea serrulata,* exhibited anti-biofilm formation activity against *Pseudomonas*, *Flavobacterium*, *Bacillus*, and *Cytophaga* species. This inhibitory activity was related to the main functional groups, including hydroxyl, amino, carbonyl, and phosphoryl functionalities, aliphatic extracts (fatty acids), and NH2 (amide I and II) extracts (Prabhakaran et al., 2012). Similarly, Cepas and López (Cepas et al., 2019) investigated the anti-biofilm activity of 675 extracts (hexane, ethyl acetate, and methanol) obtained from 225 cyanobacteria and microalgae species (11 phyla including *Cercozoa*, *Charophyta*, *Chlorophyta*, *Cryptophyta*, *Cyanobacteria*, *Euglenophyta*, *Glaucophyta*, *Haptophyta*, *Miozoa*, *Ochrophyta*, and *Rhodophyta* and two unknown species). The results showed the highest biofilm inhibition rates of algae extracts against *Candida albicans* and *Enterobacter cloacae*. Among various microalgae, *Chlorophyta* and *Charophyta* showed high minimal biofilm inhibitory concentration (MBIC) values due to their ability to produce and accumulate high amounts of bioactive compounds; in particular, lipids and polyunsaturated fatty acids (PUFAs) such as docosahexaenoic acid (Pradhan et al., 2020) and eicosapentaenoic acid (Cepas et al., 2021) possess antimicrobial and anti-biofilm activities. In addition, some algae groups exhibited particular anti-biofilm activity against certain bacteria. For instance, biofilm formation in *Enterobacter cloacae* was most susceptible to *Rhodophyta* species extracts, while biofilm formation in *Candida albicans* was influenced by extracts from *Cryptophyta*, *Euglenophyta*, and *Glaucophyta*. These observations were related to the differences in antibacterial secondary metabolites such as circular or linear lipopeptides, amino acids, fatty acids, macrolides, and amides. Similarly, the extract from green microalgae *Chlamydomonas reinhardtii* reduced the biofilm formation of *S. aureus* by approximately 89%. The presence of bioactive substances in algae extracts such as hydrocarbons, phenols, esters, 1-Heptacosanol, and Octadecyl chloride prevent the biofilm formation via the inhibition of bacteria growth, quorum quenching, and disruption of biofilm (Ghaidaa et al., 2020).

The hexane: ethyl acetate extract of brown alga *Halidrys siliquosa* from Ireland at 1.25 mg ml^{-1} and 5 mg ml^{-1} concentration had inhibition against biofilms by bacterial human pathogens such as *S. aureus* MRSA ATCC 33593 and *S. aureus* MRSA NCTC 10442, respectively (Busetti et al., 2015). Gadhi, El-Sherbiny (Gadhi et al., 2018) prepared different types of red algae *Halimeda* sp. extracts from surface and whole tissue and also prepared dried and fresh algae using methanol and hexane. The authors obtained various inhibitory activities in different approaches. The results showed that dried *Halimeda* sp. extracts, especially methanolic extract, had higher anti-proliferative activity and EPS production compared with fresh algae extracts, while the fresh *Halimeda* sp. extracts exhibited better inhibition against the biofilm formation, which might be related to differences in their metabolites due to the loss of volatile compounds, such as terpenoids and fatty acids, during the drying process. Moreover, the highest biofilm formation inhibitory was obtained in the hexane surface algae extract, which might be attributed to the presence of butanamide and piperazine derivatives. In similar investigation, Suganya and Ishwarya (Suganya et al., 2019) revealed that the ethanol extracts from brown seaweed *S. wightii* and green seaweed *Halimeda gracilis* had significant anti-biofilm formation activity up to 40–75% against gram-negative

bacteria (*E. coli*, *P. aeruginosa*, and *V. parahaemolyticus*). Moreover, the anti-QS activity of red seaweed *Asparagopsis taxiformis* extract was related to active fraction of 2-dodecanoyloxyethanesulfonate ($C_{14}H_{27}O_5S$) (Jha et al., 2013). Other red macroalgae extracts such as *Chondrus crispus* and *Sarcodiotheca gaudichaudii* also showed the downregulation of QS gene *sdiA* expression, limiting the bacterial motility, reduction of virulence factors, and biofilm formation of *Salmonella enterica* (Kulshreshtha et al., 2016). In addition, three AHL inhibitors were detected from the Korean red algae *Ahnfeltiopsis flabelliformis* (Kim et al., 2007). Batista et al. (2014) used polar (water: methanol) and non-polar (dichloromethane) extracts of different macroalgae as QS inhibitors. The authors reported that 20 polar extracts interrupted the QS of AHL producer and reporter *Chromobacterium violaceum* CV017 with an MIC value ranging from 0.28 μg ml^{-1} (*Ulva fasciata*) to 189 μg ml^{-1} (*Codium* sp.). The MIC value of non-polar extracts ranged from 69 μg ml^{-1} (*Sargassum furcatum*) to 2730 μg ml^{-1} (*Peyssonnelia capensis*).

In addition, *Candida krusei*, is one of the highest biofilm producer among *Candida* isolates which was sensitive to the acetone extract of brown seaweed *Cladostephus spongiosus* (El Zawawy et al., 2020). El Zawawy et al. (2020) investigated the inhibitory effect of different organic extracts (acetone, ethanol, and methanol) of brown alga *Cladostephus spongiosus*, red alga *Laurencia papillosa*, and green alga *Codium arabicum,* which were collected from the coastal region of Hurghada in Egypt against *Candida*. Among all extracts, acetone extract of *C. spongiosus* exhibited the highest inhibitory activity against different *Candida* species (*C. krusei*, *C. glabrata*, *C. parapsilosis,* and *C. albicans*). Acetone extract of *C. spongiosus* was composed of three main fractions, namely, 4-hydroxy-4-methyl-2-pentanone, n-hexadecenoic acid, and phenol, 2-methoxy-4-(2-propenyl), which were detected by GC–MS and were not identified in other extracts. The anti-biofilm ability of acetone extract of *C. spongiosus* attributed to these fractions and caused downregulation of hyphal-specific genes, hyphal wall protein 1 (HWP1), agglutinin-like protein 1 (ALS1), and fourth secreted aspartyl proteinase (SAP4). The authors can intercept biofilm formation and eliminate the existing biofilm. Moreover, the methanol/water extract of brown seaweed *Sargassum* sp. exhibited remarkable anti-biofilm activity (82.35%) against *S. epidermidis* at 12.5 mg ml^{-1}. The extract was mainly consisting of 2-pentadecanone, 6,10,14-trimethyl-, hexadecenoic acid, methyl ester, n-hexadecanoic acid, 1,2-benzenedicarboxylic acid, and mono (2-ethylhexyl) ester, and its anti-biofilm activity was due to the chemical interaction between extract substances and some targeted receptors (Alreshidi et al., 2023).

Furthermore, the green *Ulva lactuca* extract was considered as a renewable source of new active anti-biofilm agents. Investigation reported that the polar and non-polar extracts of green seaweeds such as *Caulerpa racemose*, *Codium* spp., and *Ulva (Enteromorpha) fasciata* had anti-QS activity (Dahms and Dobretsov, 2017). Rima and Trognon (Rima et al., 2022) explored the biofilm formation inhibition activity of extracts from various macroalgae, including green alga *U. lactuca*, brown alga *Stypocaulon scoparium*, and red alga *Pterocladiella capillacea* toward *P. aeruginosa*. Among different seaweeds, green algae extracts (cyclohexane and ethyl acetate) showed better inhibitory effect against bacterial adhesion rather than others. The same prevention was observed against *S. aureus* biofilm formation in all these three seaweed extracts (Rima et al., 2022).

5.1.2 Algae bioactive compounds as anti-biofilm agents

5.1.2.1 Algal polysaccharides

Seaweeds are good sources of carbohydrates whose content is from 5 to 75% (w/w, DW) based on the age, species, period, and harvesting site. Seaweed polysaccharides are divided into sulfated and non-sulfated forms. Green seaweeds are rich in ulvan, while brown seaweeds contain different types of polysaccharides in their cell walls, including alginate, laminaran, and fucoidan. Moreover, red macroalgae are identified by their carrageenans, agars, xylogalactans, sulphated galactans, xylans, porphyran, and floridean starch. Several research studies confirmed the biological attributes of algal carbohydrates and their derivatives (Hentati et al., 2020). The pharmacological effects of polysaccharides are related to their chemical structure, molecular weight, linkage types, and monosaccharides (Xu et al., 2017).

The first anti-biofilm algae natural substance was identified by Manefield et al. (1999), who reported that a halogenated furanone, derived from red alga *Delisea pulchra*, interfered in the gene expression of acylated homoserine lactone (AHL) and act as a quorum sensing inhibitor. Regarding the anti-biofilm activity of algae polysaccharides, alginate oligosaccharides isolated from the brown seaweed *Laminaria hyperborean* were able to decline *P. aeruginosa* biofilm biomass by weakening the EPS integrity and decomposing its structure (Powell et al., 2018). Similarly, fucoidan F85 at concentration above 250 μg ml^{-1} fully suppresses the *Streptococcus mutans* and *S. sobrinus* biofilm formation and inhibits the growth of planktonic cells (Vishwakarma and Vavilala, 2019). Some researchers have demonstrated that fucoidan is able to act as anti-biofilm forming dental plaque bacteria. The fucoidan F85 obtained from *Fucus vesiculosus* entirely ceased the biofilm production and planktonic cell growth of *S. mutans* and *S. sobrinus* at concentrations higher than 250 μg ml^{-1} (Jun et al., 2018).

5.1.2.2 Algal carotenoids

Carotenoids are lipid-soluble pigments with health benefits for human daily diets. The major carotenoids found in algae are astaxanthin, fucoxanthin, β-carotene, lutein, siphonaxanthin, zeaxanthin, violaxanthin, neoxanthin, and antheraxanthin (Generalić Mekinić et al., 2023). Recent studies were conducted to evaluate the anti-biofilm and anti-quorum sensing activity of algae carotenoids. For instance, zeaxanthin can interfere in the gene expression of quorum sensing network regulates of *P. aeruginosa* and is able to prevent *LasIR* and *RhlIR* quorum sensing system by approximately 67.8 and 66.1%, respectively, at 12 μM concentration (Gökalsın et al., 2017). Sampathkumar et al. (2019) investigated that lutein was derived from *Chlorella pyrenoidosa* to combat the biofilm formation of *P. aeruginosa*. The authors revealed that lutein exhibited strong inhibition of biofilm formation and degeneration of CSH and EPS at a concentration of 20 μg ml^{-1}. According to the docking screening, the anti-quorum sensing activity of lutein was due to its ability to interact with four proteins, including *LasI*, *LasR*, *RhlI*, and *RhlR,* taking part in the quorum sensing process through biofilm production. The highly conjugated double bond structure of lutein with phenolic and polyphenolic function groups such as gallic acid, catechin, and tannic acid provide some binding sites to interact with quorum sensing proteins. The strong hydrophobic interactions between lutein and *RhlI* protein were established. Lutein also decreased gene expression of las and *rhl* in *P. aeruginosa* PAO1, which confirmed the lutein quorum

sensing inhibitory effect (Sampathkumar et al., 2019). Moreover, the glycolipid biosurfactant isolated from *Shewanella algae* was able to reduce bio-film production by *B. cereus* (83%), *S. pneumoniae* (53%), *P. aeruginosa* (92%), *E. coli* (64%), *K. pneumoniae* (87%), and *Acinetobacter* spp. (72%) (Gharaei et al., 2022).

5.1.2.3 Algal lipids

Algae are rich in essential fatty acids, especially polyunsaturated fatty acids (PUFAs) such as docosahexaenoic (DHA), eicosapentaenoic (EPA), and linoleic (LA) (Chen et al., 2023). Moreover, algal sterols (fucosterol, clionasterol, isofucosterol, and cholesterol) are recognized as nutritional and important components with several health benefits such as anticancer, antioxidant, antiobesity, and antiviral activities. Like other bioactive compounds from algae, their lipid content and fatty acid profile are varied and depend on algae species, season and harvesting time, weather, and geographical location (Hentati et al., 2020).

Recently, it has confirmed that the *S. platensis* lipid-rich extract exhibited 80% inhibition of biofilm growth against *C. albicans* after 24 h at 0.2 mg ml^{-1}, while encapsulation of *S. platensis* lipid extracts by copper-alginate nanocarriers represented adequate anti-biofilm activity (50% inhibition at 0.1 mg ml^{-1}). *Spirulina* lipid extract was mainly containing the γ-linolenic acid and linoleic acid affecting its anti-biofilm activity (Boutin et al., 2019). Cepas and Gutiérrez-Del-Río (Cepas et al., 2021) reported that microalgae (*Scenedesmus brasiliensis* and *Enallax acutiformis*) and cyanobacteria (*Sphaerospermopsis* spp.) lipids exhibited antibacterial and anti-biofilm activity against gram-negative bacteria, gram-positive bacteria, and fungi (*K. pneumoniae*, *E. coli*, *P. aeruginosa*, *E. cloacae*, *S. aureus*, Coagulase-negative *Streptococcus*, *S. epidermidis*, *C. parapsilosis*, and *C. albicans*). The lipid-rich extracts mainly comprised sulfoquinovosyldiacylglycerol, monogalactosylmonoacylglycerol, sulfoquinovosylmonoacylglycerol, α-linolenic acid, hexadeca-4,7,10,13-tetraenoic acid (HDTA), palmitoleic acid, and lysophosphatidylcholine. However, the anti-biofilm activity of *Scenedesmus* strain extracts was related to the α-linolenic acid. α-linolenic acid is the precursor to the important long chain omega-3 fatty acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). It has been reported that the pure commercial α-linolenic acid is able to inhibit the biofilm formation by *P. aeruginosa*, *S. aureus*, and *C. albicans* at 64 mg L^{-1}. Moreover, it has been revealed that fatty acids such as palmitic acid can reduce approximately 54% of *Candida* biofilm production at concentration of 500 mg L^{-1} due to the induction of the reactive oxygen species system (Prasath et al., 2020), while palmitic acid derived from the *Oscillatoria subuliformis* extract had 54% bio-film inhibitory activity against *P. aeruginosa* (LewisOscar et al., 2018). This funding revealed that the palmitic acid leading to a reduction in biofilm formation by down regulation of abaR gene, reducing N-acyl-homoserine lactone production, resulting in interfering with the quorum sensing system (Cepas et al., 2021).

5.1.2.4 Algal phlorotannins

Phlorotannins are polyphenolic compounds mostly found in brown algae, with the structural role in cell walls and formed by the polymerization of phloroglucinol unites (Lemesheva et al., 2023). The highest amount of phlorotannins is found in the brown algae *Fucus* sp. reaching up to 12% of dry weight. Phlorotannins exhibit a wide range of molecular weight (126 Da to 650 kDa), although the majority of phlorotannins exhibit low-molecular weight in the range of 10–100 kDa. The molecular weight of phlorotannins depends on algae species, size, geographic region of growth, environmental condition (light, temperature, and water salinity), nutrient level, season, and extraction techniques (Pradhan et al., 2022). These phenolic compounds are subclassified as phloroglucinol, eckol, phlorofucofuroeckol A, phlorofucofuroeckol B, 2-phloroeckol, dieckol, 6.6-bieckol, and 8.8-bieckol (Lemesheva et al., 2023). Phlorotannins can be divided into four main groups, according to the structural linkage types between phloroglucinol units: phlorethols and fuhalols (with ether linkage), fucols (with phenyl linkage), fucophlorethols (with both ether and phenyl linkages), and phloreckols (with dibenzodioxin linkage) (Li et al., 2017).

Current knowledge of phlorotannins in brown seaweeds revealed their antibiofilm and anti-fouling activities. Their biofilm inhibition is attributed to QS suppression and prevention of bacterial adhesion (Besednova et al., 2020). Phlorotannins extracted from seaweed *Ascophyllum nodosum* exhibited anti-biofilm activity against two *E. coli* strains (serotypes O113:H21 and O154:H10) through the inhibition of cell proliferation and synthesis of exopolysaccharides in *E. coli* (Bumunang et al., 2019). Similarly, the antibiofilm effect of enzymatic extraction of polyphenols derived from *Sargassum muticum* was reported which might be related to their phlorotannins (Puspita et al., 2017). Recently, the reduction of the production of virulence factor, anti-QS, and anti-biofilm activity of phlorotannins from *Hizikia fusiforme* against *P. aeruginosa* were reported. Phlorotannins remarkably lowered pyocyanin production, leading to the disruption of QS systems, therefore decreasing the violacein production in *Chromobacterium violaceum* 12,472, which is regulated by the QS system. Moreover, phlorotannins reduced the bacterial motility, synthesis of protease, hemolysin, and suppression of the biofilm formation of *P. aeruginosa* (Tang et al., 2020).

5.2 Other applications of marine-based anti-biofilm agents

One of the possible approaches to combat biofilm formation is controlling the bacteria growth. As silver has been recognized as excellent antimicrobial agent, several studies have been focused on green synthesis of silver nanoparticles with plants and algae extracts (Negm et al., 2018). For instance, Negm and Ibrahim (Negm et al., 2018) reported that green synthesized silver nanoparticles in combination with seaweed extracts (*Ulva fasciata*, *Grateloupia* spp., *Pterocladiella capillacea,* and *Corallina mediterranea*) have antimicrobial and anti-biofilm potential against *E. coli*, *S. aureus*, *S. faecalis*, *P. aureogenosa,* and *V. damsela*. Moreover, Adebayo-Tayo et al. (2019) synthesize antibacterial and anti-biofilm activities of silver nanoparticles with the *Oscillatoria* spp. extract, which possessed significant inhibition against *S. aureus*, *E. coli*, *P. aeruginosa*, *Citrobacter* spp., *S. typhi*, and *B. cereus*. Similarly, biosynthesized silver nanoparticles using the red alga *Gelidium corneum* extract had anti-biofilm activity against *C. albicans* and *E. coli*. The highest inhibition was observed in prebiofilm treatment (0.51 μg ml^{-1}) with 81% reduction, while it was 73% for post-biofilm treatment (2.04 μg ml^{-1}) (Öztürk et al., 2020). It had reported that silver nanoparticles fabricated by Gracilaria corticata possess maximum anti-bacterial and anti-biofilm potential (88%) against *K. pneumonia* at 50 μg ml^{-1} and 100 μg ml^{-1}, respectively (Rajivgandhi et al., 2020).

Similarly, green silver nanoparticles prepared with *Sargassum myriocystum* aqueous extract had antimicrobial and anti-biofilm activity against clinical pathogens such as *P. aeruginosa* and *S. epidermidis* with the inhibition of 55.49 and 48.34% at 50 μg ml^{-1}, respectively (Balaraman et al., 2020). Danaei and Motaghi (Danaei et al., 2021) also synthesize silver nanoparticles using *Spirogyra* algae extract with effective anti-biofilm activity against *S. aureus* and *Acinetobacter baumannii*. Moreover, biosynthesized iron oxide nanoparticles were prepared using *Sargassum vulgare*, *Ulva fasciata,* and *Jania rubens* aqueous extract to investigate their biofilm inhibitory activity against gram-positive and gram-negative bacteria. Among different seaweeds, the best anti-biofilm activity was observed in *S. vulgare*, *U. fasciata*, and *J. rubens* (Salem et al., 2020). In close examination, silver nanoparticles were fabricated using *Sargassum angustifolium* (Bita et al., 2015), *Urospora* spp. (Suriya et al., 2012), and *Sargassum wightii* (Singaravelu et al., 2007). The novel biodegradable silver nanocomposite capped with k-carrageenan (Goel et al., 2019) and anti-biofilm silver nanoparticles capped with k-carrageenan (Gupta et al., 2021) exhibited anti-biofilm attributes successfully against *S. aureus* and *P. aeruginosa* (Goel et al., 2019) and *C. albicans* and *C. glabrata* (Gupta et al., 2021), respectively. However, in these studies, mostly the anti-biofilm and antibacterial activities of silver nanoparticles were discussed.

Similarly, Marzban et al. (2022) developed new antibiofilm copper nanoparticles (CuNPs) with water-soluble polysaccharides from brown seaweed, *S. vulgare* (SPs). Water-soluble *S. vulgare* polysaccharides contained fucoidan and laminaran and mainly consisted of fucose and manose (approximately 50% w/w). According to the authors' reports, Sps-CuNPs had the highest biofilm inhibition against *S. aureus* MRSA and MSSA at 100 and 50 μg/mL, respectively, while the MIC value against *S. aureus* MRSA and MSSA was approximately 250 and 150 μg/mL, respectively. The anti-biofilm activity of Sps-CuNPs was related to the high affinity of copper ions to biomolecules, such as proteins and peptides throughout carboxyle, amine, and sulfide groups and its ability to disrupt the biofilm integrity.

Moreover, treatment with orthodontic resin contains anti-biofilm agents, which has increased in recent years. Dental plaque or biofilm is characterized as a community of microorganisms embedded in a matrix of polymers that develop on tooth surface (Zhang et al., 2020). These biofilms have high tolerance to antibiotics and provide poor antibiotic penetration (Pourhajibagher et al., 2021). In addition, dental acrylic resin containing seaweeds *U. pinnatifida* (Pourhajibagher et al., 2019) and *U. lactuca* (Pourhajibagher et al., 2021) exhibited dose-dependent anti-biofilm activity against *S. mutans*. The researchers revealed that *U. lactuca* in combination with antimicrobial photodynamic therapy (aPDT) technique lower the gene expression of gtfB, gtfC, and gtfD as the virulence factors in *S. mutans*, subsequently interfering in biofilm formation (Pourhajibagher et al., 2021). Sadek and Farrag fabricated the anti-biofilm nanocomposite by *S. muticum* extract containing Zn and CuO nanoparticles, which were able to reduce the biofilm formation and adhesion of *Proteus mirabilis*, *P. aeruginosa*, and *S. aureus*.

6 Conclusion

Several microbial infections are associated with the biofilm formation. A large number of microbial species tend to communicate with others, interact with their surrounding environment, and display multicellularity, resulting in biofilm formation. Communication of microbial cells is dependent on their cell density through quorum sensing signaling phenomenon mediated by the production and secretion of AIs. The involvement of quorum sensing signaling molecules in biofilm formation has been frequently reported, which is responsible for a variety of infections, food spoilage, biofouling, and biocorrosion. However, quorum sensing systems can be applied in expanding quorum sensing inhibitors as an alternative strategy in the inhibition of particular phenotypes expression blocking cell-to-cell communication causing to control the biofilm propagation and can be of great value in the future treatment of bacterial infections. Meanwhile, natural products are an interesting substance to combat with bacterial biofilm and related infections. The results obtained by numerous investigations performed over the last decade promoted the use of marine and algae extracts and their metabolites as anti-biofilm agents. Various algae extracts inhibited the bacterial adhesion, biofilm formation, and biosynthesis of quorum sensing key enzymes. All the above studies demonstrated that marine natural products possess anti-microbial and anti-biofilm activity toward a large number of pathogens.

Experimenting with a combination of extracts or biopolymers in silver nanoparticles or dental composites also provides anti-bacterial and anti-biofilm activities and is promising for the development of anti-biofilm novel formulations. However, some studies are needed to identify the role of algae substances in anti-biofilm attributes of silver nanoparticles and composites. Moreover, the effect of various microalgae and macroalgae extracts and their other metabolites, such as pigments, are recommended. In addition, *in vivo* studies and clinical trials are necessary for treating human infections.

Author contributions

AB and NO established this concept, searched the literature, and wrote the first draft of this manuscript. MM-N improved and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ARTICLES FOR FACULTY MEMBERS

ELUCIDATION OF ANTIBIOFILM FORMATION MECHANISMS USING MARINE EXTRACTS AGAINST INFECTION AND ANTIMICROBIAL RESISTANCE (AMR)

Antibiofilm activity of marine microbial natural products: Potential peptide- and polyketide-derived molecules from marine microbes toward targeting biofilm-forming pathogens / Sukmarini, L., Atikana, A., & Hertiani, T.

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REVIEW

Antibiofilm activity of marine microbial natural products: potential peptide- and polyketide-derived molecules from marine microbes toward targeting biofilm-forming pathogens

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Abstract

Controlling and treating biofilm-related infections is challenging because of the widespread presence of multidrug-resistant microbes. Biofilm, a naturally occurring matrix of microbial aggregates, has developed intricate and diverse resistance mechanisms against many currently used antibiotics. This poses a significant problem, especially for human health, including clinically chronic infectious diseases. Thus, there is an urgent need to search for and develop new and more effective antibiotics. As the marine environment is recognized as a promising reservoir of new biologically active molecules with potential pharmacological properties, marine natural products, particularly those of microbial origin, have emerged as a promising source of antibiofilm agents. Marine microbes represent an untapped source of secondary metabolites with antimicrobial activity. Furthermore, marine natural products, owing to their self-defense mechanisms and adaptation to harsh conditions, encompass a wide range of chemical compounds, including peptides and polyketides, which are primarily found in microbes. These molecules can be exploited to provide novel and unique structures for developing alternative antibiotics as effective antibiofilm agents. This review focuses on the possible antibiofilm mechanism of these marine microbial molecules against biofilm-forming pathogens. It provides an overview of biofilm development, its recalcitrant mode of action, strategies for the development of antibiofilm agents, and their assessments. The review also revisits some selected peptides and polyketides from marine microbes reported between 2016 and 2023, highlighting their moderate and considerable antibiofilm activities. Moreover, their antibiofilm mechanisms, such as adhesion modulation/inhibition targeting biofilm-forming pathogens, quorum sensing intervention and inhibition, and extracellular polymeric substance disruption, are highlighted herein.

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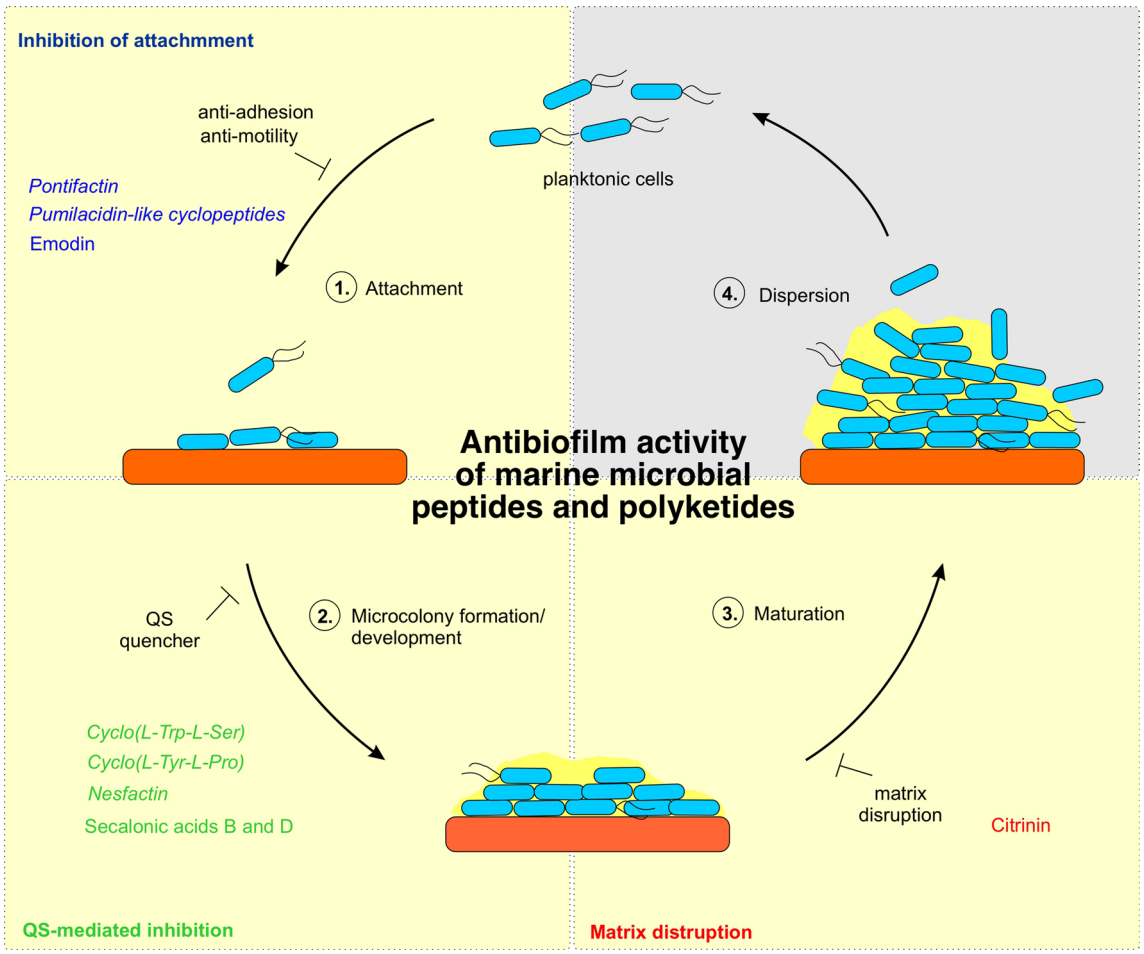
Graphical abstract



Keywords Biofilm infection · Antibiofilm mechanisms · Antibiofilm assessments · Marine microbial natural products · Peptides · Polyketides

Introduction

Nowadays, re-emerging infectious diseases remain a significant global healthcare challenge, particularly biofilm-mediated infections. These infections are often complicated by multidrug resistance (MDR), chronicity, tolerance, and persistent resistance to conventional antibiotics. Clinical biofilm infections are characterized by symptoms that persist despite repeated antibiotic therapy, often due to a weakened host immune or defense system. As a result of their adherence to contaminated surfaces and pathogenic properties, biofilm microbial colonies contribute to the occurrence of a majority of nosocomial and chronic infections, accounting for up to 65–80% of infections within the human body [1]. Microbes that are frequently involved in biofilm-associated infections include gram-positive pathogens such as *Staphylococcus epidermis*, *Staphylococcus aureus*, and other *Staphylococcus* species; gram-negative bacteria such as *Pseudomonas aeruginosa* and Enterobacteriaceae (e.g., *Escherichia coli*); and fungal pathogens such as *Candida albicans* [2, 3]. They can assemble on either implanted medical devices, such as contact lenses [4], joints and heart valve prostheses [5, 6], central venous and urinary catheters [7–10], and dental implants [11–14], or tissues, including those associated with gum periodontitis [15], bone osteomyelitis [16, 17], surgical sites, diabetes, and burn wounds [18–20]. Thus, biofilms have a profound impact on instrumental and surgical practices within healthcare, potentially leading to acute clinical complications and, in some cases, fatal consequences. Furthermore, they have broader implications for human health beyond device-related issues [21, 22].

A biofilm is a complex structure composed of bacterial (and other microbial) communities or aggregates of single or multi-/mixed-species cells (polymicrobial). These biofilms

often attach to both biotic and abiotic surfaces (e.g., soil and aquatic environments, tissue of living organisms, medical devices, and industrial facilities). This surface-attached or sessile microbial consortium is confined to a self-produced membrane structure made of extracellular polymeric substances (EPS). The polymeric matrix primarily consists of polysaccharides, proteins, nucleic acids (extracellular DNA [eDNA]), lipids, and lipopolysaccharides. Furthermore, this matrix is formed during growth, reproduction, and lysis [23, 24]. The biofilm state is commonly considered an adaptation to external stressors, promoting the metabolism, protection, and survival of microbes compared with their planktonic form in the environment. Biofilm formation can occur throughout the body. This naturally preferred and prevalent microbial lifestyle can have either beneficial or harmful effects. Biofilms have been extensively employed in biotechnological applications, such as biofuel production, surfactant synthesis, and the improvement or processing of certain foods [25]. However, they are also responsible for marine biofouling [26], metal corrosion [27], food contamination [28], and the aforementioned antimicrobial resistance and infectious diseases, particularly in a clinical setting [21].

Despite the significant health and economic burden posed by biofilm infections, no selectively approved antibiofilm drugs are currently available. Consequently, treatment options are limited, often necessitating aggressive clinical approaches, including the removal of implanted medical devices, surgical debridement, and long-term therapy involving high doses of multiple potent antibiotic combinations [29–31]. Standard or conventional antibiotic therapy can only eliminate planktonic cells, leaving sessile forms to proliferate within the biofilm and continue to disseminate or disperse once therapy is discontinued. Similarly, the host immune system struggles to resolve biofilm infections. Microbial biofilms release antigens and stimulate antibody production, but the microbes within biofilms resist these defense mechanisms. In fact, this immune response can even lead to damage to surrounding tissues. Compared with their planktonic counterparts, bacteria or microbes within biofilms exhibit significantly higher resistance (often exceeding 1000 times) to conventional antibiotics and host immune responses [21]. Hence, there is an urgent and essential need for extensive research in the quest for new effective anti-infective agents, especially novel antibiofilm compounds or strategies to inhibit pathogenic microbial biofilms. Such research could provide alternatives to currently available solutions and offer potential therapeutic options for the future.

The marine environment harbors an immense wealth of biodiversity, making marine natural products a promising source of bioactive compounds for lead compounds or drug candidates in biopharmaceuticals. These products constitute an untapped reservoir of bioactive compounds, including antimicrobial, antiviral, immunosuppressant, and cytotoxic/anticancer agents. They boast a diverse chemical repertoire encompassing fatty acids, terpenoids, peptides, and polyketides. Among these, bioactive secondary metabolites from microbes, including marine microbes, stand out as a primary source of potential anti-infective agents, particularly antibiotics [32–34]. Although antibiofilm bioactive compounds from marine microbes have not been as comprehensively examined as those from other sources (e.g., marine macroorganisms) [35], studies related to the potential of marine microbes as a source for producing antibiofilm compounds are ongoing. To date, several reviews have discussed natural products and their synthetic counterparts, including those from marine and microbial origins [36–44]. There is also an extensive body of literature, particularly focused on peptide-based natural products as promising antibiofilm agents, which has been evaluated and reviewed in other sources [43–50].

However, this review specifically highlights the potential of marine microbial peptides and polyketides as agents with antibiofilm activity against biofilm-forming pathogens. Notably, both molecules are prominent among marine natural products, known for their intricate and unique chemical structures. To create complex compounds with exotic chemical structures and enhanced biological properties, it is possible to modify them through a synthetic biology approach [51–54]. The concept of an emerging battlefield within microbial communities concerning biofilm development and the mechanisms of resistance and tolerance is discussed. Additionally, this review covers the strategies of antibiofilm agents, their screening methods and assessments, and the molecular mechanisms of potential marine microbial peptides and polyketides. These mechanisms include the disruption of EPS, quorum sensing (QS) intervention, and adhesion modulation targeting biofilm-forming pathogens.

For this review, a literature search was conducted to identify potent antibiofilm peptides and polyketides isolated from marine microbes in the past decade. We systematically examined databases such as PubMed and Scopus for relevant studies. The search was confined to studies published between 2013 and 2023, unless they contained crucial information. The primary findings from these studies were summarized. Some of the selected marine microbial peptides and polyketides reported between 2016 and 2023 are revisited. It is worth noting that their antibiofilm activity varies from moderate to considerable when compared with their promising synthetic antibiofilm analogs.

Target of antibiofilm agents: understanding the mechanisms of biofilm formation and recalcitrance, an overview

Biofilm formation

As a universal attribute shared by all microbes, especially bacteria, biofilm formation can be targeted by natural antibiofilm agents. Indeed, the high resistance of biofilms to antimicrobial agents becomes more understandable when we investigate their formation. The development of biofilms is a dynamic and intricate process, with molecular mechanisms that vary among different microbes. Essentially, biofilm formation comprises four typical stages, contingent upon the presence of a surface: initial attachment, microcolony formation, maturation, and dispersion (Fig. 1) [1, 28]. These stages are briefly discussed below.

In the initial stage, microbial cells attach to the surface both reversibly and irreversibly. This attachment is driven by hydrophobic interactions, van der Waals forces, steric interactions, and electronic interactions. During reversible attachment, microbes loosely adhere to the surface using appendages such as flagella and pili. This stage can easily transition back to the original planktonic state because it has not yet undergone the series of morphological transformations associated with differentiation. However, adhesion to abiotic surfaces is influenced by various factors in the microbial environment, including temperature, organic matter, and pH [55]. When environmental factors are altered, microbial cells secrete an extracellular matrix (EPS) through the regulation of gene expression and signaling molecules. This promotes adhesion to surfaces and leads to an irreversible attachment state. Furthermore, during this stage, the synthesis of flagella, which govern microbial cell motility, is inhibited. Serving as a multifunctional scaffold, the EPS matrix both promotes and protects the embedded microbes, creating a heterogeneous chemical and physical environment and facilitating the distribution of nutrients to resident microbes [24]. In the reversible attachment stage, the amount of EPS reaches a certain threshold, leading to a strong interaction between microbial cells and the surface. After irreversible attachment, EPS can be attached to by various microbial cells and replicated to form microcolonies—the primary unit of biofilm structure. QS primarily occurs during the microcolony formation stage, as this process of cell–cell communication enables microbes to release specific signals called autoinducers (AIs) in a population density-dependent manner. The QS mechanism involves AIs binding to response regulators and regulating multiple genes, including those responsible for biofilm development and bacterial virulence factors. *N*-acyl-homoserine lactones (AHLs), autoinducing peptides, and AI-2-based signaling molecules act as AI signal molecules synthesized by gram-negative bacteria (e.g., pathogenic *E. coli*

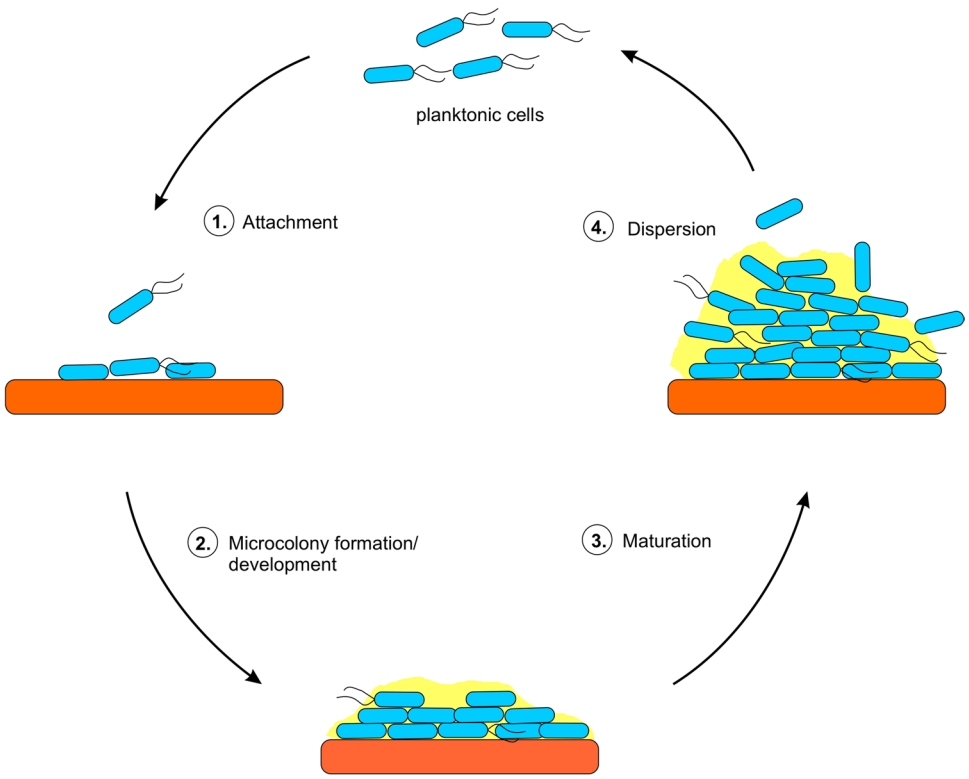


Fig. 1 Biofilm formation life cycle

and *P. aeruginosa*), gram-positive bacteria (e.g., pathogenic *S. aureus*), and both types of bacteria, respectively [56]. These initial phases, namely, initial attachment and microcolony formation, are pivotal in the development of biofilms, and targeting one or both of these steps appears to be the optimal strategy for inhibiting biofilm formation.

Moreover, the attachment of small colonies or microcolonies leads to the growth of mature biofilms with intricate three-dimensional structures. These mature biofilms feature specialized water channels and pores that facilitate the flow of nutrients and waste through the biofilm [57]. Additionally, the stability of the biofilm is reinforced by certain adhesive factors, including accumulation-associated proteins, eDNA, and polysaccharide intercellular adhesion [28]. For example, biofilm-associated proteins in *S. aureus* contribute to the formation of functional amyloids within the matrix scaffolds, enhancing the structural stability of biofilms and protecting the enclosed bacteria [58]. Mature biofilms, with their increased pathogenicity and resistance to antimicrobial agents, are challenging to disrupt, contact, or remove microbes from. Biofilm detachment often leads to infections. In the final stage of dispersion or detachment, some sessile cells are released from the biofilm surface. These cells can reattach to the existing biofilm or to other interfaces, forming new biofilms and potentially contaminating or spreading to additional surfaces, thus perpetuating the cycle. Factors that promote detachment include catabolite repression, nutrient limitation, and matrix-degrading enzymes, such as proteases. The nucleotide second messenger cyclic-di-GMP, one of the most extensively studied intracellular signaling molecules, regulates biofilm formation and dispersal by affecting flagellar motility, adhesion, and the generation of EPS [59, 60]. This final stage is considered a key driving factor in the spread of pathogens.

Biofilm-specific antibiotic recalcitrance

Biofilm formation allows pathogenic strains with complex drug resistance mechanisms to develop survival and defense strategies. Essentially, microbial biofilms resist antibiotic treatment through a combination of genetically encoded antibiotic resistance mechanisms and a reversible phenotype that imparts drug tolerance [30, 61–65]. Resistant microbes continue to grow despite the presence of antimicrobial agents that would typically inhibit their growth, whereas tolerant microbes can survive without growth or death in the presence of bactericidal antimicrobial agents. Therefore, multiple resistance and tolerance mechanisms work together, contributing to an overall increase in resistance against antimicrobial challenges. It is likely that no single mechanism can fully account for biofilm resistance/tolerance [61, 66]. Various mechanisms collectively confer a high level of resistance to antibiotics, a hallmark of biofilms. More detailed and comprehensive explanations of these mechanisms have been extensively reviewed elsewhere [63, 64, 67, 68]. Consequently, the discussion in this review aims to be concise and includes factors such as physical resistance, physiological cell status, QS, upregulation of efflux pumps, and stress-induced mutagenesis. An in-depth understanding of the molecular mechanisms involved in biofilm formation and subsequent antibiotic resistance and tolerance is crucial for developing preventive measures and effective therapies.

The physical resistance of biofilms is substantially influenced by the EPS within the biofilm matrix, which reduce the penetration of antimicrobials. The EPS acts as a physical barrier, limiting the entry and diffusion of specific antimicrobials. If the time required for an antibiotic to penetrate is longer than the duration of antibiotic therapy, slower penetration can explain antibiotic resistance. Antibiotics can bind to the anionic polysaccharides present in the EPS matrix. For instance, in *P. aeruginosa*, exopolysaccharides such as alginate, Psl, and Pel may bind to cationic antibiotics such as aminoglycosides, delaying or impeding their diffusion. Intriguingly, the eDNA matrix can bind to cationic antimicrobials, including aminoglycosides and antimicrobial peptides, creating a defensive or protective shield effect by chelating cations. According to Jennings et al. [69], the antibiotic resistance mechanisms of Pel and eDNA might be interlinked in *P. aeruginosa*. Poly-*N*-acetylglucosamine (in *S. aureus*) and α-mannans and β-glucans (in *C. albicans*) are other important examples of protective polysaccharides in biofilms [24].

The physiological status of cells in different microenvironments, such as dormant cells, is another underlying cause of biofilm-associated resistance and tolerance. Within biofilms, there exists a small, reversible subpopulation known as persister cells. These cells enter a slow- or non-growing, dormant state characterized by reduced metabolism without undergoing genetic changes. They exhibit a high tolerance to extracellular stressors, including antibiotic treatment. Because antibiotics primarily target factors related to growth, they are often less effective against the slow or dormant state of persister cells, contributing to increased resistance within biofilms. The formation of persister cells and their survival in fluctuating environmental conditions are believed to be regulated by several complex mechanisms. These mechanisms include toxin–antitoxin systems, alternative energy production, the SOS response (a global response to DNA damage), and stringent responses [70, 71]. Moreover, persister cells are highly resistant to the bactericidal effects of antibiotics but can return to a normal growth state once replication resumes under specific conditions. Thus, persistence can be considered a form of adaptive resistance associated with phenotypic variation [72]. A model *S. epidermidis* biofilm has demonstrated the importance of persister cells in conferring tolerance to antibiotics [73].

Although not a resistance mechanism in itself, cell–cell communication or QS plays a vital role in the development, formation, and functioning of biofilm communities, supporting community-level protective mechanisms. QS has been implicated in the recalcitrance of biofilms to antimicrobials. For example, the QS system has been linked to the tolerance or resistance of *P. aeruginosa* biofilms to tobramycin (*lasR* and *rhlR* systems) [74] and colistin or polymyxin E [75], *S. aureus* biofilms to rifampicin (*agr* system) [76], and *Enterococcus faecalis* biofilms to gentamicin, daptomycin, and linezolid (*fsrA*, *fsrC*, and *gelE* systems) [77]. Biofilm tolerance appears to be promoted by QS through the release of eDNA matrix via 2-n-heptyl-4-hydroxyquinoline-N-oxide mediated autolysis, as demonstrated in *P. aeruginosa* [78].

In addition to the presence of intrinsic resistance genes in certain pathogenic strains, changes in gene expression, such as the upregulation of efflux pump genes and mutagenesis, render microbes within biofilms more susceptible to drug resistance. Chromosomally encoded efflux pumps play a significant role in the intrinsic resistance of bacteria by actively transporting various antimicrobials into the extracellular space. Some of these efflux pumps belong to the category of MDR efflux pumps [79]. For example, the upregulation of MexAB-OprM and MexEF-OprN efflux pumps regulated by the transcriptional regulator BlrR in pathogenic *P. aeruginosa* biofilms has resulted in MDR to antibiotics such as tobramycin, norfloxacin, trimethoprim, tetracycline, kanamycin [80], and colistin [81]. Additionally, increased mutagenesis and a higher frequency of mutations can be induced by adaptive mutagenesis owing to the activation of stress responses during the persistence state, as mentioned earlier (e.g., SOS, oxidative stress, stringent response, or reactive oxygen species [ROS]). Rojo-Molinero et al. [82] demonstrated that the resistance observed in mutant *P. aeruginosa* populations, resulting from the overexpression of AmpC-β-lactamase or MexAB-OprM efflux pumps, provided protection to the entire bacterial biofilm, thereby preserving susceptible populations from the action of antibiotics. Thus, this mixed population of resistant and sensitive pathogenic bacteria suggests that genetically resistant mutants are localized in the surface layer of biofilms, shielding the rest of the biofilm population from antibiotics. Interestingly, the close proximity of cells within biofilms may also facilitate horizontal gene transfer, including the transfer of antimicrobial resistance genes [63, 64]. Furthermore, the presence of hydrolysis and degradation enzymes, as well as active-site metal-based chelating agents, can lead to the chemical inactivation or degradation of antibiotics [83].

Assessment of potential antibiofilm agents: a general assay system for evaluating antibiofilm activity

Discovering compounds that specifically target bacteria growing in biofilms indeed requires screening and test systems to assess their capacity for biofilm inhibition and eradication. Unlike the standard minimal inhibitory concentration (MIC) assays, which are universally used to test antimicrobial activity against planktonic cells, standardizing the number of microbial cells within a biofilm presents greater challenges. This difficulty arises not only from the heterogeneous and complex composition of biofilms but also from variations in their growth conditions and environmental influences. However, numerous antibiofilm screening assays are available and under development [84–89]. In brief, both in vitro and in vivo biofilm model systems can help determine the susceptibility of antimicrobial agents against biofilm-forming microbes, providing valuable insight into biofilm mechanisms.

In general, in vitro biofilm models can be categorized into three types: static (closed or without a nutrient supply system), dynamic (open or with a continuous nutrient supply system), and tissue culture-based models. Depending on the test system used, antibiofilm activity can be assessed in various ways, including (1) a total biomass-based assessment using methods such as crystal violet to evaluate eradication activity and (2) a viability-based assessment involving metabolism (using tetrazolium, resazurin, and fluorescent tags), cell integrity, and the ability to grow on artificial media (using colony-forming unit counts to determine the minimum eradication concentration of biofilms) to evaluate killing activity. However, when assessing antibiofilm activity, a combined evaluation of both killing and eradication is often advantageous. Moreover, these assays have been extensively described elsewhere [84, 89–93].

Microtiter plate assays are among the most extensively used in vitro model systems. In these assays, microbial biofilms grow on the bottom walls of a microtiter plate or on materials (e.g., microscopic slides, silicone, titanium, and hydroxyapatite disks) placed within a microtiter plate [94]. This assay can also aid in identifying factors involved in biofilm initiation, such as adhesins, pili, flagella, genes related to EPS production, and enzymes responsible for cGMP [93]. Recently, Hancock et al. improved the microtiter method and proposed its standardization by optimizing growth conditions for each bacterial strain and dye staining options [85, 86]. This highly useful high-throughput screening model offers a simple, cost-effective, and reproducible way to assess the biofilm inhibition and eradication abilities of antibiofilm compounds using common and

relatively inexpensive materials. Furthermore, various dynamic models replicate specific in vivo environments more closely, including those in human hosts and medical devices. However, these models come with higher costs and increased complexity in usage. One such model is a microfluidic system with a Brimor chip [95]. Additionally, tissue culture-based models allow for co-cultures of bacterial and human cells, with biofilms grown over a monolayer of host cells submerged in a medium. Although these submerged models may lack the complexity of cell types and may only mimic acute infections, recent innovations in tissue culture techniques, such as the development of host organoid (organ-like) systems, have mitigated these limitations, providing a more accurate representation of host–pathogen interactions within their in vivo microenvironment [96]. This tissue culture-based model is cost-effective, easily manipulated, highly reproducible, and amenable to high-throughput screening when compared to in vivo models. In vivo models involve the use of living organisms, including mammals (e.g., mice, rats, and rabbits) and nonmammals (e.g., nematodes and fruit flies). Nonmammalian models are often used in the initial stages of candidate selection before moving on to more expensive, labor-intensive, and ethically restricted mammalian models [93, 97].

Potential antibiofilm activity of marine natural products: antibiofilm mechanisms of marine microbial peptides and polyketides

According to Melander et al. [39], many natural products can serve as a foundation for the development of antibiofilm compounds, and alternative methods to eradicating biofilms include identifying and developing compounds that specifically target biofilm cells. These compounds typically act through one or more of the following approaches: (1) inhibition of biofilm formation (with minimal or even no toxicity toward planktonic microbial pathogens), (2) dispersion of biofilms, and (3) killing of biofilm cells (either selectively or in addition to planktonic toxicity). As mentioned above, the fundamental stages in biofilm formation have become critical targets for antibiofilm agents, particularly those inspired by nature. Furthermore, the proposed mechanisms of action for the antibiofilm activity of recently reported marine microbial peptides and polyketides may generally include the inhibition of microbial adhesion, interference with signal molecules modulating biofilm development or QS inhibition, and disruption of the EPS matrix (Table 1). Additionally, a few synthetic antibiofilm peptides and/or polyketides designed to target these mechanisms, if available or reported, are also be included for comparison.

Inhibition of microbial attachment to surfaces and colonization

Blocking the initial microbial adhesion is an effective method because it can hinder early biofilm colonization and subsequent infections caused by planktonic cells released from the biofilm. Without the critical step of surface binding, the cxsinitial colonization cannot occur. Marine microbial lipopeptide biosurfactants, such as pontifactin and pumilacidin-like cyclopeptides (Fig. 2), along with the polyketide-derived anthraquinone emodin (Fig. 3), have demonstrated antibiofilm activity by preventing pathogenic strains from attaching to the substrate, as discussed below.

Pontifactin, a lipopeptide biosurfactant, is produced by the marine bacterium *Pontibacter korlensis* strain SBK-47. The antibiofilm activity of this nonribosomal peptide antimicrobial was evaluated based on its inhibitory effects on adhesion. The activity exhibited significant concentration-dependent growth inhibition against various biofilm-forming bacterial strains. The highest antiadhesive activity, with 99% inhibition, was observed at a maximum concentration of 2 mg mL^{-1} of the compound against *Bacillus subtilis* MTCC 619, *S. aureus* MTCC 96, and *Vibrio cholerae* MTCC 3906. Conversely, bacterial growth inhibition rates ranged from 29 to 82%, indicating that this peptide could inhibit bacterial attachment through a nonbactericidal mechanism as it did not completely eliminate the bacteria [98]. Moreover, as inhibitors of microbial adhesion, biosurfactants may hinder biofilm formation by altering cell adhesion properties, including reducing bacterial cell surface hydrophobicity, as demonstrated by the surface tension reduction property of pontifactin. Owing to the amphipathic nature of their peptide structures and fatty acid moieties, lipopeptides are widely recognized as biosurfactants with antimicrobial and antiadhesive activity against pathogens [98–100]. This fatty acid or lipid moiety is introduced at the beginning of the biosynthesis of a multifunctional modular enzyme complex-based nonribosomal peptide [101]. Interestingly, the antiadhesive function of antibiofilm activity has also been demonstrated in a synthetic lipopeptide analog of battacin. Battacin is a cyclic lipopeptide (CLP) synthesized by the soil bacterium *Paneibacillus tianmunesis*. During a structure–activity relationship study of a series of battacin analogs, the chemically synthesized linearized lipopeptide battacin, named GZ3.27, prevented the attachment and eradicated biofilms against not only the plant pathogen *Pseudomonas syringae* pv. actinidiae but also the human pathogens *P. aeruginosa* (at concentrations of 10–50 μM) and *S. aureus* (at concentrations of 10–25 μM). Thus, linearizing a CLP could enhance antibiofilm activity [102]. Notably, a further cysteinylated linear lipopeptide battacin immobilized on glass, silicon, and titanium surfaces also successfully prevented *E. coli* and *P. aeruginosa* biofilm colonization, showing 98.6–99.9%

Table 1 Marine microbial peptides and polyketides with their antibiofilm activity

Mechanism of action/target inhibition	Compound	Biosynthetic class (NRPS/PKS)	Assay	Source	Target pathogenic/reference strains and biofilm inhibitory effect	References
Inhibition of microbial attachment to the surface						
Anti-adhesion	Pontifactin	Lipopeptide/NRPS	Microtiter plate, crystal violet staining	Bacterium *Pontibacter korlensis* SBK-47 (seawater)	*Bacillus subtilis* MTCC 619 *Staphylococcus aureus* MTCC 96 *Vibrio cholerae* MTCC3906 99% (2 mg mL $^{-1}$)	[98]
Anti-motility and anti-adhesion	Pumilacidin-like cyclopeptides	Lipopeptide/NRPS	Microtiter plate, crystal violet	Bacterium *Bacillus* sp. 176 (seamount)	*Pseudomonas aeruginosa B. subtilis* > 50% (0.3 mg mL^{-1})	[105]
Van der Walls interaction-mediated inhibition effect of adhesion	Emodin	PKS type II	Microtiter plate, crystal violet	Fungal symbiont *Penicillium* sp. SCSGAF 0023 (marine gorgonian coral *Dichotella gemmacea*)	*Staphylococcus aureus* ATCC 6358 > 50% (12.5 μg mL^{-1})	[110]
QS sensing inhibition						
QS-mediated pathogenicity inhibition effects of virulence factors, such as pyocyanin, elastase, and proteases; Down regulation of QS expression genes, including *la*I, *las*R, *rhl*I, *rhal*R	Cyclo (L-Trp-L-Ser)	Cyclic dipeptide/NRPS	Microtiter plate, crystal violet staining	Bacterium *Rheinheimera aquimaris* QSI02 (seawater)	*Pseudomonas aeruginosa* PAO1, 59.9% (0.2 mg mL^{-1})	[119]
QS-mediated pathogenicity inhibition effects of virulence factors, such as pyocyanin, elastase, and proteases; Down regulation of QS expression genes, including *la*I, *las*R, *rhl*I, *rhal*R	Cyclo (L-Tyr-L-Pro)	Cyclic dipeptide/NRPS	Microtiter plate, crystal violet staining	Fungus *Penicillium chrysogenum* DXY-1 (marine sediment)	*P. aeruginosa* PAO1, 48% (0.5 mg mL^{-1})	[120]

Table 1 (continued)

Mechanism of action/target inhibition	Compound	Biosynthetic class (NRPS/PKS)	Assay	Source	Target pathogenic/reference strains and biofilm inhibitory effect	References
QS-mediated pathogenicity inhibition effects on virulence factors, such as hemolysin, protease, lipase, phospholipase, esterase, elastase, rhamnolipid, alginate, and pyocyanin; Down regulation of QS expression of transcriptional QS gene agr, biofilm formation *isa*A, intracellular adhesion-related genes *ica*A, *icd*D	Nesfactin	Lipopeptide/NRPS	Confocal image analysis*;microtiter plate, crystal violet staining**	Bacterial symbiont *Nesterenkonia* sp. MSA31 (sponge *Fasciospongia cavernosa*)	*P. aeruginosa* FSPA09, 90% (75 µg mL^{-1})*; 0.1 mg mL^{-1}**	[123]
Regulates the expression of transcriptional QS gene *agr*, biofilm formation *isa*A, intracellular adhesion-related genes *ica*A, *icd*D	Secalonic acids D and B	PKS	Microtiter plate, crystal violet staining	Fungal symbiont *Penicillium* sp. SCSGAF 0023 (gorgonian coral *Dichotella gemmacea*	*Staphylococcus aureus* ATCC 6358, up to 97% (6.25 µg mL^{-1})	[110]
EPS matrix disruption						
Redox properties of molecules seem to play an important role in dispersion of the biofilm	Citrinin	PKS type I (iterative non reducing)	Microtiter plate, crystal violet staining	Fungal symbiont *Penicillium* sp. SCSGAF 0023 (gorgonian coral *Dichotella gemmacea*)	*S. aureus* ATCC 6358, 50% (12.5 µg mL^{-1})	[110]
Uncategorized/unknown yet						
None	Epicotripeptin	Cyclic tripeptide/NRPS	Microtiter plate, crystal violet staining	Fungal symbiont *Epicoccum nigrum* M13 (seagrass *Thalassia hemprichii*)	*Bacillus subtilis* ATCC6633, *S. aureus* NRRLB-767 100 µg mL^{-1}, moderate biofilm inhibitory activity against the tested Gram-positive strains (55–70% inhibition)	[134]
None	Erubescensoic acid	PKS	Microtiter plate, crystal violet staining	Fungal symbiont *Penicillium erubescens* KUFA 0220 (sponge *Neopetrosia* sp.)	*Eschericia coli* ATCC 25922 (moderate inhibition at 64 mg mL^{-1}, based on the adherence capabilities)	[135]
None	Sattahipmycin	PKS type II	Microtiter plate, crystal violet staining	Actinobacterium *Streptomyces* sp. GKU 257–1 (marine sediment)	*E. coli* NBRC 3927, 50% (15–60 µg mL^{1} with very low bactericidal effect of > 250 µg mL^{-1})	[136]

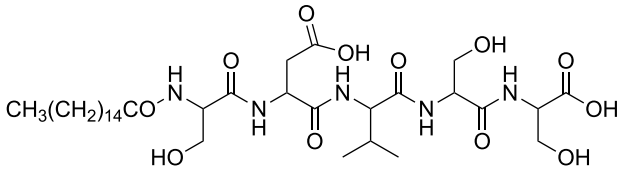
* refers to confocal image analysis

** refers to crystal violet staining

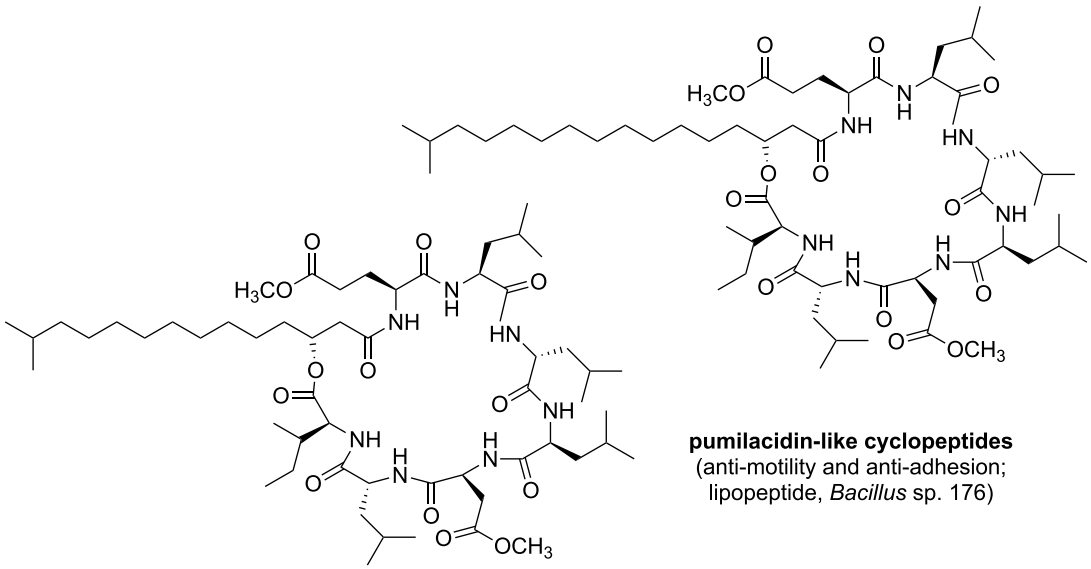
inhibition. This synthetic lipopeptide was also nonhemolytic to mouse blood cells, suggesting its promise for development as an antimicrobial coating on medical implants [103]. In addition to the lipopeptide analog, a moronecidin-like peptide is another synthetic antimicrobial peptide that has demonstrated inhibition of bacterial attachment. Moronecidin is a marine-derived antimicrobial peptide isolated from hybrid striped bass (fish). The synthetic moronecidin-like peptide was designed based on the conserved signal peptide of moronecidin using the expressed sequence tag database. With more positive charges, the moronecidin-like peptide significantly inhibited *S. aureus* ATCC 25923 attachment to surfaces at 1/16 MIC, making it more potent than natural moronecidin. Moreover, it exhibited low hemolytic activity against human red cells and cytotoxic activity against human embryonic kidneys, suggesting its potential as a novel antibiofilm agent and a template for designing new antibiotics [104].

In addition to their antiadhesive activity, Xiu et al. [105] discovered two pumilacidin-like CLPs originating from the marine bacterium *Bacillus* sp. 176 that significantly inhibited the motility of the biofilm-forming pathogen *V. alginolyticus* 178. This bacterium is a leading cause of vibriosis, which can lead to opportunistic human infections associated with raw seafood contamination. These nonribosomal CLPs (CLP1 and CLP2) differ by only one methylene group, while their distinction lies in the fatty acid chains. They share structural similarities with previously reported pumilacidins derived from endophytic bacteria in cassava [106]. *Bacillus* sp. 176, the producer of these CLPs, was isolated from the same environment as *V. alginolyticus* 178 and served as a competing bacterium, remarkably inhibiting the motility of *V. alginolyticus* 178 and reducing its attachment ability, thereby preventing biofilm formation. Interestingly, cell aggregation assays revealed that these CLPs also effectively reduced biofilm formation in *P. aeruginosa* and *B. subtilis*, in addition to *V. alginolyticus* 178, without killing the bacteria when treated at 0.3 mg mL^{-1}. These peptides downregulated the flagellar assembly genes *flgA* and *flgP* in *V. alginolyticus* 178, which are essential for motility, flagellar stability, and influence attachment and colonization [105]. Another study revealed that motility was suppressed by targeting the Na^{+}-stimulated flagellar motor component, namely MotX. Homologs of MotX were prevalent and highly conserved in various pathogenic species, suggesting the extensive potential application of CLPs as alternative antibiotics targeting bacterial motility in many biofilm-forming pathogens [107]. Moreover, significant reductions in motility were also demonstrated by the K6 peptide, a synthetic peptide designed based on the residue composition of the natural CLP polymyxin. K6 is a synthetic 9-mer peptide with a triple tryptophan motif and the ability to form nanostructured micelles. The self-assembly of K6 into nanostructured micelles was attributed to its strong inhibition of de novo biofilm formation in *P. aeruginosa* at sub-MIC concentrations of 8–16 µM, demonstrating its ability to disperse biofilms more efficiently than gentamicin by attacking the EPS matrix. This synthetic peptide also significantly decreased all motility behaviors, such as swimming, swarming, and twitching, at similar concentrations, suggesting the disruption of flagella and type IV pilus-dependent movement of *P. aeruginosa* cells. Interestingly, K6 displayed clear mixed polymicrobial *P. aeruginosa–S. aureus* infection in an in vivo mouse model of a biofilm-associated infection [108]. Similarly, the synthetic 9-mer peptide 1037, based on the human cathelicidin LL-37, directly inhibited *P. aeruginosa* biofilm growth by reducing swimming and swarming motilities, stimulating twitching motility, and suppressing the expression of genes involved in biofilm formation, including *flgB*, *flgC*, *flgD*, and *flgF*. This synthetic peptide inhibited biofilm formation by 50% at 10 and 20 µg mL^{-1} in static and dynamic flow cell assays, respectively [109].

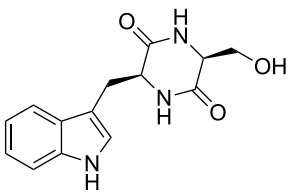
Furthermore, an anthraquinone compound, emodin, isolated from the marine gorgonian coral *Dichotella gemmacea*-associated fungus *Penicillium* sp. SCSGAF 0023 via a polyketide synthase (PKS) II pathway has exhibited potent antibiofilm activity at 12.5 µg mL^{-1}, resulting in an inhibition rate of > 50% against *S. aureus* ATCC 6538 [110, 111]. Similarly, emodin derived from plant secondary metabolites has demonstrated reduced biofilm formation in *S. aureus* MSSA/ATCC 6538 by > 70% at 10 µg mL^{-1} [112]. Furthermore, emodin has demonstrated in vitro inhibition of biofilm formation in *Streptococcus mutans*, the pathogenic bacteria responsible for dental caries, by nearly 90% at 5 µg mL^{-1} [113]. Emodin achieves this by preventing the adhesion of sessile cells, hindering biofilm formation, and causing membrane disruption in pathogenic cells. This aromatic polyketide can penetrate phospholipid bilayers, strongly influencing van der Waals interactions between hydrocarbon chains of phospholipids, destabilizing membrane bilayers by promoting nonbilayer phases, and disrupting the fluidity of cell membranes [113, 114]. Another anthraquinone derivative, aloe-emodin, has also exhibited inhibition activity against *S. aureus* biofilm, particularly at the initial attachment stage of biofilm development. It achieves this by inhibiting the generation of extracellular proteins and the accumulation of polysaccharide intracellular adhesin on the cell surface [115]. Similarly, a synthetic peptide, P1, derived from an antifreeze protein reduced biofilm formation in *S. mutans* by 75% at 25 µg mL^{-1} and that of *S. oralis* and *S. aureus* by 25–80% at 5 µg mL^{-1}. Notably, the P1 peptide can bind directly to *S. mutans* without bactericidal activity, leading to irregular biofilms with disconnected cell aggregates and an easily detachable EPS matrix. Being positively charged, P1 appears to affect interactions between eDNA and EPS in *S. mutans*, altering the surface cell charge to hinder bonding



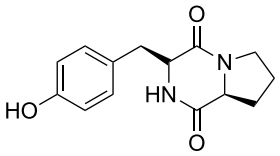
pontifactin
(anti-adhesion; lipopeptide, *Pontibacter korlensis* SBK-47)



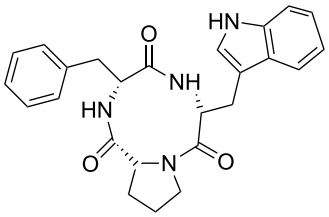
pumilacidin-like cyclopeptides
(anti-motility and anti-adhesion; lipopeptide, *Bacillus* sp. 176)



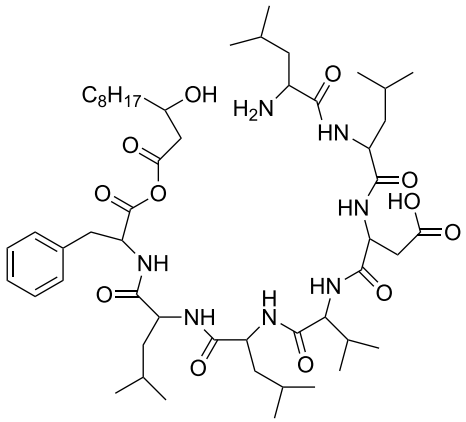
cyclo (L-Trp-L-Ser)
(QS inhibitor; cyclodipeptide, *Rheinheimera aquimaris* QSI02)



cyclo (L-Tyr-L-Pro)
(QS inhibitor; cyclodipeptide, *Penicillium chrysogenum* DXY-1)

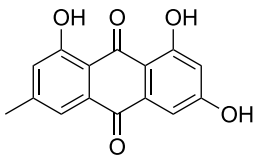


epicotripeptin
(cyclic tripeptide, *Epicoccum nigrum* M13)

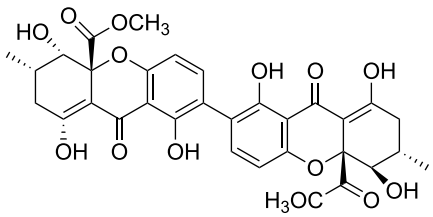


nesfactin
(QS inhibitor; lipopeptide, *Nesterenkonia* sp. MSA31)

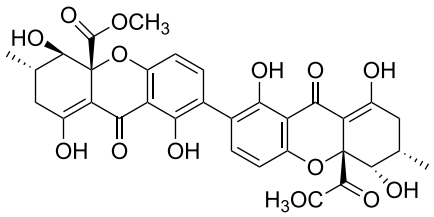
Fig. 2 Marine microbial peptides with antibiofilm activity



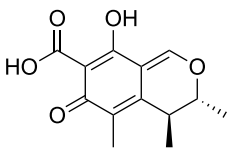
emodin
(van der Walls interaction-mediated of adhesion; polyketide, *Penicillium* sp. SCSSGAF0023)



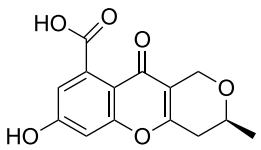
secalonic acid D
(polyketide, *Penicillium* SCSGAF 0023)



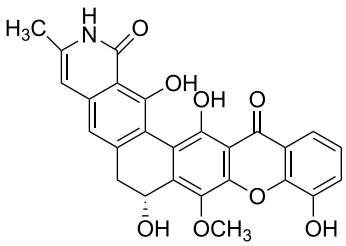
secalonic acid B
(polyketide, *Penicillium* SCSGAF 0023)



citrinin
(biofilm dispersion; polyketide, *Penicillium* sp. SCSGAF0023)



erubescencoic acid
(polyketide, *Penicillium erubescens* KUFA 0220)



sattahipmycin
(polyketide, *Streptomyces* sp. GKU 257-1)

Fig. 3 Marine microbial polyketides with antibiofilm activity

and disrupt stability. Ultimately, this prevents bacterial attachment to surfaces and the connection between microcolonies in the biofilm [116].

QS inhibition or interference with signal molecules modulating biofilm development

Unlike traditional antibiotics that suppress or kill microbial growth, QS inhibitors or quenchers can impede bacterial infection by specifically targeting QS-regulated

pathogenicity, including the expression of virulence factors, biofilm development, migration, and secretion regulation. Inhibiting QS presents an intriguing and innovative approach to combat microbial infections without accelerating MDR. Therefore, targeting QS can be an effective strategy to restrain biofilm formation [38, 56]. Some recently reported QS quenchers from marine microbial peptides and polyketides include cyclodipeptides, nesfactin (Fig. 2), and secalonic acids B and D (Fig. 3).

As the smallest cyclic peptides, cyclodipeptide diketopiperazines (DKPs) are recognized as a group of QS inhibitors that influence cell–cell signaling and represent a novel class of QS signal molecules [117]. This DKP group can be formed as truncated side products by nonribosomal peptide synthetases (NRPS) during the synthesis of longer peptides and is widespread in bacteria and fungi [118]. Two marine microbial cyclodipeptides, one is the cyclo (L-Trp-L-Ser) from a gram-negative bacterium and the other is the cyclo (L-Tyr-L-Pro) from a fungus, have been identified as QS inhibitors that affect the bacterial QS system in *P. aeruginosa*; both of these compounds were investigated by Zhu et al. [119, 120]. The QS system in pathogenic *P. aeruginosa* regulates biofilm formation and the production of virulence factors, including pyocyanin, elastase, and protease. The biofilm secreted by *P. aeruginosa* is a highly complex matrix formed on the surface of bacteria and is regulated by multiple genes. At least three QS signaling systems have been reported in *P. aeruginosa*. The Las and Rhl systems are acyl-homoserine lactone (AHL)-dependent systems, utilizing the *N*-3-oxo-dodecanoyl-homoserine lactone (3-oxo-C_{12}HSL) and *N*-butanoyl-homoserine lactone (C_4HSL) signal molecules, whereas the *Pseudomonas quinolone signal* (PQS) system employs 2-heptyl-3-hydroxy-4-quinolone as a signaling molecule. Because the QS system is organized hierarchically, the Las system occupies the top position in this signaling pathway and modulates both the Rhl and quinolone signaling systems. The Las system comprises the transcriptional activator LasR and the AHL synthase LasI, which regulate the synthesis of the 3-oxo-C_{12}HSL signal molecule—a virulence determinant—and the production of virulence factors, such as elastase and proteases. The Rhl system includes the transcriptional activator RhlR and the synthase RhlI, which oversee C_4HSL synthesis and the production of rhamnolipids, elastase, pyocyanin, and siderophores. These systems are further influenced by PQS, increasing the complexity of the QS network [121, 122].

In the first study, cyclo (L-Trp-L-Ser), isolated from the marine gram-negative bacterium *Rheinheimera aquimaris* QSI02, was found to exhibit anti-QS activity against *Chromobacterium violaceum* CV026 and *P. aeruginosa* PA01. This cyclodipeptide suppressed *P. aeruginosa* PA01 biofilms by 59.9% at 0.2 mg mL^{-1} and simultaneously inhibited the production of the pathogen's virulence factors, pyocyanin and elastase, by 65% and 40%, respectively [119]. In silico molecular docking and molecular dynamic investigations have suggested that cyclo (L-Trp-L-Ser) likely exerts QS inhibition activity by destabilizing the LasR receptor due to the absence of the long side chain in the LasR-mediated QS system of *P. aeruginosa* [119].

Furthermore, another cyclodipeptide, DKP cyclo (L-Tyr-L-Pro), isolated from the marine fungus *Penicillium chrysogenum* DXY − 1, was identified. Similar to its anti-QS activity against *Chromobacterium violaceum* CV026 and *P. aeruginosa* PA01, cyclo (L-Tyr-L-Pro) also exhibited antibiofilm activity against *P. aeruginosa* PA01 [120]. The peptide reduced biofilm formation in *P. aeruginosa* PA01 to 48% at 0.5 mg mL^{-1}. Additionally, it decreased the QS-mediated pyocyanin production, protease activity, and elastase activity of *P. aeruginosa* PA01 by 41%, 20%, and 32%, respectively, at the same sub-minimum inhibitory concentration. Moreover, the QS-related gene expression levels of *P. aeruginosa* PA01, including *lasI*, *lasR*, *rhlI*, and *rhlR*, were considerably reduced by 43%, 46%, 54%, and 64%, respectively. In silico molecular docking results indicated that this cyclodipeptide could obstruct the effect of the QS AI or natural ligand through competitive binding to the same pocket of the receptor protein LasR of *P. aeruginosa*, leading to the inhibition of the QS system [120].

Lipopeptide nesfactin is another recent marine microbial-derived QS quencher for *P. aeruginosa* biofilms. This NRPS-derived molecule was isolated from the marine sponge *Fasciospongia cavernosa*-associated bacterium *Nesterenkonia* sp. MSA31. Nesfactin inhibited biofilm formation in MDR *P. aeruginosa* FSPA02 by 90% at 75 μg mL^{-1} without inhibiting cell growth, as confirmed via confocal image analysis, and at 0.1 mg mL^{-1} based on a microtiter plate-based assay. Additionally, AHL molecules C4HSL and 3-oxo-C12HSL were degraded at 50 μg mL^{-1}. The nesfactin-coated catheter also demonstrated a significant reduction in biofilm formation. Further investigations revealed that this linear peptide effectively inhibited QS-mediated virulence factors (hemolysin, protease, (phospho)lipase, esterase, elastase, rhamnolipid, alginate, and pyocyanin) and motility [123, 124]. The binding of nesfactin to the receptor proteins RhlB and AlgR, rather than LasR, appeared to play an important role in QS inhibition [124].

Moreover, secalonic acid D and its epimer, secalonic acid B, demonstrated a QS-mediated biofilm inhibitory effect on other pathogens, such as *S. aureus*. These PKS-based compounds were isolated from the aforementioned marine-derived fungus, *Penicillium* sp. SCSGAF 0023. These compounds inhibited *S. aureus* ATCC 6358 biofilm formation by > 90% at 6.25 μg mL^{-1} without reducing cell growth [110]. Secalonic acid D regulates the transcriptional expression of several genes, such as *agr* (the QS gene), *isaA*, *icaA*, and *icaD* (biofilm regulator genes), associated with biofilm

formation in both planktonic and biofilm states. In planktonic cells, the compound markedly upregulated the transcriptional expression of, among others, the QS gene (*agr*), biofilm regulator genes (*isaA* and *icaD*), and protease genes, resulting in the inhibition of biofilm formation, whereas the expression levels of *icaA*, *icaD*, and *sigB* were downregulated in the biofilm state, leading to inhibited maturation [110, 125, 126].

Compared with the aforementioned natural QS inhibitors, two recent synthetic peptides were found to be more effective than DKPs and quite as effective as nesfactin, inhibiting biofilm formation in *P. aeruginosa* PAO1 by 50–66.2% at 0.1 mg mL^{-1}. These small 6-mer cationic peptides designed based on host defense peptide sequences, named LIVRRK and LIVRHK, downregulated the gene expression levels of QS-related virulence factors such as *lasI*, *lasR*, *rhlI*, and *rhlR*. The synthetic peptide LIVRHK considerably downregulated the gene expression levels of *lasI*, *lasR*, *rhlI*, and *rhlR* by 40.6%, 41.4%, 76.7%, and 43%, respectively, whereas LIVRRK downregulated the gene expression levels of *lasI*, *lasR*, *rhlI*, and *rhlR* by 59.6%, 73.6%, 86.9%, and 74.4%, respectively. Moreover, the production of pyocyanin, protease, and rhamnolipids by both synthetic peptides was also significantly decreased without inhibiting pathogen growth [127].

Biofilm matrix disruption

The biofilm matrix is a crucial target for destabilizing or disassembling biofilms in the search for novel strategies or antibiofilm agents to combat biofilm infections. In addition to anthraquinone emodin and secalonic acids B and D, the marine fungal symbiont *Penicillium* sp. SCSGAF 0023 can produce a polyketide citrinin through a PKS type I pathway (Fig. 3). Similar to emodin, fungal citrinin exhibited a biofilm formation inhibitory effect of > 50% at 12.5 μg mL^{-1} against *S. aureus* ATCC 6358 [110]. Citrinin, a mycotoxin with broad biological activity, possesses redox properties that likely play important roles in its function. Several studies in various cell systems also appear to support the finding that citrinin can generally induce the production of ROS [128, 129]. Intriguingly, Ooi et al. [130] reported that redox-active molecules can eradicate staphylococcal biofilms. The antibiofilm mode of action seemed to involve the disruption of the biofilm matrix. However, further studies are needed to unravel the in-depth molecular mechanism.

Moreover, a series of synthetic peptide-based prodrugs, cephalosporin-3'-diazeniumdiolates (C3Ds), have demonstrated biofilm dispersion linked to the generation of the redox-active molecule nitric oxide (NO) [131–133]. These synthetic prodrugs are composed of a peptide antibiotic, cephalosporin, which contains a β-lactam ring and is linked to a diazeniumdiolate NO donor. They selectively release NO following a reaction with the bacteria-specific enzyme β-lactamase and induce the dispersion of *P. aeruginosa* biofilms in vitro by inhibiting up to 91% at 5–100 μM [132]. Further development of C3Ds has recently yielded the most active compound, named AMINOPIP2-ceftazidime, which could reduce ceftazidime-resistant *P. aeruginosa* biofilms in vitro and showed efficacy equivalent to ceftazidime in a murine *P. aeruginosa* respiratory infection model [133].

Miscellaneous

Studies have also reported marine-derived microbial peptides and polyketides that were previously unknown or underwent further investigation for their antibiofilm activity. These compounds include a new cyclic tripeptide called epicotripeptin derived from the sea grass-associated endophytic fungus *Epicoccum nigrum* M13 (Fig. 2) [134], new polyketides erubescensoic acid derived from the sponge-associated fungus *Penicillium erubescens* KUFA0220 [135], and polycyclic xanthone sattahipmycin derived from sediment-derived *Streptomyces* sp. GKU 257−1 (Fig. 3) [136]. Epicotripeptin demonstrated moderate antibiofilm activity against gram-positive strains of clinical isolates *S. aureus* and *B. subtilis*, with an inhibition rate of 55–70% at 0.1 mg mL^{-1}, whereas erubescensoic acid and sattahipmycin impeded biofilm formation in *E. coli* ATCC 25922 and *E. coli* NBRC 3972, respectively. Sattahipmycin, a PKS II pathway-based polycyclic xanthone, inhibited approximately 50% of biofilm formation in *E. coli* at 15−60 μg mL^{-1}, with a very low bactericidal effect at > 250 μg mL^{-1}, but not *S. aureus* [136]. However, the mechanism of action of their antibiofilm activity remains unclear at present.

Conclusion and future directions

Briefly, the increasing prevalence of antibiotic resistance and recalcitrance in pathogenic microbes, coupled with the fact that many clinically significant infections are caused by intrinsic microbial biofilms, underscores the urgent need for the discovery and development of specific antibiofilm agents. Furthermore, it is worth noting that no antibiofilm molecules have been approved for clinical or medical use to date. Marine natural products, particularly those of microbial origin, have long been recognized as valuable sources of drug leads and therapeutic agents due to their diverse structures and broad-spectrum bioactivities, including antibiotics. However, marine microbial natural products with antibiofilm activity remain largely unexplored. Recently, a limited number of marine microbial peptides and polyketides—a total of 12 compounds comprising 6 nonribosomal peptides and 6 polyketides—have been identified and reported to exhibit in vitro antibiofilm potential. These compounds serve as a

foundation for potential therapeutic or antibiotic alternatives. Although they are promising as antibiofilm agents that could combat bacterial infections through various mechanisms and targets, including inhibiting microbial attachment, QS, and disrupting the biofilm matrix, their potency may need improvement compared with some available synthetic compounds.

Recent advancements in (meta)genomics and metabolomics have significantly accelerated the opportunities for discovering novel marine microbial natural products, including those with antibiofilm properties. It is highly likely that more peptide- and polyketide-based compounds with antibiofilm potential will be explored and developed in the foreseeable future [137–139]. There is still ample room for improvement and innovation in this field. Progress in synthetic methodologies, particularly in the context of synthetic peptides, synthetic biology, recombinant production, and the automation of screening processes, will continue to drive down the manufacturing costs of these compounds. The modular structure of nonribosomal peptides and polyketides makes them intriguing candidates for re-engineering using synthetic biology approaches to create analogs with enhanced activity and/or yield [54]. Given their inherent feasibility, which allows for rapid and extensive structure–activity relationship studies for lead optimization while minimizing the likelihood of side effects, these efforts may result in higher success rates in clinical trials. However, it is crucial to emphasize that further in vivo and clinical evaluations are urgently required. To advance the development of these compounds as semisynthetic or synthetic drugs, the exploitation of natural motifs and processes in drug discovery and design has been facilitated by in silico computational methods. The use of in silico computer-aided drug design approaches, such as structure-based virtual screening and neural networks in automated drug discovery methods, coupled with experimental in vitro and in vivo techniques, may aid in identifying these molecules and confirming their drug-like or lead-like chemical properties [140, 141]. Furthermore, Haney et al. [142] demonstrated the prospect of applying computer-based quantitative structure–activity relationship methodologies for identifying synthetic antibiofilm peptides. The method has successfully assisted in the identification of specific peptides with antibiofilm activity in vitro against methicillin-resistant *S. aureus* biofilms and has proven effective in an in vivo mouse model of cutaneous abscess infections. Interestingly, in addition to in vitro biofilm models of microtiter plate biofilm assays and in vivo models of nonmammalian and mammalian model organisms, human tissue culture-based biofilm models, the so-called human organoids, are available, including the recent rise of organoid-on-a-chip models for evaluating antibiofilm agents. The latter model offers the benefits of an in vitro technique that resembles human physiology while maintaining the high-throughput features addressing the logistical, ethical, and accuracy issues in mimicking human physiology [92].

Therefore, the discovery and development of new antibiofilm drugs from marine microbial natural products, particularly peptides and polyketides, and their semisynthetic and synthetic derivatives, are notably creditable.

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Declarations

Conflict of interest The authors declare no conflict of interest in this study.

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ARTICLES FOR FACULTY MEMBERS

ELUCIDATION OF ANTIBIOFILM FORMATION MECHANISMS USING MARINE EXTRACTS AGAINST INFECTION AND ANTIMICROBIAL RESISTANCE (AMR)

Anti-biofilm extracts and molecules from the marine environment / Caudal, F., Roullier, C., Rodrigues, S., Dufour, A., Artigaud, S., Le Blay, G., Bazire, A., & Petek, S.

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Review

Anti-Biofilm Extracts and Molecules from the Marine Environment

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Abstract: Pathogenic bacteria and their biofilms are involved in many diseases and represent a major public health problem, including the development of antibiotic resistance. These biofilms are known to cause chronic infections for which conventional antibiotic treatments are often ineffective. The search for new molecules and innovative solutions to combat these pathogens and their biofilms has therefore become an urgent need. The use of molecules with anti-biofilm activity would be a potential solution to these problems. The marine world is rich in micro- and macro-organisms capable of producing secondary metabolites with original skeletons. An interest in the chemical strategies used by some of these organisms to regulate and/or protect themselves against pathogenic bacteria and their biofilms could lead to the development of bioinspired, eco-responsible solutions. Through this original review, we listed and sorted the various molecules and extracts from marine organisms that have been described in the literature as having strictly anti-biofilm activity, without bactericidal activity.

Keywords: biofilm; marine natural products; bacteria; anti-biofilm

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1. Introduction

Antimicrobial resistance (AMR) is a global public health problem, limiting therapeutic strategies against bacterial infections. The World Health Organization (WHO) has identified this issue as a priority, as it is estimated that drug-resistant infections contributed to nearly 5 million deaths in 2019 [1]. The Organization for Economic Cooperation and Development (OECD) indicates that resistance to antibiotics of last resort could increase twofold by 2035, highlighting the urgent need for robust antimicrobial stewardship practices and also for increased research of novel compounds.

The situation could be even worse, as most studies of AMR fail to take into account that bacteria often adopt a biofilm lifestyle in their environment and when causing infections. Bacterial biofilms are communities of cells located at interfaces, embedded into a self-produced matrix composed of exopolysaccharides, proteins, lipids, and extracellular DNA. The matrix contributes to protection from the environment by providing a relatively impermeable physical barrier to toxic substances, including antibiotics [2]. The multilayered organization of cells enhances this protection, as only peripheral cells are usually exposed to external agents [3]. Persistent cells found in the deeper layers of the biofilm are less sensitive to antibiotics, due to their mechanism of action which generally targets actively growing bacteria [4].

The overuse of antibiotics leads to an increase in their concentration in the environment of human activities. Their increasing concentration is problematic because they have been shown to induce biofilm formation, leading to an adaptive response of bacteria [5–7] and potentially to gene transfer from animal to human pathogens [8].

Cell proximity in biofilm structures allows two types of communication between bacteria: (i) genetic, through horizontal gene transfer favoring the exchange of antibiotic resistance genes [9], and (ii) chemical, through the perception of small molecules, allowing the estimation of population density to perform joint actions, a phenomenon known as quorum sensing (QS) [10]. QS-controlled phenotypes include bacterial virulence and regulation of biofilm formation, and QS is therefore an interesting therapeutic target, as its short-circuiting (quorum quenching) can impair biofilm formation and/or reduce virulence mechanisms [11].

The discovery of new molecules and innovative solutions to prevent biofilm formation or disrupt biofilms of pathogenic bacteria has become critical. The use of molecules with anti-biofilm activity, inhibiting the pathways that regulate virulence and biofilm formation, would be a potential solution to these problems, rather than trying to eradicate bacteria through the use of "traditional" antibiotics.

For many years, research in the pharmaceutical industry focused primarily on natural products from the terrestrial world, which were generally easier to access. However, in the middle of the 20th century, technological and technical advances in diving and remotely operated vehicles such as ROVs (remotely operated underwater vehicles), made it easier to explore the marine world [12], with the promise of new bioactive molecules, as 70% of Earth's surface is covered by oceans and seas.

The marine world is teeming with micro- and macro-organisms capable of producing secondary metabolites with original skeletons and interesting activities. Numerous research teams are aware of this, and are interested in the chemical strategies used by some of these organisms to regulate and/or protect themselves from pathogenic bacteria and their biofilms, in order to develop bioinspired, eco-responsible solutions. Several strategies are used by organisms to limit pathogenic bacterial biofilms, including inhibiting microbial growth, interfering with bacterial communication, disrupting adhesion processes, or destroying pre-formed biofilms, as in the case of matrix polymer-degrading enzymes.

We focused this review on articles describing molecules or extracts from marine organisms with specific anti-biofilm or anti-QS activities. This approach is quite original compared to other existing reviews, as we avoided all molecules with antibacterial properties that consequently prevent the establishment of biofilm. Using "molecules", "biofilm", "antibiofilm", "anti-biofilm", and "marine" as keywords for our PubMed® search, we found 71 articles that met this criterion between 2009 and 2023. Many articles were excluded because they also reported anti-biofilm molecules with antibacterial activities. We decided not to include these results because of a potential bias: if a certain proportion of bacteria is killed, then less biofilm is formed, making it difficult to conclude that the molecule is strictly anti-biofilm.

The first section presents the active extracts for which the active molecule was not found or isolated, followed by a presentation of the purified and identified molecules. Finally, the various results reported here are discussed.

2. Anti-Biofilm Compounds and Quorum-Sensing Inhibitors

Among the different articles dealing with pure anti-biofilm activities (without antibacterial activity), we found 20 articles presenting the activity of extracts and supernatants, and 51 articles presenting the results of purified molecules from marine organisms. These will be treated separately as they imply different issues and development perspectives.

2.1. Extracts and Culture Supernatants

Several publications described extracts or culture supernatants of macro- or microorganisms with anti-biofilm activities, for which the active molecules have not yet been

described. From a review of these articles, the most common models for biofilms were those from *Staphylococcus aureus* and *Pseudomonas aeruginosa*, two pathogens with major public health issues. Indeed, they are involved in numerous multi-resistant chronic infections. In most cases, the nature of the active molecules in these extracts or culture supernatants was not identified. This may be explained by the fact that, in some cases, purification leads to activity loss as several compounds in the mixture might have synergistic effects. Therefore, culture supernatants are directly tested or only after a first round of purification to separate organic and aqueous fractions.

Bakkiyaraj et al. used methanolic extracts from *Streptomyces akiyoshiensis*, an actinomycete associated with the coral *Acropora digitifera*, against various strains of *S. aureus*, including methicillin-resistant strains and or clinical strains. These extracts showed anti-biofilm activity at MBIC = 0.1 mg/mL (MBIC: Minimum Biofilm Inhibiting Concentration) and was able to inhibit intestinal colonization in the nematode *Caenorhabditis elegans* [13].

Leetanasaksakul et al. showed an anti-biofilm activity against *S. aureus* and *Escherichia coli* biofilms from 13 and 10 marine actinomycetes culture supernatants, respectively, out of 101. Interestingly, those that were active on *E. coli* biofilm were not active on *S. aureus* and vice versa. They showed a significant reduction of more than 60% of the biofilm. Analysis of the culture supernatants showed that most actinomycetes secrete non-toxic anti-biofilms metabolites with varying degrees of proteolytic activity. Non-toxicity towards bacteria is an important feature, as it prevents them from developing resistance. Out of the 23 active culture supernatants, only 4 also showed antibacterial activity [14].

The fungus *Blastobotrys parvus* PPR3 isolated from a mangrove wood sample (*Avicennia marina*) also showed promising activity. The crude extract of PPR3 reduced various virulence characteristics of *P. aeruginosa*, in particular pyocyanin, elastase, protease, and chitinase production, as well as motility, biofilm formation, exopolysaccharide, and alginate production. The authors were able to demonstrate an interaction with *P. aeruginosa* LuxR type receptors, suggesting an inhibition of QS [15].

Extracts derived from three algae, *Ulva lactuca*, *Halopteris scoparia* (ex *Stypocaulon scoparium*), and *Pterocladiella capillacea*, were prepared by successive macerations with different solvents (cyclohexane, dichloromethane, ethyl acetate, and methanol). Extracts obtained with cyclohexane and ethyl acetate showed *P. aeruginosa* biofilm inhibitory activity, but with different mechanisms of action [16]. In a second study, the team looked at the effect of the same extracts on *S. aureus.* Here the four extracts showed inhibition in *S. aureus* biofilm formation, with action on adhesion and proliferation stages [17].

From a red seaweed, *Gracilaria changii*, Muthukrishnan et al. showed a strong anti-biofilm and anti-QS activity against *Vibrio campbellii* BB120. The crude methanol extract showed activity at 1 µg/mL, with a decrease in biofilm formation and inhibition of violacein production by *C. violaceum* [18].

Still based on seaweed extracts, this time from three different algae, *Chaetomorpha aerea*, *Agardhiella subulata*, and *Hypnea cornuta*, anti-biofilm activities were searched for against diverse marine pathogens. The tests on different *Vibrio* species and *Listonella anguillarum* showed anti-adhesive properties of the extracts with modification of hydrophobicity levels and cell surface charges. They also demonstrated the lack of toxicity of these extracts on aquaculture [19].

Wang et al. exhibited some interesting anti-biofilm activity from several extracts of coastal mangroves of Mayotte against a clinical strain of *P. aeruginosa*. Three of the twenty-three extracts showed more than 50% inhibition of biofilm formation [20].

A really interesting review of marine algae-derived anti-biofilm compounds by Behzadnia et al. showed that, with these different extracts and the molecule that will be present in the next section, and the one they presented, algae should be a really promising source of anti-biofilm compounds. These compounds could be very useful for human, animal, and environmental health [21].

Using methanolic extracts of different parts (tentacle, disc, and whole body) of Haddon's sea anemone, *Stichodactyla haddoni*, collected in the Persian Gulf, Hamayeli et al.

showed a predominance of aliphatic compounds with anti-biofilm activity against *Bacillus cereus* and *P. aeruginosa* [22].

In 2021, the same team extracted metabolites from two sponges, *Psammocinia* sp. and *Hyattella* sp., using a mix of two organic solvents, and tested their anti-biofilm activity against six bacteria: *P. aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *E. coli*, *S. aureus*, and *B. cereus*. Both extracts showed significant effects, probably due to the presence of phenolic compounds, butanedioic acid, propanoic acid, and benzene-acetaldehyde, without however identifying the active molecule(s) [23].

Methanolic extract of the sponge *Agelas dispar* was shown to inhibit biofilm formation and destroy biofilm of *Candida krusei* (ATCC6258), *C. glabrata* (ATCC 2001), and *C. parapsilosis*. It appears that this extract causes changes in the cytoplasmic membrane and/or changes in the cell wall [24].

Various sponge extracts isolated from Wallis were tested for their anti-biofilm activities, particularly against the marine pathogen *Vibrio harveyi* ORM4. Twenty-eight different genera were tested and seven of them showed anti-biofilm activities. Four different extracts from the genus *Hyrtios* were among the most efficient with up to 93.61% inhibition of biofilm formation [25].

Some studies came very close to identifying an active molecule. Balasubramanian et al. (2017) were able to demonstrate the activity of *Streptomyces* sp. SBT343, a sponge-associated actinomycete, on different strains of *Staphylococcus* [26]. In their subsequent study, they succeeded in purifying the SKC3 compound and carried out initial characterization works, but without obtaining the exact structure. At concentrations ranging between 3.95 and 31.25 µg/mL, SKC3 inhibited *S. epidermidis* biofilm formation. Analysis of the transcriptome of treated bacteria revealed a negative effect on central metabolism, notably carbon flux, but also amino acid, lipid, and energy metabolism [27].

Bacteria belonging to the *Pseudoalteromonas* genus are sources of numerous anti-biofilm metabolites, identified or not. The culture supernatant of *Pseudoalteromonas haloplanktis* TAC125, isolated in Antarctica, inhibits the biofilm of *S. epidermidis* [28]. The mode of action has not yet been fully elucidated, but the molecule is suspected to act as an AI-2 agonist or as a ligand targeting the AI-2 receptor, AI-2 being a universal language for interspecies communication. Moreover, the molecule appears to be produced at all stages of bacterial growth and under a wide variety of experimental conditions [29]. The team subsequently succeeded in identifying a pentadecanal, a long-chain fatty aldehyde, which acts on the AI-2 pathway [30], and then tested the activity of derivatives of this pentadecanal and showed an increase in activity with pentadecanoic acid [31].

The same research team, still using Antarctic marine bacteria, showed anti-biofilm activity on ESKAPE bacteria, which are a major health issue. Interestingly, these four culture supernatants did not exhibit any antimicrobial activity but acted on biofilm formation and pre-formed biofilms, mainly of *S. aureus*, *K. pneumoniae*, and *P. aeruginosa* [32].

Among 86 heterotrophic marine bacteria, Doghri et al. identified the *Pseudomonas* sp. IV2006 strain, the culture supernatant of which inhibited the biofilm of another marine bacterium, *Flavobacterium* sp. II2003. The supernatant altered the surface properties of the glass, making it more hydrophilic and alkaline, thus significantly reducing bacterial adhesion. The supernatant was also active against biofilms of human pathogens such as *S. aureus*, *P. aeruginosa*, and *Yersina enterocolitica* [33].

Enzymes are another family of molecules with the potential to perform interesting activities. An interesting activity of a stony coral, *Montipora foliosa*, a supernatant on the pathogen *Stenothrophomonas maltophilia*, was shown in the article by Peters et al. A group of metalloproteases responsible for anti-biofilm activity was identified by proteomic analysis of this active supernatant [34].

This first section on active extracts or culture supernatants highlights the diversity of their origins in the marine world, whether from macro-organisms such as sponges or anemones, or from micro-organisms such as algae, bacteria, or fungi. The study of marine diversity is therefore a promising avenue for research of active natural compounds.

Most of the teams have not yet gone as far as to purify the active molecule, but this section includes very recent papers, published in the last five years, and the rest of the story may not yet have been published or is likely still in progress. There are a number of additional factors that can complicate further studies, such as the limited availability of molecules, particularly those extracted from marine macro-organisms, which may be available in limited quantities, or the non-homogeneous production of metabolites by a micro-organism, depending on the culture conditions.

The use of culture supernatants or extracts saves time in the search for anti-biofilm molecules. In fact, this use can be seen as a screening to see where the anti-biofilm molecules are. Bio-guided purification can then be used to move from the fractions to the active molecule in a more or less timely manner. This saves a lot of time compared to purifying and then testing every single molecule produced by an organism. Extracts from sponges are also readily available and are usually made from freeze-dried material, allowing their chemical diversity to be studied and conserved. For bacteria and algae, culture supernatants are often reproducible and available in larger quantities.

However, the demonstration of activity in an extract or culture supernatant does not necessarily mean that the active molecule will be easy to purify and characterize. In fact, it often turns out that this activity may be due to several molecules in the extract or culture supernatant, or to a synergy of molecules that lose their activity once separated. Extracts from sponges or cnidarians are often available in limited quantities, so characterization of the active molecule(s) may be hampered by the problem of accessing larger quantities.

The second section of this review describes the identified molecules that have been isolated and characterized from marine organisms and that have anti-biofilm and/or anti-QS activities.

2.2. Active Compounds

In addition to the numerous interesting extracts described in the literature, it is possible to find more or less purified molecules whose modes of action have sometimes been demonstrated. Table 1 describes the various non-biocidal molecules found in the literature. The compounds are numbered and their structures are shown in Figures 1–5.

Table 1. Non-biocidal anti-biofilm molecules reported in the literature. Compounds are grouped by family, and for each one the producing organism and target organism(s) are indicated. When modes of action are known or assumed, they are described at the end of the table. Each type of organism is distinguished by the color associated with the box (Producer organisms: **bacteria**, **fungi**, **sponge**, **cnidarian**, **alga**, **other invertebrate**).

Compound Family	Compound Number	Compound Name	Producing Organisms	Target Organisms	Mechanisms of Action	Reference
Peptides and proteins	Unknown structure	Cyclic lipopeptide	*Pseudomonas* sp. TAD1S	*S. aureus*	Surfactant	[35]
	Unknown structure	Alterocin	*Pseudoalteromonas* sp. 3J6	*P. aeruginosa; E. coli; S. enterica; Vibrio* sp. D01; *Paracoccus* sp. 4M6	Impact on bacterial adhesion	[36–38]
	Unknown structure	P004	*Pseudoalteromonas* sp. IIIA004	*Roseovarius* sp. VA014		[39]
	1	*cis*-cyclo(Leucyl-Tyrosyl)	Sponge associated *Penicllium* sp.	*S. epidermidis*		[40]
	Unknown structure	Scyreprocin	*Scylla paramamosain*	*Candida albicans* and *C. neoformans*		[41]

Table 1. *Cont.*

Compound Family	Compound Number	Compound Name	Producing Organisms	Target Organisms	Mechanisms of Action	Reference
Peptides and proteins	**2**	Paracentrin 1	*Paracentrotus lividus*	*S. epidermidis* DSM 3269; *S. aureus* ATCC 29213; *P. aeruginosa*		[42–44]
	Unknown structure	Catasan	*Psychrobacter* sp. TAE2020	*S. epidermidis* RP62A	Reduces biofilm biomass and modifies its structure	[45]
	3	Nesfactin	*Nesterenkonia* sp. MAS31 isolated from *Fasciospongia cavernosa*	*P. aeruginosa*	Quenches QS via LasR	[46]
	4	Cyclo(*L*-Trp-*L*-Ser)	*Rheinheimera aquimaris*	*Chromobacterium violaceum* and *P. aeruginosa* PAO1	Decreases production of violacein, exhibits pyocyanin production, swimming motility, adhesion, and biofilm formation	[47]
Phenolic compounds	**5**	2,4-di-tert-butylphenol	*Vibrio alginolyticus* G16	*S. marcescens*	Impacts production of virulence factor via QS	[48]
	6	Methyl benzoate	*Pseudomonas aeruginosa* CBMGL12 isolated from coral *Favites* sp.	*S. aureus* MTCC96	Diminishes virulence and biofilm phenotypes, seems to target the QS	[49]
	7	Methyl phenylacetate				
Alkaloids	**8**	Psammaplin A	*Aplysinella rhax*	*P. aeruginosa*	Inhibits production of elastase and QS	[50]
	9	Bisaprasin				
	10	Ageloxime D	*Agelas nakamurai*	*S. epidermidis*		[51]
	11	Maipomycin A	*Kibdelosporangium phytohabitans* XY-R10	*Actinobacter baumannii* and *P. aeruginosa*	Iron chelator	[52]
	12	Isonaamine D	*Leucetta chagosensis*	*V. harveyi*	Inhibitor activity on all three QS pathways	[53]
	13	Isonaamidine A				
	14	2,2-*bis*(6-bromo-1*H*-indol-3-yl)ethanamine	*Didemnum candidum*, and *Orina* spp.	*S. aureus* CH 10850 and *S. aureus* ATCC 29213		[54,55]
	15	2,2-*bis*(6-fluoro-1*H*-indol-3-yl)ethanamine				
	16	Makaluvamine A	*Zyzzya fuliginosa*	*Streptococcus mutans*		[56]
	17	Makaluvamine F				
	18	Mavaluvamine G	*Histodermella* sp.			
	19	Meridianin D	*Aplidium meridianum*	*S. aureus*		[57,58]
	20	Collismycin C	*Streptomyces* sp. MC025	*S. aureus*		[59]

Table 1. *Cont.*

Compound Family	Compound Number	Compound Name	Producing Organisms	Target Organisms	Mechanisms of Action	Reference
Terpenoids	**21**	α-bisabolol	*Padina gymnospora*	*Serratia marcescens*	Inhibits prodigiosin and protease production, and acts on bacterial motility and hemolysin production	[60]
	22	Dolabellanes	*Dictyota* sp.	*Pseudoalteromonas* sp.		[61]
	23					
	24					
	25	Dictyol C				
	26	Dictyol L	*Dictyota pinnatifida*	*P. aeruginosa*		[62]
	27	Knightal	*Eunicea knighti*	*Chromobacterium violaceum*, *S. aureus*, *V. harveyi* and *P. aeruginosa*	Anti-QS activity	[63,64]
	28	11(*R*)-hydroxy-12(20)-en-knightal				
	29	11(*R*)-hydroxy-12(20)-en-knightol acetate				
	30	Phorbaketal B	*Phorbas* sp.	*S. aureus*	Inhibition in expression of the biofilm-related hemolysin gene *hla* and the *nuc1* nuclease gene	[65]
	31	Phorbaketal C				
	32	Ophiobolin K	*Emericella variecolor*	*Mycobacterium smegmatis*		[66]
	33	6-*epi*-ophiobolin K				
	34	6-*epi*-ophiobolin G				
	35	Siphonocholin	*Siphonochalina siphonella*	*C. violaceum* and *P. aeruginosa*	Altered production of elastase, total protease, pyocyanin, chitinase and exopolysaccharides	[67]
	36	Halistanol sulfate A	*Petromica ciocalyptoides*	*S. mutans*		[68]
	37	5-episinuleptolide	*Sinularia leptoclados*	*A. baumannii* ATCC 19606, BAA747, 29115, 68704, D4	Diminish production of the extracellular polysaccharide poly-β-(1,6)-N-acetylglucosamine (PNAG)	[69]
	38	5-octylfuran-2(5H)-one	*Streptomyces* sp.	*E. coli* K12, *P. aeruginosa* PAO1 and methicillin-resistant *Staphylococcus aureus*	Matrix destruction and interference with AI-2 mediated QS system	[70]

Table 1. *Cont.*

Compound Family	Compound Number	Compound Name	Producing Organisms	Target Organisms	Mechanisms of Action	Reference
Fatty acids and derivatives	**39**	(9Z)-9-octadecenal	*Streptomyces griseoincarnatus* HK 12	*S. aureus* and *P. aeruginosa*	(13Z)-13-octadecenal is thought to target the quorum sensing system by binding 3-oxo-C12 HSL in *P. aeruginosa*	[71]
	40	Arachic acid				
	41	Erucic acid				
	42	(13Z)-13-octadecenale				
	43	Tetracosanoic acid				
	44	4-Phenylbutanoic acid	*Bacillus pumilus* S6-15	*P. aeruginosa*, *B. indicus* MTCC5559 and *B. pumilus* MTCC5560		[72,73]
	45	Stearidonic acid (18:4 n-3)	Various marine origins	*Candida albicans* and *C. dubliniensis*	Oxidative stress	[74]
	46	Eicosapentaenoic acid (20:5 n-3)				
	47	Docosapentaenoic acid (22:5 n-3)				
	48	Docosahexaenoic acid (22:6 n-3)				
	49	Mevalonolactone	*Sordariales associated to Mycale magnirhaphidifera*	*S. epidermidis*		[75]
	50	Myristic acid	*Mycale contarenii*	*S. aureus* methicillin susceptible and resistant, *L. monocytogenes*	Repress transcription of *fnbA* and *fnbB* genes, fibronectin-binding protein, and *icaADBC* operon (polysaccharide intercellular adhesin)	[76]
	51	Oleic acid				
	52	Lyngbyoic acid	*Lyngbya* sp.	*P. aeruginosa* PaO1	Inhibits biofilm formation (biovolume) and QS pathways	[77]
	53	Benderadienne				
	54	Pentadecanal	*P. haloplanktis* TAC125	*S. epidermidis*	Impair biofilm formation	[31]
Polysaccharides	Unknown structure	A101	*Vibrio* sp. QY101	Wide range of Gram positive and negative		[78]
	55	Fucoidan	*Fucus vesiculosus*	*S. mutans* and *S. sobrinus*	Only active on biofilm formation	[79]
	56	MO245	*Vibrio alginolyticus* sp.	*P. aeruginosa* PaO1 and *V. harveyi* DSM19623	Leads to abiotic and bacterial surface modification	[80]
	57	Monomeric units of α-D-galactopyranosyl-(1→2)-glycerol-phosphate (1800 kDa)	*B. licheniformis* associated with *Spongia officinalis*	*E. coli* PHL628, *P. fluorescences*	Reduces cell surface hydrophobicity	[81]

Table 1. *Cont.*

Compound Family	Compound Number	Compound Name	Producing Organisms	Target Organisms	Mechanisms of Action	Reference
Polyketides	**58**	Hygrocin C	*Streptomyces* sp. SCSGAA 0027	*S. aureus* and *B. amyloliquefaciens* SCSGAB0082	Reduces matrix formation, decreases surface hydrophobicity, impacts on bacterial flagellar system	[82]
	59	Secalonic acid D	*Penicillium* sp. SCSGAF0023 (CCTCC M 2012507)	*S. aureus*	Targets genes associated to biofilm formation: *agr*, *isaA*, *icaA*, and *icaD*	[83]
	60	Tetracenomycin D	*Streptomyces* sp. EG1	*S. aureus* and *E. coli*	Target biofilm forming protein (ClfB in *S. aureus* and CSgG in *E. coli*)	[84]
	61	Resistomycin				
	62	Resistoflavin				

As with culture supernatants and extracts, the diversity of producing organisms is notable.

This table includes seven different families of molecules: peptides and proteins, phenolic compounds, alkaloids, terpenoids, fatty acids and derivatives, polysaccharides, and polyketides. The target micro-organisms mainly studied are of the *Pseudomonas* or *Staphylococcus* genus. The mode of action is specified when it is described in the articles, but it is not always known precisely.

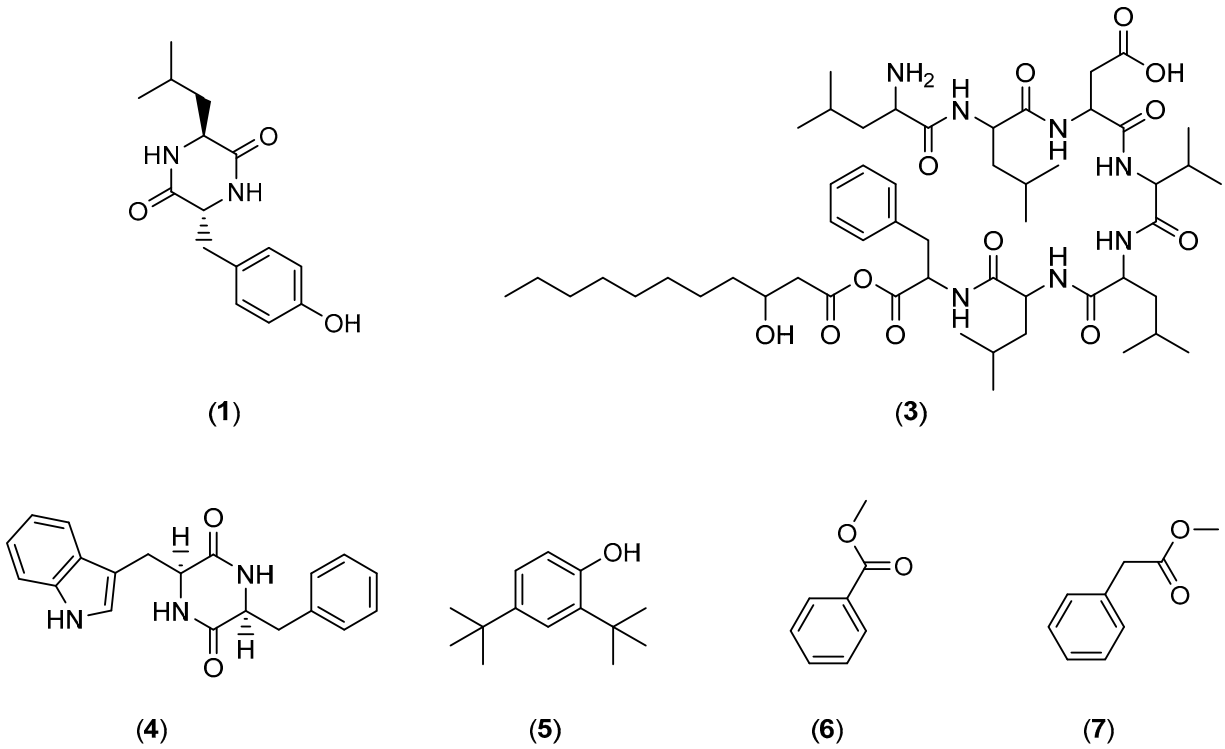

Figure 1. Chemical structures of cis-cyclo(Leucyl-Tyrosyl) (**1**), nesfactin (**3**), cyclo(*L*-Trp-*L*-Ser) (**4**), 2,4-di-tert-butylphenol (**5**), methyl benzoate (**6**), and methyl phenylacetate (**7**).

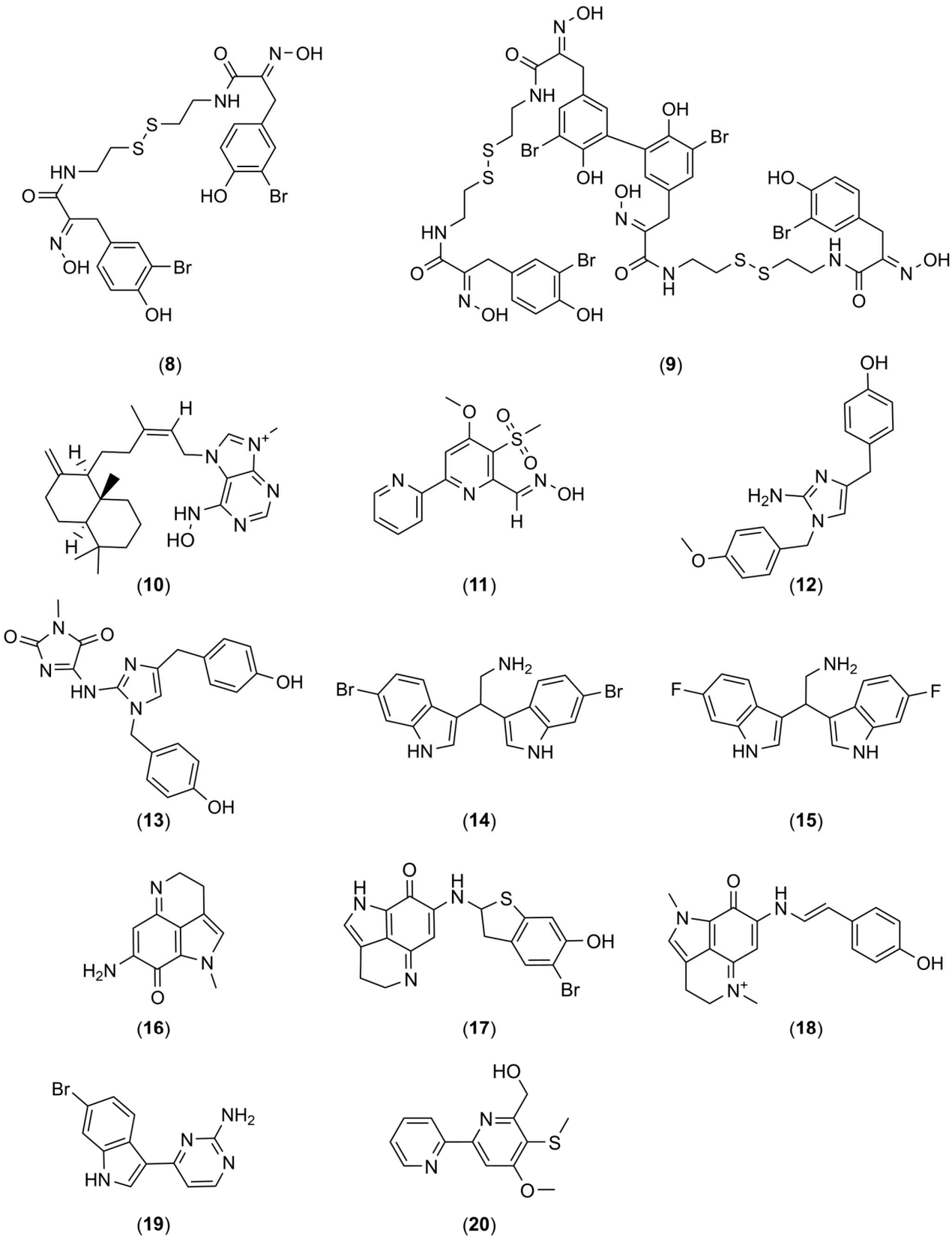

Figure 2. Chemical structures of psammaplin A (**8**), bisaprasin (**9**), ageloxime D (**10**), maipomycin A (**11**), isonaamine D (**12**), isonaamidine A (**13**), 2,2-*bis*(6-bromo-1*H*-indol-3-yl)ethanamine (**14**), 2,2-*bis*(6-fluoro-1*H*-indol-3-yl)ethanamine (**15**), makaluvamine A (**16**), makaluvamine F (**17**), makaluvamine G (**18**), meridianin D (**19**), and collismycin C (**20**).

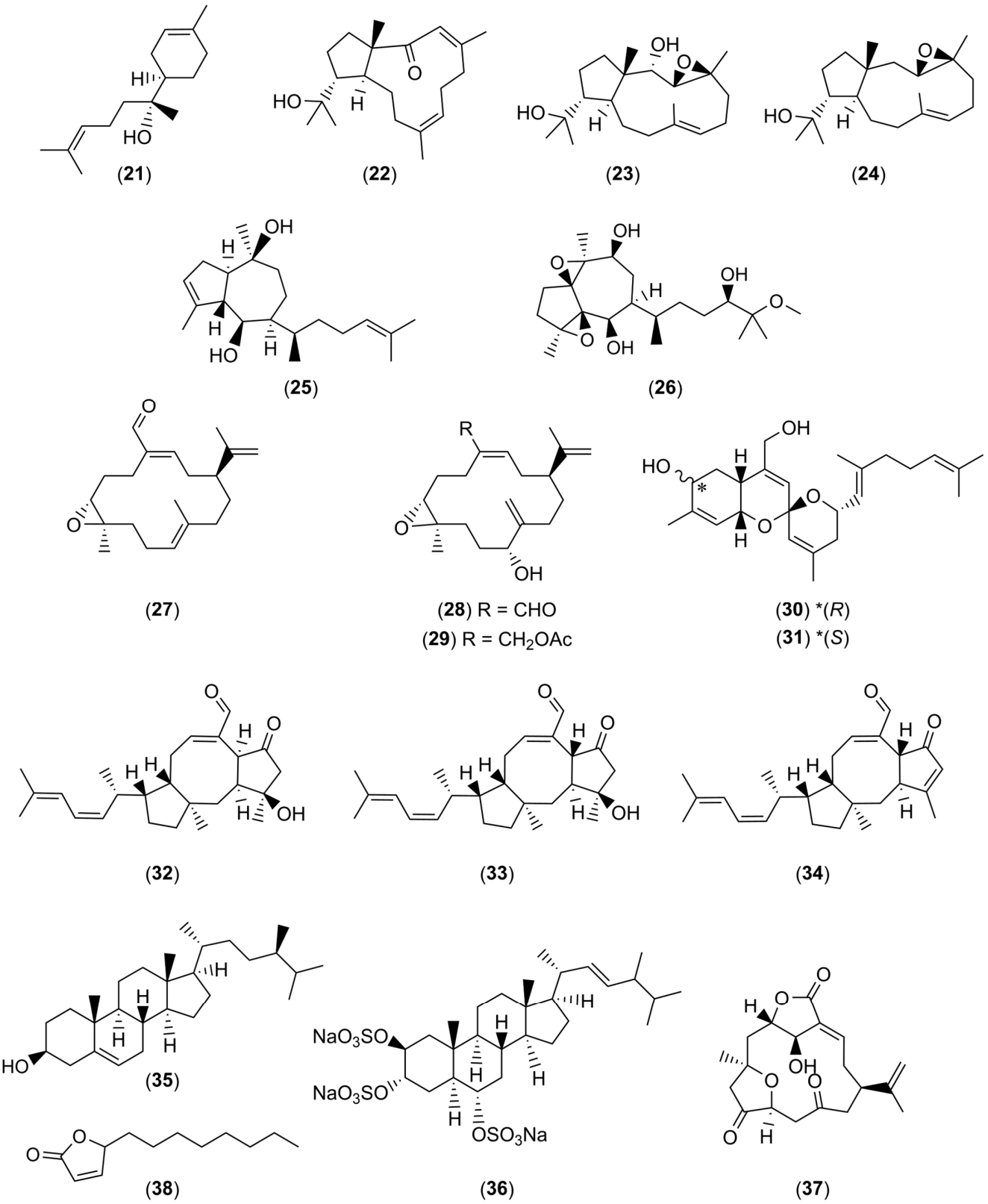

Figure 3. Chemical structures α-bisabolol (**21**), three dolabellanes (**22**–**24**), dictyol C (**25**), dictyol L (**26**), knightal (**27**), 11(R)-hydroxy-12(20)-en-knightal (**28**), 11(R)-hydroxy-12(20)-en-knightol acetate (**29**), phorbaketal B (**30**), phorbaketal C (**31**), ophiobolin K (**32**), 6-epi-ophiobolin K (**33**), 6-epi-ophiobolin G (**34**), siphonocholin (**35**), halistanol sulfate A (**36**), 5-episinuleptolide (**37**), and 5-octylfuran-2(5H)-one (**38**).

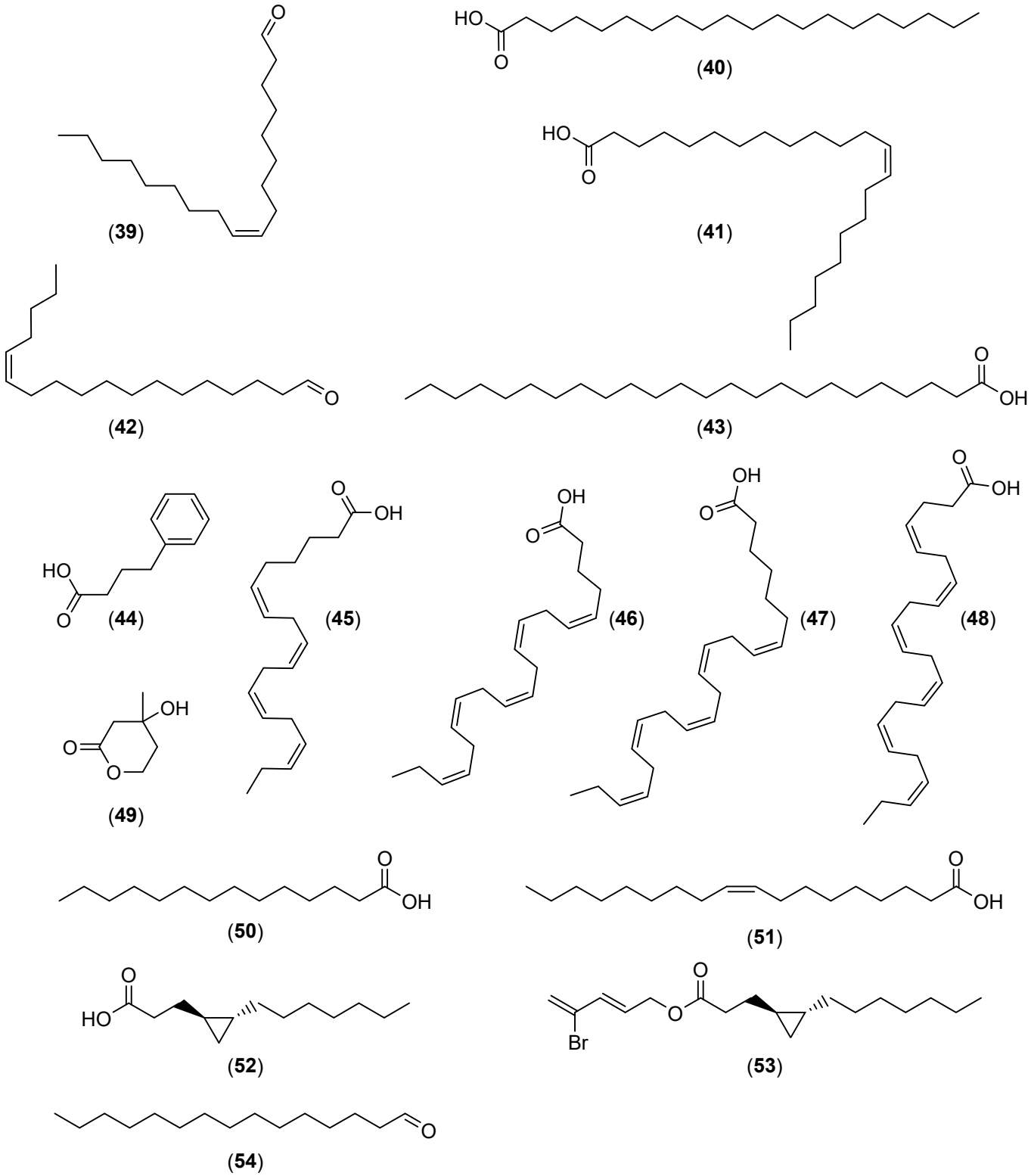

Figure 4. Chemical structures of (9Z)-9-octadecenal (**39**), arachic acid (**40**), erucic acid (**41**), (13Z)-13-octadecenale (**42**), tetracosanoic acid (**43**), 4-Phenylbutanoic acid (**44**), stearidonic acid (18:4 n-3) (**45**), eicosapentaenoic acid (20:5 n-3) (**46**), docosapentaenoic acid (22:5 n-3) (**47**), docosahexaenoic acid (22:6 n-3) (**48**), mevalonolactone (**49**), myristic acid (**50**), oleic acid (**51**), lyngbyoic acid (**52**), benderadienne (**53**), and pentadecanal (**54**).

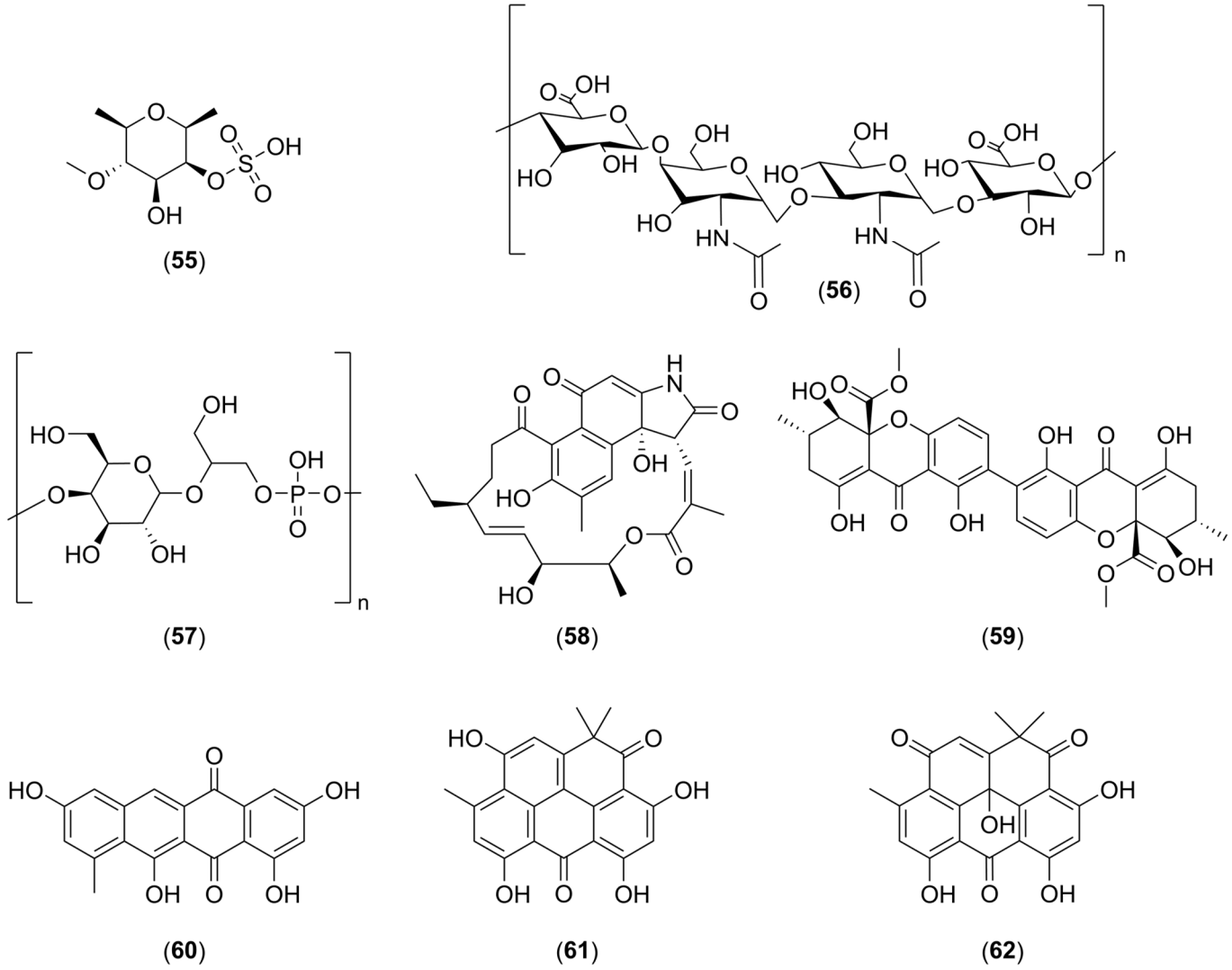

Figure 5. Chemical structures of fucoidan (**55**), MO245 (**56**), α-D-galactopyranosyl-(1→2)-glycerol-phosphate (**57**), hygrocin C (**58**), secalonic acid D (**59**), tetracenomycin D (**60**), resistomycin (**61**), and resistoflavin (**62**).

3. Discussion

Through our review, we showed a great diversity of anti-biofilm molecules produced in the marine world: peptides and proteins, phenolic compounds, alkaloids, terpenoids, fatty acids and derivatives, and polyketides. We also showed that many marine organisms are potential producers of anti-biofilm molecules: bacteria, fungi, algae, and invertebrates (sponges, corals, echinoderm, mollusks, ascidians, etc.). Active supernatants or extracts have also been determined, for which the active molecule(s) have not yet been identified.

Bacteria are the most studied, accounting for nearly 40% of the producers studied, probably due to their abundance in the environment and also because they represent a convenient renewable and sustainable resource to exploit. Access to larger quantities of molecules is facilitated. In terms of ecology, they are in constant competition with each other to occupy environmental niches crucial for their survival, which probably explains their great capacity to produce anti-biofilm molecules. Marine fungi also compete with bacteria and therefore have the ability to inhibit biofilm, accounting for nearly 15% of identified producers.

Filter-feeding organisms such as sponges and mollusks are also widely studied, as they are in constant contact with bacteria. Sponges are really interesting, especially re-

garding the intriguing chemical skeletons of their metabolites. From the point of view of anti-biofilm research, sponges have the advantage of being sessile organisms, producing numerous metabolites that enable them to control the bacteria that colonize them, probably by repelling some via anti-biofilm metabolites or attracting others for symbiosis [85].

Finally, some organisms, such as algae, have developed strategies to avoid colonization of their thallus, also making them ideal sites to search for anti-biofilm compounds.

For many macro-organisms, however, it is their association with bacteria to form the so-called holobiont that is probably at the origin of the production of active metabolites. Thus, the part played by bacterial metabolites is certainly largely underestimated, and it is often difficult to know whether it is one of the two organisms that produces the molecule, or whether it is the association of the two that makes it possible. Cultivation of bacteria isolated from macro-organisms is therefore no guarantee of success in recovering activity.

Of the sixty molecules tested, just over a third had activity on several pathogens, which is all the more interesting given that biofilms are generally mixed with at least two types of bacteria. The other molecules should not be ruled out, as they may not yet have been tested on other pathogens.

Figure 6, which groups together the years of publication of the articles reviewed in this study, also shows the recent interest in this field.

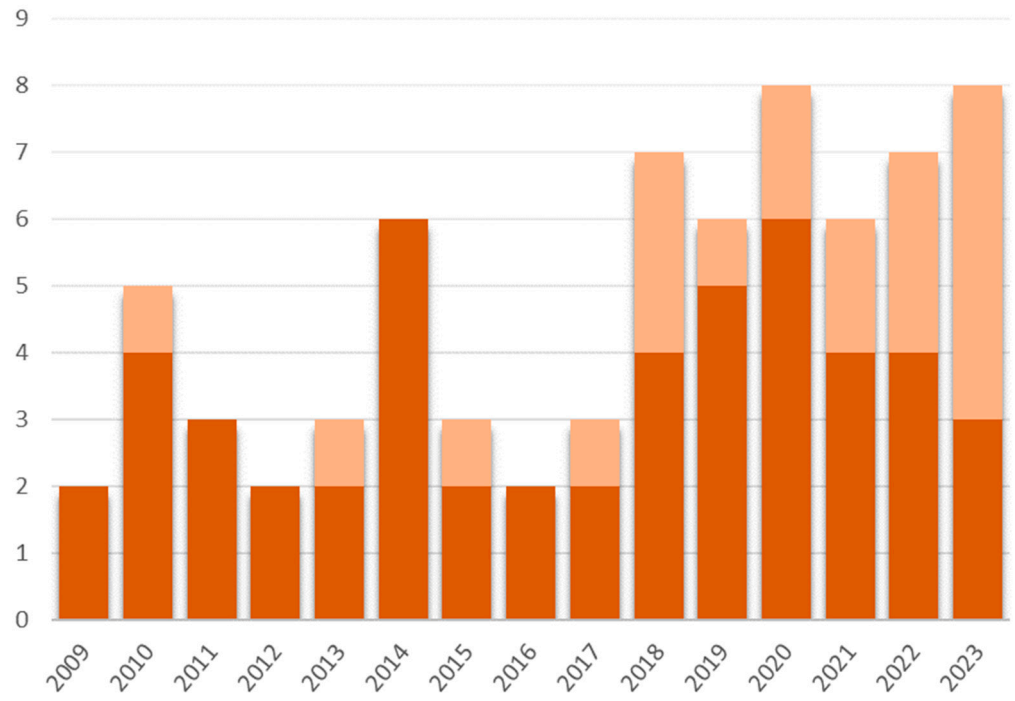

Figure 6. Number of publications reviewed in the present study, by year, presenting extracts (pale orange) or pure molecules (bright orange) with strictly anti-biofilm action.

Most studies on anti-biofilm activities of marine natural resources have been published in the last 15 years with more than 60% of articles after 2016 (Figure 6). The most recent articles even present extracts or culture supernatants for which the molecules have not yet been described.

Despite the fact that these compounds are of marine origin, the majority of the authors have tested their activities against biofilms of human pathogenic bacteria such as *E. coli*, *Salmonella*, and *Streptococcus mutans*, and in 50% of cases against *P. aeruginosa* and *S. aureus*, which are models for bacterial biofilm studies. The most targeted marine bacterial genus is *Vibrio*, to which belong the main pathogens in marine environments.

P. aeruginosa and *Staphylococcus* were chosen because of the wealth of data available on these strains and because they are a major problem in health care. In fact, they are used

as models in many areas of research, and the QS in *P. aeruginosa* was one of the first to be described, along with that of *Vibrio*. Moreover, a large amount of molecular data on biofilm formation mechanisms is available, making it easier to understand the mechanisms of action of the identified anti-biofilm molecules.

To take our synthesis work one step further, we decided to compare the molecules using Datawarrior ® software version 5.5.0 [86]. The structural similarities were first studied and the clusters identified logically represented the different classes of molecules described. However, no structural similarities were shown, which could not explain their shared anti-biofilm activities. This can be explained by the diversity of modes of action that anti-biofilm molecules can have. In fact, even though they are grouped under a single name, their actions can be totally different. As mentioned earlier, they can affect bacterial communication, disrupt the adhesion process, or degrade matrix polymers.

Based on their structures, by calculating different parameters of these molecules such as logP or Druglikeness (DL) score, those presenting the best potential for drug development could be highlighted. In fact, logP is a good indicator of bioavailability of molecules in the human body. As an example, for good oral absorption, values of less than 5 are usually preferred. The Druglikeness score is based on the presence of different fragments of the molecule compared to a collection of fragments of commercial drugs and compounds. A positive score indicates that the molecule under investigation contains mostly fragments found in marketed drugs. Out of the 50 molecules described, only 8 have a clogP of less than 5 and a positive Druglikeness score. Toxicity tests are not always performed in studies, but could be used to discriminate between a larger number of candidate drugs. It is important to note that logP is included in the calculation of the Druglikeness score, but sometimes the LD score is reduced by the lipophilicity of certain molecules. However, with current galenic formulation techniques, and depending on the intended application, these molecules should not be excluded (Table 2).

However, these assumptions are theoretical and would require laboratory testing to determine the actual toxicity of the compounds. Indeed, among the eight molecules that could be considered as potential best candidate drugs, there are the makaluvamines, which are nonetheless known for their toxicity [87,88].

Table 2. Analysis of the logP values and Druglikeness scores of the molecules using Datawarrior® software version 5.5.0. (In blue: molecules with logP < 5 and Druglikeness score > 0).

Compound Family.	Compound Number	Compound Name	LogP	Druglikeness Score
Peptides and proteins	**1**	*cis*-cyclo(Leucyl-Tyrosyl)	**1.1773**	**4.294**
	2	Paracentrin 1	/	/
	3	Nesfactin	4.0566	−31.67
	4	Cyclo(*L*-Trp-*L*-Ser)	1.8772	4.4232
Phenolic compounds	**5**	2,4-di-*tert*-butylphenol	4.4777	−5.276
	6	Methyl benzoate	1.5726	−3.9278
	7	Methyl phenylacetate	1.5707	−6.9825
Alkaloids	**8**	Psammaplin A	**4.2446**	**1.5181**
	9	Bisaprasin	8.4888	1.5181
	10	Ageloxime D	3.0262	−5.0562
	11	Maipomycin A		
	12	Isonaamine D	**2.4565**	**2.5205**
	13	Isonaamidine A	**1.6476**	**4.386**

Table 2. *Cont.*

Compound Family.	Compound Number	Compound Name	LogP	Druglikeness Score
Alkaloids	**14**	2,2-*bis*(6-bromo-1*H*-indol-3-yl)ethanamine	4.1514	−1.8628
	15	2,2-*bis*(6-fluoro-1*H*-indol-3-yl)ethanamine	2.9026	−1.4128
	16	Makaluvamine A	**−0.3414**	**3.1635**
	17	Makaluvamine F	**2.2402**	**2.5254**
	18	Makaluvamine G	**1.0552**	**3.189**
	19	Meridianin D	2.3034	−2.0575
	20	Collismycin C	1.3629	−1.2477
Terpenoids	**21**	α-bisabolol	4.4711	−1.4665
	22	Dolabellanes	5.4304	−3.5032
	23		4.0308	−1.2618
	24		5.0526	−1.8279
	25	Dictyol C	4.0017	−1.8996
	26	Dictyol L	1.1555	−2.9689
	27	Knightal	7.087	−20.275
	28	11(*R*)-hydroxy-12(20)-en-knightal	5.0026	−20.636
	29	11(*R*)-hydroxy-12(20)-en-knightol acetate	5.4872	−16.924
	30	Phorbaketal B	5.0073	−0.61496
	31	Phorbaketal C	5.0073	−0.61496
	32	Ophiobolin K	5.5062	0.094351
	33	6-*epi*-ophiobolin K	5.5062	0.094351
	34	6-*epi*-ophiobolin G	6.3296	−3.2017
	35	Siphonocholin	7.4008	−8.1908
	36	Halistanol sulfate A	1.5225	−5.4372
	37	5-episinuleptolide	1.6808	−17.833
	38	5-octylfuran-2(5*H*)-one	3.2099	−21.892
Fatty acids and derivatives	**39**	(9*Z*)-9-octadecenal	6.8564	−26.022
	40	Arachic acid	7.8801	−25.216
	41	Erucic acid	8.5367	−28.971
	42	(13*Z*)-13-octadecenale	6.8564	−17.802
	43	Tetracosanoic acid	9.6977	−25.216
	44	4-Phenylbutanoic acid	2.0516	−6.2653
	45	Stearidonic acid (18:4 n-3)	5.9625	−19.501
	46	Eicosapentaenoic acid (20:5 n-3)	6.6191	−14.291
	47	Docosapentaenoic acid (22:5 n-3)	7.5279	−20.741
	48	Docosahexaenoic acid (22:6 n-3)	7.2757	−10.83
	49	Mevalonolactone	−0.2323	−0.032673

Table 2. *Cont.*

Compound Family.	Compound Number	Compound Name	LogP	Druglikeness Score
Fatty acids and derivatives	**50**	Myristic acid	5.1537	−25.216
	51	Oleic acid	6.7191	−28.971
	52	Lyngbyoic acid	3.9235	−18.267
	53	Benderadienne	6.2758	−26.52
	54	Pentadecanal	5.7454	−22.307
Polysaccharides	**55**	Fucoidan	−2.6337	−0.043172
	56	MO245	NA	NA
	57	Monomeric units of α-D-galactopyranosyl-(1→2)-glycerol-phosphate	NA	NA
Polyketides	**58**	Hygrocin C	**2.9757**	**2.234**
	59	Secalonic acid D	1.2992	−1.54
	60	Tetracenomycin D	3.1889	−1.1275
	61	Resistomycin	3.7044	−3.2806
	62	Resistoflavin	2.2781	−1.5295

Our review of marine molecules with anti-biofilm activity shows that many teams have discovered anti-biofilm extracts or molecules with bactericidal activity, while others have failed to mention them in detail. If a molecule has a bactericidal effect, it can de facto prevent the appearance of biofilm, but it may not have a curative effect. To truly speak of an anti-biofilm effect, it would be necessary to systematically define whether the dose used has an antibacterial effect, both in preventing and in curing pre-formed biofilm.

One of the major difficulties in research in this field is purification. In fact, many extracts lose their activity after purification. The quantities of molecules extracted may be too small to perform the necessary tests, the interaction between several molecules may be essential, or unsuitable solvents may be used. The importance of culture media is paramount in the production of molecules of interest. In the case of bacterial production, we have seen a very wide variety of media used, making it difficult to harmonize results and predict the type of molecules produced.

Methods for assessing biofilm formation are varied, with some teams using microplates to form a biofilm at the air–liquid interface, while others assess biofilms formed in microfluidics, magnetic beads, or plots. With such a wide variety of media, techniques, and solvents used for purification, there are countless opportunities for discovery or, conversely, lack of discovery.

The standardization required to harmonize results seems difficult to achieve, except perhaps in a large company, but it not be desirable, because it would ultimately limit the discoveries that fundamental science has to offer.

All these data show that there is still a lot of work to be done on marine anti-biofilm molecules and that this field has significantly evolved over the last 15 years.

4. Conclusions

Antibiotics are currently the main therapeutic solution used to combat bacterial infections. However, their massive and abusive use over the last 60 years has led to the development of multi-resistant bacteria, which are found all over the world, regardless of species.

The presence of bacteria in the form of biofilms leads to chronic and persistent infections, which in turn leads to the massive use of antibiotics. There is therefore an urgent

need to find molecules with anti-biofilm activity that would limit their formation and help the immune system to fight the infection.

As shown above, natural marine products are a major source of metabolites with original skeletons, many of which have yet to be discovered. These secondary metabolites are an important source of potential drug candidates. By linking the various disciplines of fundamental research such as analytical chemistry, organic chemistry, and microbiology with knowledge of ecosystems, particularly chemical ecology, it becomes easier to find molecules of interest. The risk of rediscovery is always present, but has been reduced by the emergence of various techniques derived from analytical chemistry, such as metabolomics and the use of molecular networks.

This review highlighted the importance of distinguishing strict anti-biofilm molecules or extracts from those with antibacterial activities. It is astonishing to find only around sixty strict anti-biofilm molecules over more than a decade. Combining anti-biofilm and bactericidal tests is therefore of real importance. Bacteria and fungi appear to be interesting sources in the field of anti-biofilm molecules, not only because of the durability of their source, but also because of the possibility of accessing their genomes. Indeed, biosynthetic pathways of molecules of interest can therefore be studied, allowing an improvement in their production through biotechnological engineering.

The druggable aspect is interesting, but should not put an end to studies on less druggable molecules, given the subsequent possibilities for galenic formulation to improve bioavailability.

It is already possible to observe promising molecules showing activity on highly problematic multi-resistant bacteria such as *S. aureus* and *P. aeruginosa*, without showing any activity on the growth of planktonic bacteria. The information that is generally lacking relates more to the modes of action of these molecules, which can be very wide-ranging.

These compounds, which are generally active at low concentrations, should have negligible or no side-effects on patients, animals, or the environment, and should make it possible to limit antibiotic resistance linked to selection pressure.

It would be interesting to work on the terminology of the term "anti-biofilm" and add categories according to the mode of action of the molecules, if this is known. Furthermore, the distinction between antibiotics and anti-biofilms seems essential at a time when antibiotic resistance is such a major issue. A biocidal activity test would therefore seem to be an essential prerequisite for any research into anti-biofilm activity.

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ARTICLES FOR FACULTY MEMBERS

ELUCIDATION OF ANTIBIOFILM FORMATION MECHANISMS USING MARINE EXTRACTS AGAINST INFECTION AND ANTIMICROBIAL RESISTANCE (AMR)

High reduction of staphylococcal biofilm by aqueous extract from marine sponge-isolated Enterobacter sp. / Nunes, S. de O., Rosa, H. da S., Canellas, A. L. B., Romanos, M. T. V., dos Santos, K. R. N., Muricy, G., Oelemann, W. M. R., & Laport, M. S.

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Original Article

High reduction of staphylococcal biofilm by aqueous extract from marine sponge-isolated *Enterobacter* sp.

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ABSTRACT

Staphylococcus aureus and *Staphylococcus epidermidis* are among the most important bacterial species responsible for biofilm formation on indwelling medical devices, including orthopaedic implants. The increasing resistance to antimicrobials, partly attributed to the ability to form biofilms, is a challenge for the development of new antimicrobial agents. In this study, the cell-free supernatant obtained from sponge-associated *Enterobacter* strain 84.3 culture inhibited biofilm formation (>65%) and dissociated mature biofilm (>85%) formed by *S. aureus* and *S. epidermidis* strains. The culture supernatant was subjected to solvent partitioning and the aqueous extract presented a concentration-dependent antibiofilm activity for each strain with a minimum biofilm eradication concentration (MBEC) ranging from 16 to 256 μg/mL. The effect of the aqueous extract on mature *S. aureus* biofilm was analyzed by confocal scanning laser microscopy, showing a significant reduction of the biofilm layer as well as diminished interactions among the cells. This extract is not toxic for mammalian cells (L929 cell line). Studies targeting substances with antibiofilm activity gained significant attention in recent years due to difficult-to-treat biofilm infections. Here, sponge-associated *Enterobacter* 84.3 proved to be a source of substances capable of eradicating staphylococcal biofilm, with potential medical use in the future.

1. Introduction

Staphylococci are currently the major agents of medical device-related infections [1,2]. In orthopaedic prosthetic infections, the most commonly Gram-positive cocci isolated are *Staphylococcus aureus* and coagulase-negative staphylococci (CNS) [3]. *S. aureus* strains causing implant infections show high rates of antimicrobial resistance, and there is an alarming increase of antimicrobial resistance observed in other species, such as *Staphylococcus epidermidis* [1,3,4]. Implant infection causing bacteria generally form biofilms, in which bacterial aggregates tightly adhere to the biomaterial surface and are responsible for the persistence of implant infections. Biofilm serves as a bacterial strategy to evade the host's immune system and is often a source of bacterial spread to other body sites. Biofilms show an inherent resistance to antimicrobials [5,6] and an increased rate of horizontal genetic transfer leading to the acquisition and spread of multidrug-resistance [1–3].

Today there are no therapies that effectively target staphylococcal biofilms, and none of the known modulators of virulence has been approved yet for clinical use [6]. This indicates the need for new antibiofilm molecules. Marine sponge-associated bacteria represent a still underexploited source of biodiversity able to synthesize a broad range of bioactive substances, including antibiofilm agents with good performance against clinical isolates [7–10].

In a previous study, we built a collection of culturable marine sponge-associated bacteria isolated from samples of the marine sponge *Oscarella* spp. from Cabo Frio, SE Brazil [11]. In search of potential antimicrobial substances, 85 bacterial isolates were tested against indicator bacteria of medical importance in agar

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diffusion assays, where cells experience *quorum sensing*. Twenty-seven (31.7%) of them showed inhibitory activity against at least one clinical indicator strain assayed, including some biofilm producers [11]. Aiming to find substances that eradicate biofilm rather than bacterial growth, supernatants of the remaining 58 strains grown under planktonic conditions were evaluated for antimicrobial activity against the reference strains *S. aureus* ATCC 29213, *S. aureus* ATCC 25923 and *S. epidermidis* ATCC 35984. The cell-free supernatant from *Enterobacter* strain 84.3 showed no inhibitory activity against the staphylococcal strains (data not shown). Thus, this strain was selected as potential producer of antibiofilm agents, because these agents may impose a weaker selective pressure for the development of drug resistance relative to current antibiotics. Besides, antibiofilm substances may be co-administered with antibiotics and some promising results *in vitro* have been reported [12]. Therefore, in this study *Enterobacter* strain 84.3 was screened for the capacity to inhibit biofilm formation and to dissociate mature biofilm of *Staphylococcus* spp. reference strains as well as clinical strains.

2. Materials and methods

2.1. Bacterial strain and culture condition

Sponge-associated *Enterobacter* strain 84.3 (MK780772.1) was isolated from the Brazilian sponge *Oscarella* spp. as previously described [11]. Cell-free culture supernatants were prepared by inoculating 10^7 cells of *Enterobacter* 84.3 in 3 mL of Brain Heart Infusion (BHI) medium (Difco, MI, USA). After incubation at 25 °C for 24 h, 22 mL of BHI containing 1% glucose were added and the culture further incubated at 25 °C for 48 h. After centrifugation at 4000×*g* for 20 min (Eppendorf centrifuge 5804 R, Hamburg, Germany) the supernatant was sterilized by filtration (Millipore 0.22 μm) and kept at 4–7 °C until use.

Six strains classified as strong biofilm producers were included in the study as indicator strains and positive control for the antibiofilm assays: three reference strains of the American Type Culture Collection (ATCC), *S. aureus* ATCC 29213, *S. aureus* ATCC 25923 and *S. epidermidis* ATCC 35984; and three clinical strains isolated from orthopaedic prosthetic infections, *S. aureus*1117a and *S. aureus*11123a, and from surgical site infection, *S. epidermidis* 24 [13,14]. All *Staphylococcus* strains were grown on BHI agar for 18 h at 37 °C or in BHI broth supplemented with 1% glucose (BHIg).

2.2. Metabolite extraction

Metabolite extraction was performed as previously described [15] with minor modifications. Briefly, total biomass obtained from a 500 mL culture in BHI medium was centrifuged (11,000 × g for 15 min). The supernatant was collected and thoroughly mixed with 250 mL of ethyl acetate (Tedia, OH, USA) in a separation funnel and partitioned overnight into ethyl acetate and aqueous phases. After removal of the upper organic phase, partition of the aqueous phase was repeated twice with 250 mL each of ethyl acetate. The pooled organic phases were evaporated to dryness in a rotary vacuum evaporator (HeiVap, Heidolph, Schwabach, Germany) and the weight was determined. The delipidated aqueous phase was filtered (Millipore 0.22 μm), lyophilized and weighed. For the assays, the lipid extract was dissolved in BHIg supplemented with 10% dimethyl sulfoxide (DMSO) at a concentration of 5 mg/mL and the lyophilized aqueous residue at the same concentration in BHIg medium.

2.3. Antibiofilm activity

2.3.1. Cell-free supernatant

Marine *Enterobacter* culture supernatant was evaluated for its capacity to either inhibit biofilm formation or to disrupt biofilm previously formed by the reference and clinical strains. Antibiofilm activity was performed as previously described [16] with modifications. Briefly, in the wells of a 96-well microtiter plate (TPP, Trasadingen, Switzerland) 20 μL of the indicator strain culture (~10^8 CFU/mL) were mixed with either 180 μL of the cell-free supernatant or 180 μL of BHIg medium (negative control). All tests were carried out in triplicate and the plate was incubated aerobically for 24 h at 37 °C. After incubation, the wells were washed three times with phosphate-buffered saline (PBS; pH 7.4). The remaining attached bacteria were fixed by exposure to hot air at 60 °C for 60 min, and the wells were stained with 0.2% crystal violet for 15 min at room temperature. The plate was air-dried, and the dye retained by adherent cells was dissolved in 95% ethanol. The optical density of each well was measured at 570 nm using a microtiter plate reader (model 680, Bio-Rad Laboratories, Hertfordshire, UK). The inhibition of biofilm formation of each strain was expressed as percentage of biofilm reduction, by applying the following formula: $(OD_{control} - OD_{sample}/OD_{control}) \times 100$. Three independent experiments were performed in triplicate and therefore each data point was averaged from a total of nine values obtained and the standard deviation (SD) was calculated.

Enterobacter 84.3 supernatant was also evaluated for its capacity to dissociate mature biofilm produced by the reference strains. As mentioned above, three independent experiments were carried out in triplicate. Twenty microliters of indicator bacteria (~10^8 CFU/mL) in 180 μL of BHIg were loaded into wells of a microtiter plate and incubated at 37 °C for 24 h. Planktonic cells were removed by washing the plate three times with PBS. To the remaining preformed staphylococcal biofilm, 200 μL of the cell-free supernatant were added per well. Control samples of each staphylococcal biofilm were kept without the addition of cell-free supernatant. After another 24 h at 37 °C the plates were again washed in PBS, heat-fixed and stained as described above. The ability to dissociate biofilm was also expressed as antibiofilm activity (%), by applying the above-mentioned formula.

2.3.2. Extracts

To determine the antibiofilm activity, the extracts were tested in serial dilutions ranging from 1024 to 1 μg/mL in BHIg medium and used for antibiofilm activity assays as described above with few modifications. In a 96-well microtiter plate containing 20 μL (~10^8 CFU/mL) of each reference strain culture was inoculated with the BHIg containing the diluted extracts or in BHIg without addition of the extracts (negative control wells). All tests were carried out in triplicate and the plate was incubated aerobically for 24 h at 37 °C. After incubation plates were treated as described in section *2.3.1*.

The extracts diluted in BHIg were also evaluated for their capacity to dissociate mature biofilm of the reference strains, as described for the cell-free supernatant in the previous section.

The minimum biofilm eradication concentration (MBEC) was determined considering the lowest concentration of each extract capable of inhibiting biofilm formation or dissociating mature biofilm calculated by applying the formula shown in section *2.3.1*.

2.3.3. Statistical analysis

For biofilm production, statistical analyses were performed on raw optical density data of three biological replicates. An unpaired Student's t test was performed to determine the *p*-values between control and treatment by GraphPad Prism 8 (GraphPad Software,

Inc., CA, USA). Results with a p-value ≤ 0.05 were considered statistically significant.

2.4. Confocal microscopic observation of antibiofilm activity

In order to directly observe the multicellular structures in the biofilm in the presence or absence of bioactive extract, Confocal Laser Scanning Microscopy (CLSM), with specific fluorescent markers using the Filmtracer™ LIVE/DEAD™ Biofilm Viability Kit (Thermo Fisher Scientific, MA, USA) was performed according to the instructions of the manufacturer. As previously described, aliquots of overnight culture (~10^8 CFU/mL) of *S. aureus* ATCC 25923 strain were distributed in chamber slides (Nunc Inc., IL, USA) for CLSM observations. After incubation at 37 °C for 24 h, bacteria that remained in suspension were removed by aspiration and the remaining adherent cells on the slide were washed with sterile distilled water and treated with the bioactive extract at the previously established MBEC. Untreated biofilm control was included on each slide. After heat fixation, cells were incubated with SYTO 9 and propidium iodide nucleic acid stains provided in the Filmtracer™ LIVE/DEAD™ Biofilm Viability Kit. Samples were observed on a Zeiss Axioplan microscope (Carl Zeiss, Jena, Germany) equipped for fluorescence microscopy. Live bacteria with intact cell membranes show green fluorescence and dead bacteria with compromised membranes show red fluorescence.

2.5. Cytotoxicity bioassays

A microassay for cytotoxicity in the L929 cell line (mouse fibroblast) was performed with the method known as "dye-uptake", using neutral red dye [17], with minor modifications. One-hundred microliters of L929 cell line suspension at a concentration of 5×10^5 cells/mL were seeded in 96-well microplates. Cells were incubated for 24 h at 37 °C in a 5% CO2 atmosphere to allow attachment. The active extract was added to the cell culture at concentrations ranging from 500 μg/mL to 7.8 μg/mL, and the cells were incubated for 24 h at 37 °C in a 5% CO_2 atmosphere. Then, 100 μL of 0.01% neutral red solution were added for 2 h at 37 °C in a 5% CO_2 atmosphere. After incubation, the medium was removed and the cell monolayers washed with PBS and the dye incorporated by the viable cells was eluted using a mixture of methanol/acetic acid/water (50:1:49). The dye uptake was determined by measuring the optical density of the eluate at 490 nm in an automatic spectrophotometer (ELx800TM, Bio-TeK Instruments, Inc., VT, USA). The 50% cytotoxic concentration (CC_{50}) was defined as the concentration of the extract which caused 50% reduction in the number of viable cells.

3. Results

3.1. Effects of enterobacter 84.3 supernatants on biofilms

Antibiofilm activity was evaluated by microtiter plate assay using the cell-free supernatants obtained from *Enterobacter* strain 84.3 cultures. The results indicated that the marine strain produces substances able to reduce biofilm formation and to dissociate mature biofilm formed by the tested indicator strains. For all reference strains, the cell-free supernatant inhibited biofilm formation at a high percentage, with a maximum value of 93.4% for *S. aureus* ATCC 25923, followed by 93.0% for *S. aureus* ATCC 29213 and of 89.0% for *S. epidermidis* ATCC 35984 (Fig. 1A–C). Biofilm formation by the three clinical strains isolated from orthopaedic prosthetic or surgical site infections was also inhibited and ranged from 80.1% for *S. aureus* 11123a over 82.9% for *S. aureus* 1117a to 66.2% for *S. epidermidis* 24 (Fig. 1D–F).

In addition to inhibiting biofilm formation, the *Enterobacter* 84.3 culture supernatant showed a high capacity to dissociate mature biofilm formed by *Staphylococcus* spp. strains. The biofilm eradication levels were similar to those observed for inhibitory activity of biofilm formation. Eradication activity was 86.1% for *S. aureus* ATCC 25923, 85.3% for *S. aureus* ATCC 29213 and 76.1% for *S. epidermidis* ATCC 35984 (Fig. 2).

3.2. Antibiofilm activity of aqueous extract

To investigate whether the antibiofilm activity of cell-free supernatant from *Enterobacter* 84.3 culture might be due to the lipidic or aqueous fraction, the respective fractions were evaluated at different concentrations. No inhibition or dissociation effect on the biofilms was observed for the lipid extract. However, the aqueous extract acted by inhibiting and dissociating biofilms of the reference strains at all concentrations tested (from 1 to 1024 μg/mL) and antibiofilm activity rates and the MBEC are listed in Table 1.

For all reference strains, the aqueous extract inhibited biofilm formation at a high percentage (Fig. 3A–C). *S. aureus* ATCC 25923 biofilm formation was inhibited by 94.0% in the presence of the 16 μg/mL of the aqueous extract and it was dissociated by 85.3% when treated with 32 μg/mL of the same bioactive extract. Furthermore, a strong inhibitory effect (≥77%) on biofilms has also been observed against *S. aureus* ATCC 29213 and *S. epidermidis* ATCC 35984, as well as promising disruption rates (≥68%) of the mature biofilms.

The aqueous extract showed a concentration-dependent antibiofilm activity both for the inhibitory effect on biofilm formation and for the dissociation effect of mature biofilm from *S. aureus* and *S. epidermidis*. Fig. 3D represents this observation on the mature biofilm of *S. aureus* ATCC 25923. The effect of aqueous extract at 32 μg/mL on pre-formed *S. aureus* ATCC 25923 biofilm was also visualized on glass surfaces by confocal laser scanning microscopic imaging. This approach confirmed that the aqueous extract significantly reduced the biofilm biomass, average thickness, and substrate coverage as compared to the untreated control (Fig. 4), thus having a major effect on mature biofilm.

3.3. Cytotoxicity of the active extract

The aqueous extract was subjected to cytotoxicity assay in order against the mammalian L929 cell line. When the cells were treated with concentration from 7.8 to 500 μg/mL, the observed CC_{50} was higher than the maximum concentration tested (where CC_{50} stands for 50% cytotoxic concentration, defined as the concentration required to reduce the cell number by 50% compared with that for the untreated controls). These results suggest that the antibiofilm substance present in the aqueous extract from *Enterobacter* 84.3 is not toxic for L929 cells. Thus, as the CC_{50} for mammalian cells was higher than the observed MBEC (16–128 μg/mL) for *S. aureus* strains, the use of this substance for inhibiting or dissociate biofilms could be recommended in the future.

4. Discussion

Staphylococci are currently the most common cause of nosocomial infections, mainly related to orthopaedic devices on which the bacteria form biofilm [3–5]. Staphylococcal biofilms are preserved from host defenses and often display dramatic decrease in antimicrobial susceptibility, resulting frequently in the development of persistent and chronic infections [3,5].

In the past years many antibiofilm agents have been identified from various sources, including from sponge-associated bacteria, which represent promising producers of substances for

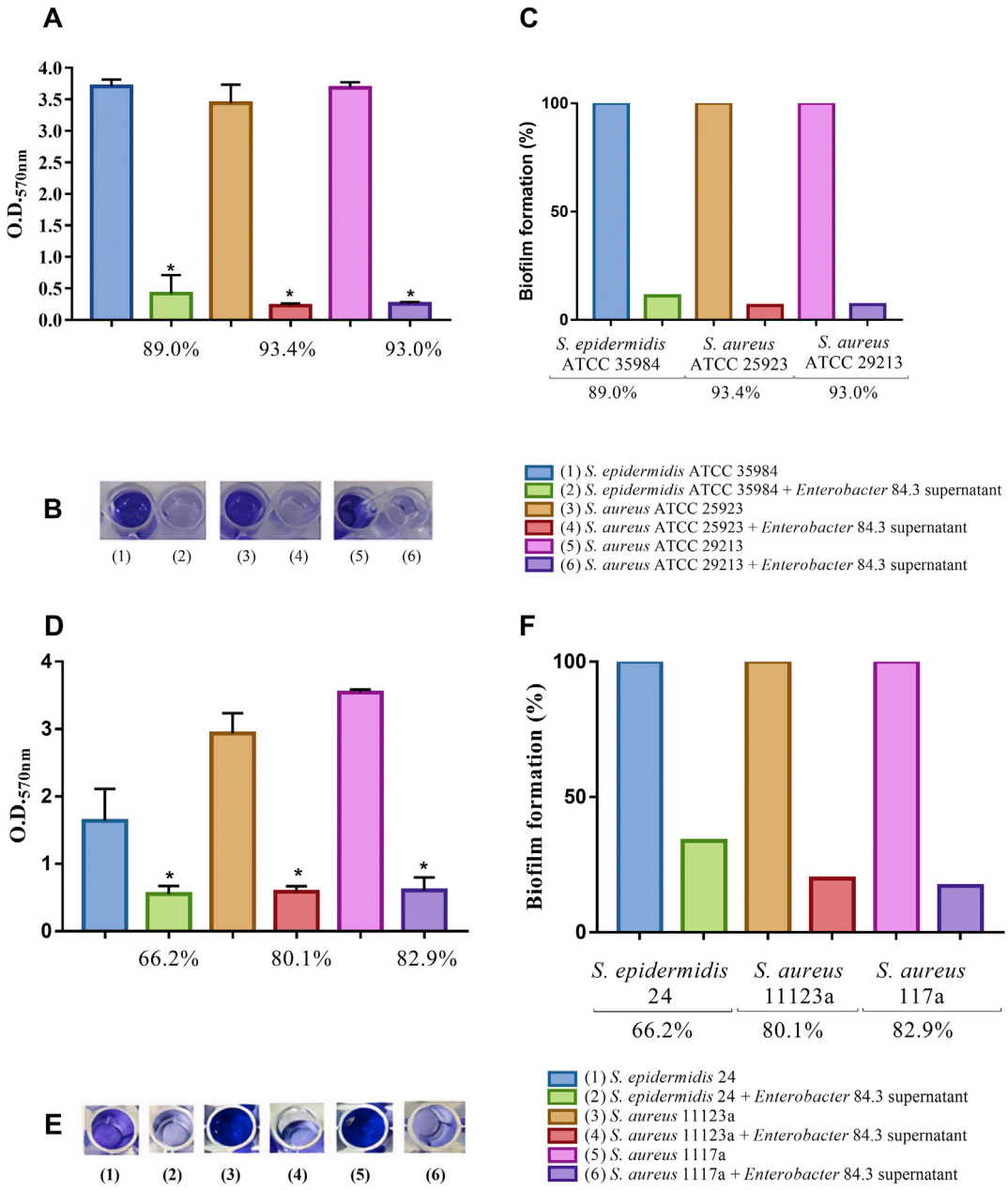

Fig. 1. Antibiofilm activity of the cell-free supernatant from *Enterobacter* sp. 84.3 culture on *Staphylococcus* spp. biofilm formation. Biofilm produced in absence of supernatant was used as control. The inhibition of biofilm formation is expressed as optical density of each well measured at 570 nm (A and D), *$p < 0.0001$. The ratio of biofilm absorbance/planktonic absorbance was calculated and this value used to calculate the "biofilm formation" in percentage on the y axis (C and F). Wells of 96-well plate showing biofilms in the absence and presence of supernatant after crystal violet staining (B and E). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

biotechnological and pharmaceutical applications [7–10,18–21]. However, none of them has entered the market. Hence, there is a large unmet need for the development of antibiofilm formulations to tackle this problem [7].

In a previous study, antibacterial tests were performed with bacteria isolated from *Oscarella* sponges and their cell-free culture supernatants [11], and the marine strain *Enterobacter* 84.3 was selected because no bacteriostatic or bactericidal activity against the target strains were observed, in spite of strong antibiofilm activity. The current study aimed to analyze for the first time the antibiofilm activities of sponge-associated *Enterobacter* 84.3 on staphylococcal biofilms. Our findings showed that *Enterobacter* 84.3 substances were particularly effective at both inhibiting biofilm development and disaggregating the mature biofilm, but without killing the *Staphylococcus* strains or inhibiting their growth. *S. aureus and S. epidermidis* infections associated with biofilm development are estimated to reach 250,000 cases per year in the USA with a mortality rate of up to 25%. These infections represent an important social and economic burden worldwide [2]. The interest in the development of innovative approaches for prevention and treatment of staphylococcal adhesion and biofilm formation capabilities has therefore increased. A viable approach should target the staphylococcal adhesive properties without affecting bacterial viability in order to avoid the rapid appearance of escape mutants [21,22].

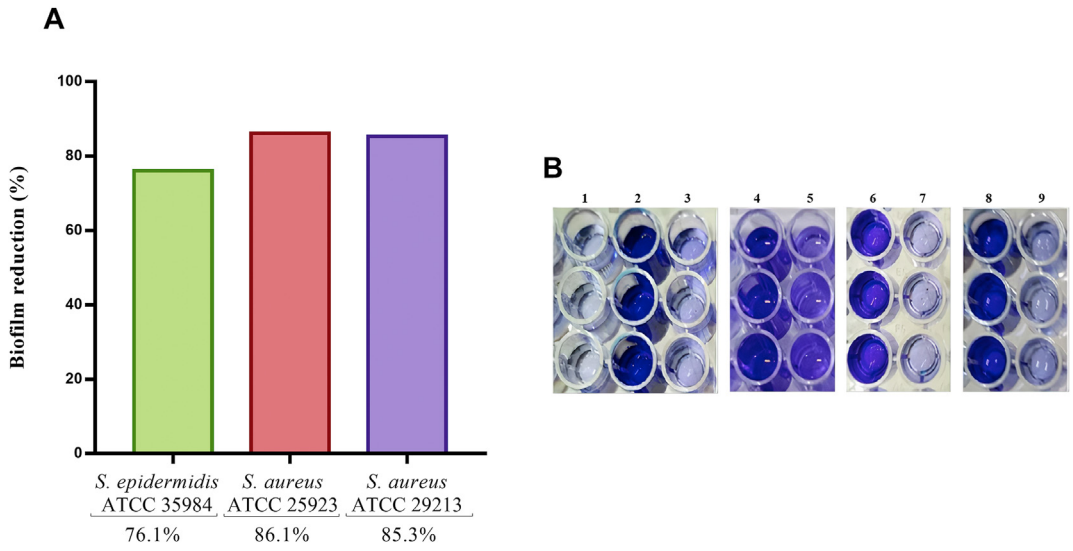

Fig. 2. Antibiofilm activity of the cell-free supernatant from *Enterobacter* sp. 84.3 culture on mature biofilms. Percentages of biofilm biomass reduction of reference staphylococcal strains (A). Wells of 96-well plate showing mature biofilms untreated and treated after crystal violet staining (B). Control wells: 1. sterile growth medium (BHIg); 2. *S. aureus* ATCC 25923, indicator strain producing strong biofilm; 3. only cell-free supernatant. Test wells: 4. *S. aureus* ATCC 25923; 5. *S. aureus* ATCC 25923 treated with cell-free supernatant; 6. *S. aureus* ATCC 29213; 7. *S. aureus* ATCC 29213 treated with cell-free supernatant; 8. *S. epidermidis* ATCC 35984; 9. *S. epidermidis* ATCC 35984 treated with cell-free supernatant. Values below the wells 5, 7 and 9 show the percentages of mature biofilm biomass reduction. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Antibiofilm activity of the aqueous extract from *Enterobacter* sp. 84.3 culture on staphylococcal biofilm.

Strains	Antibiofilm activity	
	Inhibitory rate[a] (MBEC[b] - µg/mL)	
	Inhibition	Dissociation
S. aureus ATCC 25923	94.0% (16)	85.3% (32)
S. aureus ATCC 29213	87.3% (64)	79.6% (128)
S. epidermidis ATCC 35984	77.0% (128)	68.2% (256)

[a] Biofilm reduction rate (%).
[b] Minimum Biofilm Eradication Concentration (MBEC).

Our results showed high rates of antibiofilm activity (from 66% to 94%), including clinical strains isolated from orthopaedic prosthetic and surgical site infections [13,14]. Sayem and colleagues [10] reported that cell-free supernatants obtained from sponge associated *Bacillus licheniformis* SP1 showed inhibition effects of almost 90% on biofilm formation by *S. aureus*. Interestingly, *S. aureus* biofilms were more affected than *S. epidermidis* biofilms, as also observed in our study. In general, the cell-free supernatant from *Enterobacter* 84.3 culture inhibited *S. aureus* biofilm formation by more than 75%, while acting on *S. epidermidis* biofilm at a 10% lower rate. This result could be explained by differences in the extracellular matrix components of each strain, which comprise a complex mixture of polysaccharides, lipids, extracellular DNA (eDNA) and proteins [1,20]. Studies have revealed how distinct constituents of the biofilm matrix contribute to its architectural stability and functionality. These findings will provide the basis for developing novel therapeutics that can effectively target components of the biofilm matrix and modulate biofilm stability [1,5,23].

In this study, we observed that the biofilm degradation activity from *Enterobacter* 84.3 was caused by water-soluble substances. The aqueous extract presented minimum biofilm eradication concentration values smaller than the CC_{50} observed for L929 cells (higher than 500 µg/mL). These are some of the features that make it of great biotechnological interest.

Confocal laser scanning microscopy images also confirmed that the bioactive extract was able to dissociate the mature biofilm. This characteristic shows that the antibiofilm agent could be applied both for prevention of formation and removal of mature biofilm on indwelling medical devices. In addition, its use could also be employed in a combination therapy with antibiotics of interest, since the bacterial cells will end up unprotected from the extracellular matrix (biofilm) [21,22]. This model of action was also proposed for pentadecanoic acid, a molecule derived from pentadecanal produced by the Antarctic bacterium *Pseudoalteromonas haloplanktis* TAC125 [21,24]. Pentadecanoic acid is a good candidate for combined therapies with conventional antibiotics, because it prevented staphylococcal biofilm formation and positively modulated the antimicrobial activity of vancomycin [21].

In the case of infections related to orthopaedic prosthetic biofilm, the cost is very high since it involves long hospitalization time, surgery to change the prosthesis and antimicrobial administration as prophylaxis agents of nosocomial infections [3]. Biofilm dispersal agents generally interfere with chemical pathways or processes, such as *quorum sensing*, which are required for bacteria to maintain the mode of existence as biofilm [5,25]. As dispersed cells are generally more susceptible to antimicrobial treatment than biofilm-residing cells, this strategy has recently become an intense area of study [1,12,21].

Based on the findings, we hypothesize that antibiofilm substances present in aqueous extract might interfere with the cell surface, thus influencing cell–cell interactions as pre-requisite for biofilm formation [23], or with other steps of biofilm assembly. Since the antibiofilm substances are found in the aqueous phase, they might be polysaccharides. Polysaccharides have been previously reported to produce anti-adherence effects between microorganisms and surfaces [10,23].

In conclusion, the cell-free culture supernatant from sponge-associated *Enterobacter* 84.3 contains water-soluble molecules that can provide a tool for better exploration of novel antibiofilm substances. Inhibiting biofilm formation and dissociating mature biofilm of strains of *S. aureus* and *S. epidermidis* without affecting their growth represent a special feature of the antibiofilm activity

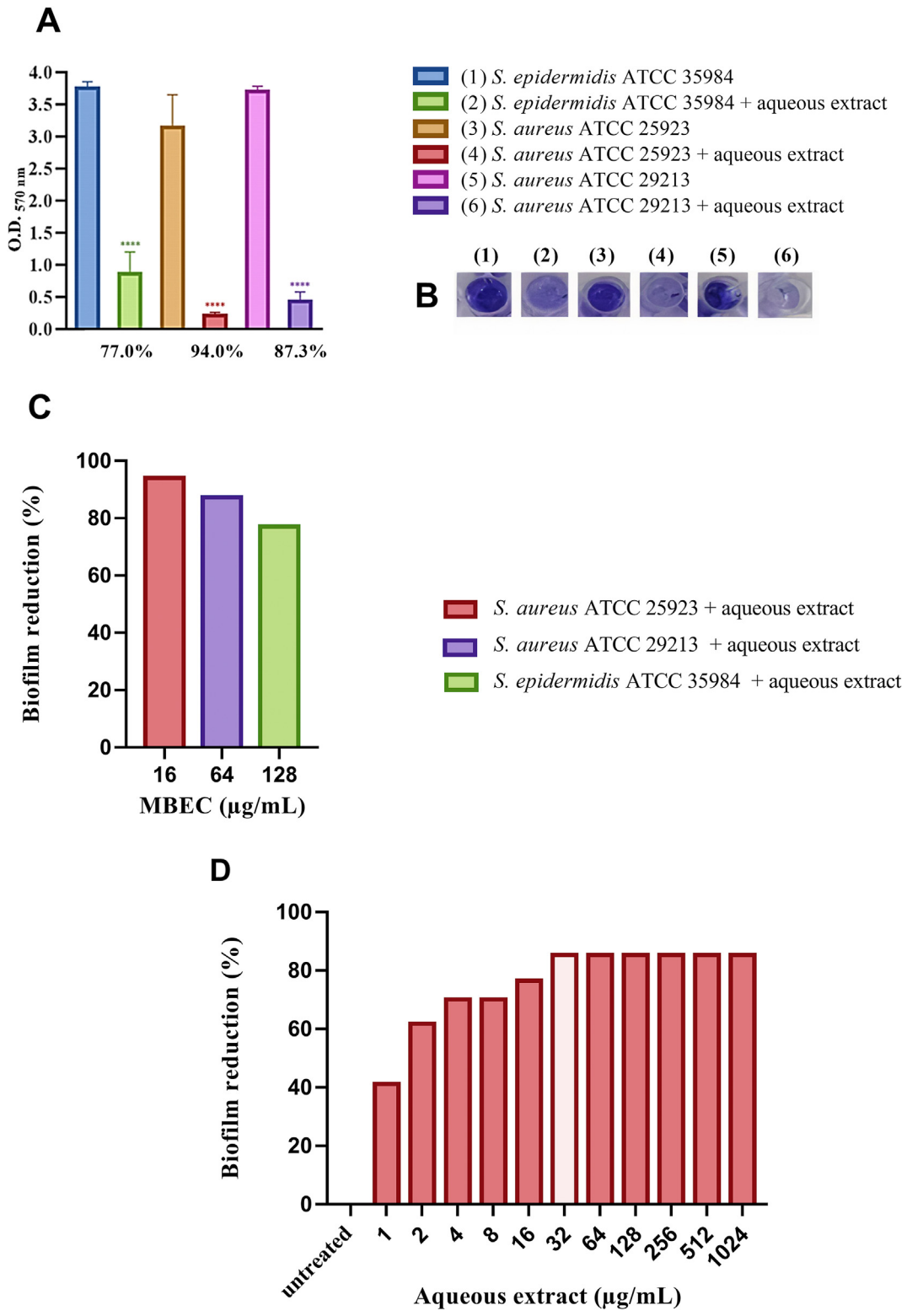

Fig. 3. Antibiofilm activity of the aqueous extract from *Enterobacter* sp. 84.3 culture on *Staphylococcus* spp. biofilm formation. The assays were performed at minimum biofilm eradication concentration (MBEC) for each reference strain and the activity is concentration-dependent. The inhibition of biofilm formation is expressed as optical density of each well measured at 570 nm (A), *$p < 0.0001$. Wells of 96-well plate showing biofilms in the absence and presence of aqueous extract after crystal violet staining (B). The absorbance values were used to calculate the antibiofilm activity in percentage applying the formula shown in section *2.3.1*(C). MBEC was defined at 32 µg/mL on pre-formed *S. aureus* ATCC 25923 biofilm (D). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

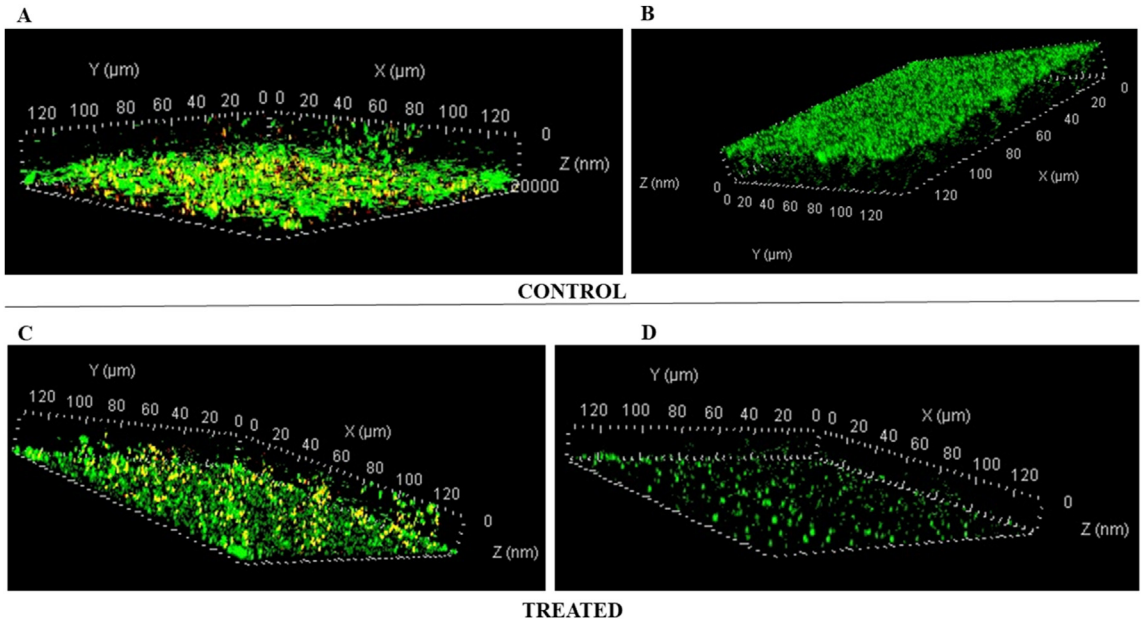

Fig. 4. Representative confocal laser scanning microscopy images of *S. aureus* ATCC 25923 biofilm in the presence of the bioactive aqueous extract (32 μg/mL), visualized by fluorescence vital dye. 3D read out of the stained biofilm: (top) untreated biofilm control, and (bottom) treated biofilm. Biofilm incubated with SYTO 9 and propidium iodide nucleic acid stains (A and C) and with FilmTracer stain (B and D). Green cells and clusters indicate bacterial cells with intact membranes (live). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

described in this report. Further research on such bioactive aqueous extract might help developing new antibiofilm agents active against staphylococcal biofilms on indwelling medical devices.

Declaration of competing interest

No conflict of interest is declared.

Acknowledgements

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ARTICLES FOR FACULTY MEMBERS

ELUCIDATION OF ANTIBIOFILM FORMATION MECHANISMS USING MARINE EXTRACTS AGAINST INFECTION AND ANTIMICROBIAL RESISTANCE (AMR)

Marine-derived bioactive materials as antibiofilm and antivirulence agents / Jeong, G. J., Khan, F., Tabassum, N., Cho, K. J., & Kim, Y. M.

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Trends in Biotechnology

Review

Marine-derived bioactive materials as antibiofilm and antivirulence agents

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Microbial infections are major human health issues, and, recently, the mortality rate owing to bacterial and fungal infections has been increasing. In addition to intrinsic and extrinsic antimicrobial resistance mechanisms, biofilm formation is a key adaptive resistance mechanism. Several bioactive compounds from marine organisms have been identified for use in biofilm therapy owing to their structural complexity, biocompatibility, and economic viability. In this review, we discuss recent trends in the application of marine natural compounds, marine-bioinspired nanomaterials, and marine polymer conjugates as possible therapeutic agents for controlling biofilms and virulence factors. We also comprehensively discuss the mechanisms underlying biofilm formation and inhibition of virulence factors by marine-derived materials and propose possible applications of novel and effective antibiofilm and antivirulence agents.

Highlights

Microbial infections are the most significant threats to human health, and there has been a recent increase in the number of deaths exclusively attributed to bacterial and fungal infections.

The development of biofilms by bacterial and fungal pathogens has been identified as a key adaptive antimicrobial resistance (AMR) mechanism, in addition to intrinsic and extrinsic AMR.

Various bioactive molecules have been isolated from marine organisms for use in biofilm therapies. These compounds are structurally complex, biocompatible, and economically viable.

Studies have demonstrated that bioactive chemicals obtained from marine sources, marine-bioinspired nanomaterials, and marine polymer-antibiotic conjugates can control the biofilms and virulence factors of microbial pathogens.

Biofilm infection: significance and control

Biofilm (see Glossary)-related health issues have a global economic burden of approximately USD 500 billion annually [1]. The National Institutes of Health in the USA believes biofilms cause more than 80% of microbial infections in humans, whereas the Centers for Disease Control and Prevention estimates that biofilms cause 60% of hospital-acquired infections [2]. Approximately USD 1 billion is projected to be the annual cost of biofilm-associated catheter infections worldwide [1]. Thus, biofilm-related issues are a global concern for human health, and their widespread impact across various sectors has led to increased awareness of the importance of biofilm control. Biofilm is a population of microorganisms that adheres to the surface of biotic or abiotic substances and may be composed of a single or numerous species. Biofilm formation is an important virulence mechanism in various pathogens, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus mutans*, and *Candida albicans* [3–6]. The process by which biofilms form comprises a series of phases that begins with the formation of aggregates on a surface by **planktonic cells**, followed by the adhesion and production of extracellular polymeric substances (EPS) [7]. EPS consist of lipids, proteins, polysaccharides, and extracellular DNA (eDNA) and are responsible for anchoring biofilm cells and keeping them intact. Additionally, EPS are responsible for stabilizing biofilm cells via a series of intensive interactions, including cell–cell communication, gene transfer, and development of synergistic microconsortia [8]. **Quorum sensing (QS)**, a cell–cell communication process, plays an important role in biofilm formation and virulence factor production. The QS system regulates gene expression by detecting changes in cell density [9]. In particular, the QS system of Gram-negative bacteria is based on the autoinducer (AI) acylated homoserine lactone and governs the synthesis of many virulence factors, such as pyocyanin, lectin, elastase, and protease [10,11]. Furthermore, the QS system affects the formation of biofilms and protects them against external stresses, such as host immunity and antibiotic treatment [12,13]. In addition, shear stress, a physical force related to flow conditions, increases the concentration of signaling molecules in biofilms, and the QS response to

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these signaling molecules is the expression of genes involved in adhesion, contributing to stronger cell adhesion [14,15].

Therefore, strategies to control biofilms (Box 1) have become a crucial research topic. One of the most significant obstacles preventing the effective treatment of biofilm infections is the fact that biofilm cells exhibit several distinct morphological, biochemical, metabolic, and genetic properties that are different from those of planktonic cells. Enhanced resistance to antimicrobial drugs is a consequence of these characteristics of biofilm cells and their environments [16]. The EPS layer of biofilms limits the penetration of existing antibiotics and reduces their effectiveness [17,18]. Different concentration gradients of oxygen, nutrients, ions, and chemicals result in phenotypic heterogeneity within biofilms, with cells located in the bottom layer of biofilms having lower metabolic activity than cells located in the top surface layer, thereby contributing to antibiotic resistance [19,20]. The formation of persister cells is another mechanism of antibiotic resistance. Persister cells within biofilms enter a dormant state, allowing them to survive under stress conditions and not be targeted by antibiotics [21,22]. Moreover, standard antibacterial treatments are primarily directed against planktonic cells and may not be effective against pathogenic biofilms, which are estimated to be 1000 times more resistant than planktonic cells [1]. Furthermore, antibiotic misuse contributes to the development of drug resistance and exacerbates biofilm-related diseases. Exposure of microorganisms to subinhibitory concentrations of antibiotics increases or decreases their ability to form biofilms and susceptibility to antibiotics [23,24]. Therefore, novel methods to control biofilms require further exploration. In general, natural products are more structurally complex and 3D than synthetic drugs, making them beneficial for creating antibiofilm therapies. These products are uncommon in screening libraries based on synthetic compounds

Glossary

Biofilm: microorganism communities with complex structures that adhere to biotic or abiotic surfaces. These cells are encased by biofilm matrixes, which are self-produced polymeric substances.
Nanoparticles (NPs): materials with a 1–100 nm diameter have better surface or interface characteristics than the bulk. These qualities provide them with excellent optical, biological, electrical, thermal, and magnetic properties.
Planktonic cells: microorganisms that float freely. The adherent or sessile mode is the opposite of the planktonic form.
Polymer-antibiotic conjugates: substances that function as a drug delivery system by binding an antibiotic to a polymer carrier. These conjugates encase the drug in a polymer material and deliver it to the desired target.
Quorum sensing (QS): a cell–cell communication process that causes fluctuations in gene expression in response to changes in cell population density and adverse environmental cues. This process produces, releases, and detects autoinducers, which are extracellular signaling molecules, and subsequently acts on them at the population level.

Box 1. Antibiofilm and antivirulence strategies

Several antibiofilm and antivirulence strategies have been proposed (Figure I).

- EPS in biofilms plays a role in protecting microorganisms from various antibiotics; therefore, numerous biofilm treatment strategies have targeted the EPS matrix [95,96]. Biofilm dispersants, such as β-glucosidase, amylase, protease, and nuclease enzymes, destroy EPS, resulting in the dispersal of biofilm cells (Figure IA).
- Early-stage biofilm formation is significantly influenced by bacterial adhesion, which plays a significant role in development. Abiotic surface features and bacterial surface appendages such as flagella and fimbria all have a role in determining the degree to which bacteria adhere to one another. Therefore, antiadhesion strategies include the modification of abiotic surfaces in a certain manner to restrict the adherence of cells by preventing the surface binding of flagella and fimbria (Figure IB) [97,98].
- Since discovering that the QS system is responsible for the regulation of biofilm development and generation of virulence factors in certain microbes, QS inhibitors have been developed to alleviate the QS system (Figure IC) [11,35]. QS inhibitors reduce biofilm formation by (i) inactivating AIs, (ii) inhibiting AI production, (iii) inhibiting intercellular signaling using AI antagonists, and (iv) inhibiting signaling cascades by interfering with response regulators.
- Some antibiofilm drugs prevent the replication of Gram-positive bacteria by inhibiting the biosynthesis of wall teichoic acid and lipoteichoic acid [99]. Surface adhesion caused by electrostatic interactions of charged amino acids in the wall, including teichoic acid, and lipoteichoic acid, leads to biofilm development (Figure ID).
- As the second messenger cyclic di-GMP (c-di-GMP) plays a role in biofilm development in some microbes, certain drugs inhibit biofilm formation by interfering with c-di-GMP production (Figure IE) [100–102]. Diguanylate cyclases (DGCs) and phosphodiesterases (PDEs) control biofilm development by detecting diverse input signals. Therefore, newly discovered antibiofilm drugs should target DGCs and PDEs.
- Nanomaterials represent a potential antibiofilm strategy because they can direct antibiotics to the EPS site and release them in a controlled manner (Figure IF) [18,47]. Furthermore, because of their high surface area-to-volume ratio, NPs can readily interact with microbial cells [44]. Nanomaterials that inhibit biofilms demonstrate various antibiofilm actions, such as generation of ROS, disruption of signal transduction pathways, inhibition of protein synthesis, and destruction of DNA [103].
- A combination of natural products and conventional antibiotics prevents biofilm formation (Figure IG) [104–106]. More specifically, a treatment strategy that involves combining an antibacterial agent with a biofilm dispersal agent makes it possible for biofilm dispersal, which in turn encourages the antimicrobial agent to penetrate biofilm cells.

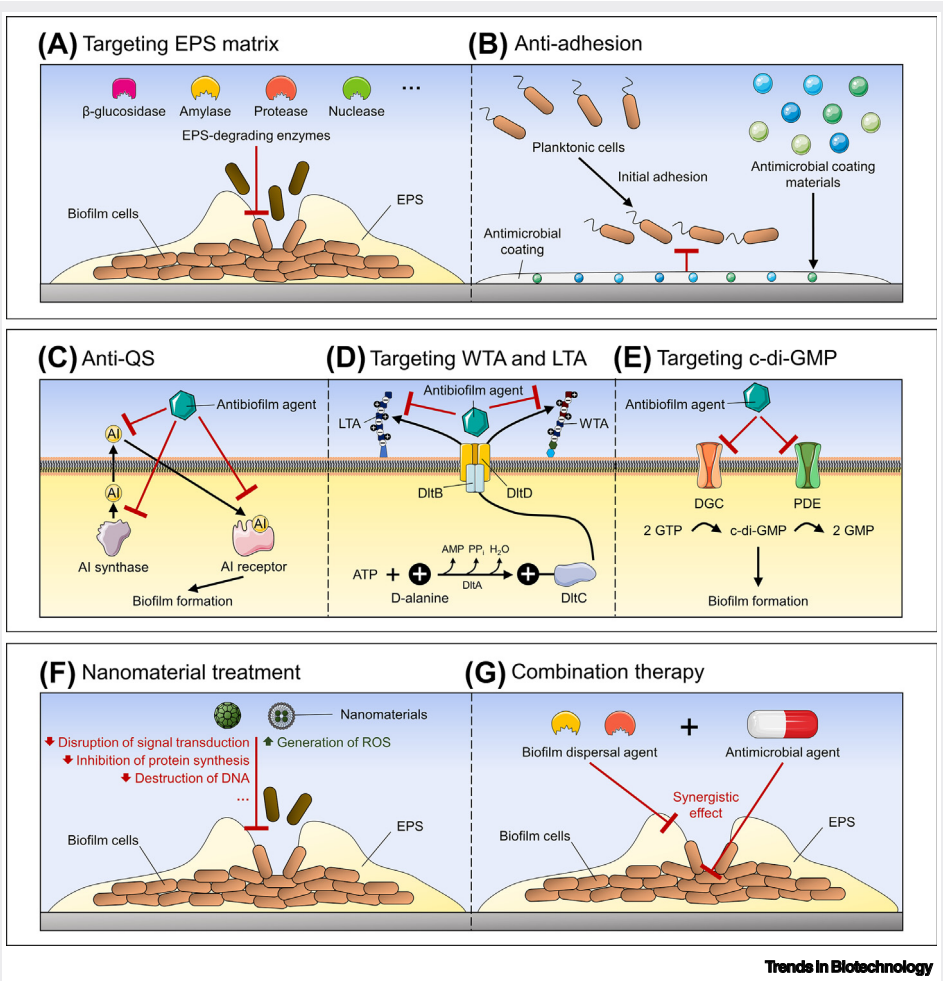

Figure I. Antibiofilm and antivirulence strategies. (A) Targeting EPS matrix, (B) antiadhesion, (C) anti-QS, (D) inhibiting biosynthesis of WTA and LTA, (E) interfering with c-di-GMP signaling, (F) nanomaterial treatment, and (G) combination therapy. Abbreviations: AI, autoinducer; c-di-GMP, cyclic di-GMP; DGC, diguanylate cyclase; EPS, extracellular polymeric substances; LTA, lipoteichoic acid; PDE, phosphodiesterase; QS, quorum sensing; ROS, reactive oxygen species; WTA, wall teichoic acid.

[25] and destroy pathogenic biofilms and sensitize biofilm cells to existing antibiotics; thus, they can be used for the development of new antibiofilm agents.

Trends in marine-derived materials: development of marine drugs

Oceans occupy over 70% of the Earth's surface but are mostly unexplored and likely contain undiscovered resources and valuable commodities. Owing to its complex and diversified environment, which includes extremes in temperature, pressure, solar radiation, salt, and pH, the ocean serves as a habitat for a wide variety of organisms, resulting in significant biological diversity [26]. Marine organisms are a rich source of chemical compounds stemming from their enormous biological diversity [27]. In the past, researchers focused their efforts on marine habitats that are highly biodiverse, which significantly contributed to the search for novel species and metabolites. However, recently, a portion of this research conducted in the pharmaceutical industry has focused on the marine environment as a source of novel bioactive compounds.

Multiple perspectives may be used to explain why marine-derived materials often have therapeutic effects and are used as drug materials. From a structural perspective, marine organisms have evolved distinct metabolic processes and can survive and even thrive in harsh settings. Consequently, marine species vary physiologically and produce different metabolites from those of terrestrial organisms [28]. Marine natural compounds (MNCs) not only vary considerably but also possess distinctive structures that may assist in predicting and assigning their antimicrobial properties. This can be accomplished using *in silico* molecular docking against virulence factors and signaling molecules that are generated by microbial pathogens [28–30]. Discovering new antimicrobial active molecules is often difficult because of the frequent isolation of known compounds that overlap with existing compounds. However, the marine environment provides a vast pool of resources that could potentially be used for the development of effective new drugs against antibiotic-resistant microbial pathogens. Moreover, MNCs with high bioavailability can potentially be transformed into effective drugs against infectious disorders. Liu and colleagues investigated the physicochemical features of MNCs with antibacterial activity against drug-resistant bacteria and found that 60% of these MNCs were orally available [31]. The ability to collect marine organisms from deeper parts of the ocean and conduct in-depth research is more feasible now, owing to developments in analytical methods, engineering strategies, machine learning, screening procedures, and deep ocean probing. From an economic perspective, the global market for marine-derived pharmaceuticals was estimated at USD 9749.46 million in 2022 and is expected to reach USD 17 194.38 million by 2028 [32]. Consequently, research on marine-derived materials for the development of marine-derived drugs is increasing.

This review comprehensively discusses the application of materials originating from marine sources to treat pathogenic microbial infections and reduce virulence. MNCs, marine-bioinspired **nanoparticles (NPs)**, and marine **polymer-antibiotic conjugates** are all included in the collection of materials that are referred to as 'those of marine origin' in the conceptual definition of marine-derived materials.

Antibiofilm, antivirulence, and anti-QS agents based on marine-derived materials

Several studies have used marine-derived materials to develop novel antibiofilm drugs (Figure 1, Key figure); however, the mechanisms involved are not completely understood. Indirect assessments based on transcriptome, metabolite, proteome, and molecular docking studies have provided evidence confirming their antibiofilm mechanism. Marine-derived materials can regulate biofilm development and eliminate both early-stage and mature biofilms.

Marine natural compounds

Extracting and identifying pharmacologically active chemicals from marine organisms is a possible method for drug discovery [33]. The process of discovering new drugs based on natural products begins with biological screening of crude extracts, followed by the isolation of active pure molecules and identification of their biological attributes [34]. Research focusing on pure compounds can uncover interactions between natural molecules and target proteins, which enables a more in-depth analysis of biological activities than research focusing on crude extracts. Consequently, many researchers have successfully extracted and purified MNCs from marine organisms and revealed the mechanisms by which they inhibit biofilm formation (Figure 1A and Table 1).

For example, Chang and colleagues identified tyrosol in *Penicillium chrysogenum* DXY-1 isolated from marine sediments, which inhibited the biofilm formation and QS-regulated virulence factor production of *P. aeruginosa* without inhibiting planktonic growth at concentrations ranging from 0.1 to 0.5 mg/ml [35]. Molecular docking analysis showed that tyrosol bound to the DNA-binding domain of *Chromobacterium violaceum* QS receptor CviR and induced modification of

Key figure

Antibiofilm and antivirulence activity of marine-derived materials

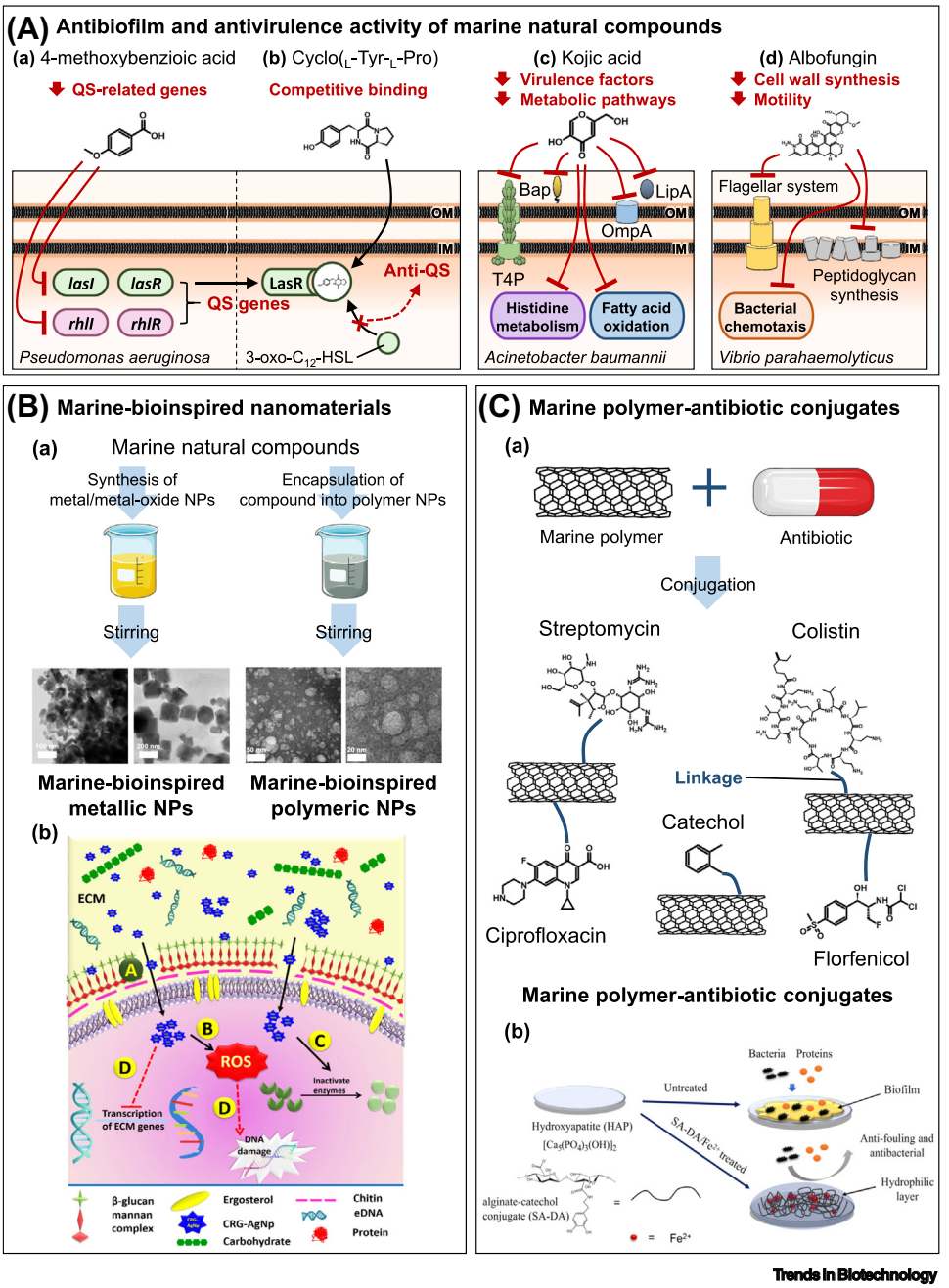

Figure 1. (A) MNCs. (a) 4-Methoxybenzioic acid downregulated QS-related genes in *Pseudomonas aeruginosa* [36]. (b) Cyclo(L-Tyr-L-Pro) blocked the effect of QS AIs through competitive binding to same pocket of receptor protein LasR in *P. aeruginosa* [38]. (c) Transcriptome analysis showed that kojic acid interfered with fimbriae assembly and virulence factor

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the active receptor protein. Danaraj and colleagues reported that 4-methoxybenzioic acid purified from *Halodule pinifolia* exhibited antibiofilm and antivirulence activities by downregulating the transcription levels of QS genes (*lasI*, *lasR*, *rhlI*, and *rhlR*) in *P. aeruginosa* at a concentration of 62.5 μg/ml (Figure 1Aa) [36]. Zhang and colleagues reported that maipomycin A, a metabolite of *Kibdelosporangium phytohabitans*, exhibited antibiofilm activity due to its ability to chelate iron [37]. In addition, biofilm formation by *Acinetobacter baumannii* was reduced in the presence of maipomycin A but was partially restored by the addition of exogenous iron. Yu and colleagues reported that cyclo($_L$-Tyr-$_L$-Pro) purified from the extract of *P. chrysogenum* and administered to *P. aeruginosa* at a concentration of 0.5 mg/ml reduced biofilm formation and production of virulence factors (pyocyanin, elastase, and proteases) by downregulating the expression of QS genes (*lasI*, *lasR*, *rhlI*, and *rhlR*) [38]. Furthermore, an *in silico* study showed that cyclo($_L$-Tyr-$_L$-Pro) inhibited the effects of QS AIs by competitively binding to the same pocket of LasR in *P. aeruginosa* (Figure 1Ab). Chang and colleagues identified chrysin exhibiting anti-QS activity from *P. chrysogenum*, which inhibited QS by competing with AIs to bind to the same pocket of CviR in *C. violaceum* [39]. Circular dichroism analysis revealed that chrysin bound to CviR and changed its structure, preventing the binding of the AI to CviR and blocking the transcription of downstream genes, thereby suppressing QS activity. Li and colleagues purified kojic acid following solid fermentation of *Aspergillus flavus* isolated from a marine sponge and showed that the kojic acid had a significant inhibitory effect on *A. baumannii* biofilms at a concentration of 6 mM [40]. Transcriptomic and metabolomic analyses showed that kojic acid treatment disrupted fimbriae assembly, virulence factor production, and amino acid and fatty acid metabolism, suggesting a synergistic effect in delaying biofilm formation of *A. baumannii* (Figure 1Ac). She and colleagues isolated albofungin and albofungin A from *Streptomyces chrestomyceticus* and found that they could eradicate penicillin- and cephalosporin-resistant *Vibrio parahaemolyticus* biofilms at concentrations of 0.06 and 0.06 μg/ml, respectively [41]. Furthermore, proteomic analysis demonstrated that albofungin treatment downregulated proteins involved in peptidoglycan biosynthesis, bacterial chemotaxis, and flagellar assembly pathways, thereby identifying the antibiofilm mechanism of albofungin (Figure 1Ad).

Kumla and colleagues and Machado and colleagues screened the antibiofilm activity of several MNCs isolated from a marine sponge [42,43]. Several MNCs isolated from marine organisms demonstrate antibiofilm activity and can control biofilm formation and virulence factors of pathogens at low concentrations (below 100 μg/ml). The antibiofilm activity of the MNCs was further demonstrated on the basis of molecular docking analysis, confirmation of QS gene expression, and transcriptome and metabolite analyses.

production in *Acinetobacter baumannii*. Metabolomic analysis indicated that kojic acid perturbed amino acid and fatty acid metabolism [40]. (d) Proteomic analysis showed that albofungin downregulated proteins involved in peptidoglycan biosynthesis, bacterial chemotaxis, and flagellar assembly in *Vibrio parahaemolyticus* [41]. (B) (a) Marine-bioinspired metallic/polymeric NPs. (b) Kappa-carrageenan-AgNPs destroyed *Candida* biofilm matrix and penetrated it by creating pores [60]. They exhibited antibiofilm activity by suppressing the expression of ECM-producing genes and generating ROS inside cells to damage DNA. Reprinted, with permission, from [60]. Copyright © 2021, Elsevier B.V. (C) (a) Marine polymer-antibiotic conjugates. (b) Hydroxyapatite-coated with alginate-catechol conjugates/Fe^{2+} showed antibiofilm activity against *Streptococcus mutans* due to its highly hydrophilic properties [74]. The alginate-catechol conjugates contained many hydrophilic carboxyl and hydroxyl groups, which inhibited the attachment of *S. mutans* to the hydroxyapatite surface. Reprinted, with permission, from [74]. Copyright © 2022, American Chemical Society. Downside red arrows indicate 'downregulation'. Abbreviations: AgNPs, silver nanoparticles; AI, autoinducer; Bap, biofilm-associated protein; ECM, extracellular matrix; eDNA, extracellular DNA; IM, inner membrane; LipA, lipoyl synthase; MNCs, marine natural compounds; NPs, nanoparticles; OM, outer membrane; OmpA, outer membrane protein A; QS, quorum sensing; ROS, reactive oxygen species; T4P, type IV pili.

Table 1. Antibiofilm and antivirulence activity of marine natural compounds

Compounds	Sources	Chemical classifications	Target pathogens[a]	Active concentrations[b]	Biofilm reduction[c]	Functions	Refs
Tyrosol	*Penicillium chrysogenum* DXY-1	Peptides	*Pseudomonas aeruginosa*	0.1–0.5 mg/ml	NA[d]	Antibacterial activity Antibiofilm activity Anti-QS activity	[35]
4-Methoxybenzoic acid	*Halodule pinifolia*	Carboxylic acids	*P. aeruginosa*	62.5 μg/ml	>78.29%	Antibacterial activity Antibiofilm activity Anti-QS activity	[36]
Meleagrin	*Emericella dentata*	Alkaloids	*Staphylococcus aureus*	0.05 mg/ml	87.1%	Antibacterial activity Antifungal activity Antibiofilm activity	[88]
Vioxanthin	*Monanchora unguiculata*	Xanthophylls	*S. aureus* *Enterococcus faecalis*	4 μg/ml	99.69%–99.73%	Antibacterial activity Antibiofilm activity	[42]
Maipomycin A	*Kibdelosporangium phytohabitans* XY-R10	Alkaloids	*Acinetobacter baumannii* *P. aeruginosa*	8–32 μg/ml	82.6%–84.3%	Antibacterial activity Antibiofilm activity	[37]
8-*O*-metyltetrangomycin	*Spirostella* sp.	Polyketides	Methicillin-resistant *S. aureus*	0.5–8 μg/ml	52.85%–86.64%	Antibacterial activity Antibiofilm activity Antihemolysis activity	[107]
Aspulvinones R	*Mycale* sp.	Butenolides	*S. aureus* *E. faecalis*	8 μg/ml	95.62%–97.57%	Antibacterial activity Antibiofilm activity	[43]
Aspulvinones S				8 μg/ml	91.81%–94.48%		
Cyclo($_L$-Tyr-$_L$-Pro)	*P. chrysogenum* DXY-1	Peptides	*P. aeruginosa*	0.5 mg/ml	48%	Antibacterial activity Antibiofilm activity Anti-QS activity	[38]
Chrysin	*P. chrysogenum* DXY-1	Flavonoids	*P. aeruginosa*	40 μg/ml	42.4%	Antibiofilm activity Anti-QS activity Antimotility activity	[39]
Kojic acid	South China Sea sponge	Pyrans	*A. baumannii*	6 mM	Mostly destroyed	Antibiofilm activity Anti-QS activity Antimotility activity	[40]

Table 1. (continued)

Compounds	Sources	Chemical classifications	Target pathogens[a]	Active concentrations[b]	Biofilm reduction[c]	Functions	Refs
Albofungin	*Streptomyces chrestomyceticus* BCC 24770	Polyketides	*Vibrio parahaemolyticus V. alginolyticus*	0.06 μg/ml	>90%	Antibacterial activity Antibiofilm activity	[41]
Albofungin A				0.06 μg/ml	>90%		
N-hexadecanoic acid	*Callyspongia diffusa*	Fatty acids	*P. aeruginosa*	100 μg/ml	60%	Antibacterial activity Antibiofilm activity	[89]

[a]The biofilm test for all pathogenic microorganisms was performed *in vitro*.
[b]Biofilm reduction concentration.
[c]Reduction compared with nontreatment.
[d]Not available.

Marine-bioinspired nanomaterials

Nanotechnology has become increasingly popular as an alternative method for treating biofilm infections. NPs are <100 nm in size and have a high surface-to-volume ratio that facilitates their interaction with microbial cells [44]. They also have the capacity to act as drug carriers with high bioavailability owing to their targeted delivery to cells [45]. NPs interact with biofilms in three stages: (i) transport substances proximal to biofilms, (ii) adhere to biofilm surfaces, and (iii) move within biofilms [46]. Therefore, NPs loaded with an antimicrobial drug can release the drug in a controlled manner within the biofilm, producing more effective antimicrobial action against the microbial cells [18,47].

Green chemistry technologies have recently been attracting attention owing to their numerous benefits, including environmental friendliness, minimal preparation costs, renewable resources, and no hazardous by-products [44]. Green-synthesized NPs are manufactured using natural extracts or compounds derived from the organisms, such as reducing agents for metal and nonmetal ions [48]. However, in green chemistry, the synthesis of NPs using natural products is an entirely *in situ* chemical process; thus, NPs have various advantages owing to the use of products from organisms, such as nontoxicity, biocompatibility, and high stability [49,50]. Marine bioactive substances have been used as reducing, capping, and stabilizing agents in the synthesis of green-produced NPs for various biological applications [48,51,52]. This section focuses on the antibiofilm and antivirulence activities of the marine-bioinspired nanomaterials, which include metallic NPs and polymeric NPs synthesized using MNCs (Figure 1Ba and Table 2). Table S1 in the supplemental information online summarizes the physicochemical characteristics of the described marine-bioinspired nanomaterials.

Khan and colleagues synthesized fucoidan-gold nanoparticles (AuNPs) by reducing ionic gold (Au^{3+}) using fucoidan (a negatively charged polymer derived from brown algae) that inhibited biofilm formation, virulence factors (rhamnolipid, pyocyanin, pyoverdine, hemolysins, and protease), and motility in *P. aeruginosa* [53]. Tabassum and colleagues showed that fucoidan-AuNPs not only eradicated early-stage and mature monospecies biofilms of *C. albicans*, *S. aureus*, and *S. mutans* but also suppressed dual-species biofilms of *C. albicans* with *S. aureus* or *S. mutans* [54]. Fucoidan was shown to have antibiofilm and anti-QS activities, and its antibiofilm action was effective against *Proteus vulgaris* and *Salmonella enterica* biofilm formations at a concentration of 25 μg/ml [55]. The antibiofilm activity of fucoidan-AuNPs may be due to the synergistic or additive impact of capped fucoidan and AuNPs, although there is no evidence to

Table 2. Antibiofilm and antivirulence activity of marine-bioinspired nanomaterials

Types of nanomaterials	Compounds	Sources	Target pathogens[a]	Active concentrations[b]	Biofilm reduction[c]	Functions	Refs
AuNPs	Fucoidan	Brown algae	*Pseudomonas aeruginosa*	128–256 μg/ml	84%–86%	Antibacterial activity Antibiofilm activity Antivirulence activity Antimotility activity	[53]
AuNPs	Chitosan oligosaccharide	Crustaceans	*P. aeruginosa*	512 μg/ml	NA[d]	Antibacterial activity Antibiofilm activity Antivirulence activity Antimotility activity	[56]
AuNPs	Laminarin	Brown algae	*Aeromonas hydrophila*	100 μg/ml	56%	Antibacterial activity Antibiofilm activity	[59]
AgNPs	Kappa-carrageenan	Red algae	*Candida albicans* *C. glabrata*	~300 μg/ml	>80%	Antifungal activity Antibiofilm activity	[60]
AgNPs	Chitosan	Crustaceans	Methicillin-sensitive *Staphylococcus aureus* Methicillin-resistant *S. aureus* *Klebsiella pneumoniae* Carbapenemase *P. aeruginosa* Polymyxin-resistant *Escherichia coli*	12.5–50 μg/ml	>90%	Antibacterial activity Antibiofilm activity	[57]
Chitosan nanoparticles	Phloroglucinol	Brown algae	*C. albicans* *S. aureus* *K. pneumoniae* *Streptococcus mutans* *C. albicans-K. pneumoniae/S. aureus/S. mutans*[e]	1024 μg/ml	86%–92%	Antibacterial activity Antifungal activity Antibiofilm activity	[61]
AuNPs	Chitosan	Crustaceans	*P. aeruginosa*	125 μl/ml	76.1%	Antibacterial activity Antibiofilm activity	[58]
AuNPs	Fucoidan	Brown algae	*S. aureus* *C. albicans* *C. albicans-S. aureus/S. mutans*[e]	512 μg/ml	NA	Antibacterial activity Antifungal activity Antibiofilm activity	[54]
AuNPs	Phloroglucinol	Brown algae	*S. aureus* *C. albicans* *C. albicans-S. aureus*[e]	2048 μg/ml	NA	Antibacterial activity Antifungal activity Antibiofilm activity	[62]

[a]The biofilm test for all pathogenic microorganisms was performed *in vitro*.
[b]Biofilm reduction concentration.
[c]Reduction compared with nontreatment.
[d]Not available.
[e]Polymicrobial biofilm.

support this hypothesis. Several studies have been conducted on the synthesis of NPs using the representative marine-derived polymer chitosan and its derivatives. For example, Khan and colleagues reported that AuNPs-capped chitosan oligosaccharides reduced biofilm formation and virulence factors in *P. aeruginosa*, similar to previous studies on fucoidan-AuNPs [56]. Dos Santos and colleagues used chitosan to synthesize silver nanoparticles (AgNPs) of various sizes, and the smallest AgNPs showed the highest antibiofilm activity [57]. Additionally, Sokary and colleagues also used chitosan to synthesize AuNPs, which reduced biofilm formation by *P. aeruginosa* [58]. Several studies have shown that the antibiofilm activity of chitosan is due to its polycationic properties, which enable interactions with negatively charged biofilm components. Vijayakumar and colleagues synthesized AuNPs using the marine polysaccharide laminarin, which inhibited biofilm formation by *Aeromonas hydrophila* [59]. These results were attributed to the fact that laminarin-AuNPs inhibited the production of EPS and hydrophobicity of *A. hydrophila*. Gupta and colleagues investigated the antibiofilm activity of AgNP-capped kappa-carrageenan, a marine sulfated polysaccharide, against *C. albicans* and *C. glabrata* [60]. Kappa-carrageenan-AgNPs inhibited and eradicated *Candida* biofilm by generating reactive oxygen species (ROS) and modulating the composition of the extracellular matrix (Figure 1Bb). The same research team incorporated phloroglucinol into chitosan NPs and AuNPs and investigated their ability to inhibit monospecies and dual-species biofilm formation [61,62].

Marine-bioinspired nanomaterials have been synthesized by reducing metal ions (Au and Ag) with MNCs or using polymers (chitosan) as carriers for MNCs. Some studies have reported that marine-bioinspired nanomaterials exhibit better antibiofilm activity than MNC alone or metal-aqueous solutions used to synthesize metallic NPs [57,59]. By contrast, in a study by Sokary and colleagues, chitosan alone showed higher antibiofilm activity than chitosan-capped NPs [58]. Researchers have conducted studies to control polymicrobial or host-mimicking biofilms [54,61,62].

Marine polymer-antibiotic conjugates

Several drawbacks, including cytotoxic effects, limited stability, drug resistance, and water insolubility, are associated with antibiofilm drugs that are currently available. These constraints have been overcome by developing a method that allows the controlled release of antibiofilm drugs by loading them into biopolymers [63]. Biocompatibility, nontoxicity, and biodegradability are advantages of biopolymers, which are becoming important materials for drug loading [49,64]. Chitin, chitosan, alginate, hyaluronic acid, carrageenan, collagen, and gelatin are examples of marine polymers with practical applications in various industries [65–69]. Therefore, marine polymers have been conjugated with antibiotics in several studies aiming to achieve antibiofilm capabilities superior to those of currently available antibiotics (Figure 1Ca and Table 3). This paragraph addresses marine polymer-antibiotic conjugates, which are molecules produced by covalently linking antibiotics with marine polymers.

Li and colleagues synthesized chitosan oligosaccharide-streptomycin conjugates as antibiofilm agents by covalently linking the amino group of chitosan oligosaccharides to the aldehyde group of streptomycin [70]. The chitosan oligosaccharide-streptomycin conjugates downregulated the *pelA* and *algD* genes involved in exopolysaccharide biosynthesis in *P. aeruginosa* and did not induce the activation of the MexX–MexY drug efflux pump system that is activated by streptomycin, thus inhibiting the development of streptomycin resistance. Stokniene and colleagues developed alginate oligosaccharide-colistin conjugates with ester or amide linkages to improve the antibiotic efficacy of colistin [71]. Alginate oligosaccharide-colistin conjugate treatment was found to significantly reduce the biomass of *P. aeruginosa* biofilms

Table 3. Marine polymer-antibiotic conjugates as antibiofilm and antivirulence agents

Marine-derived polymers	Antibiotics	Target pathogens[a]	Active concentrations[b]	Biofilm reduction[c]	Functions	Refs
Chitosan oligosaccharide	Streptomycin	*Pseudomonas aeruginosa*	250 μg/ml	>70%	Antibiofilm activity	[70]
Alginate oligosaccharide	Colistin	*P. aeruginosa*	1–2 μg/ml	NA[d]	Antibacterial activity Antibiofilm activity	[71]
Chitosan oligosaccharide	Florfenicol	*P. aeruginosa* *Staphylococcus aureus* *Salmonella enterica*	250 μg/ml	NA	Antibacterial activity Antibiofilm activity	[72]
Hyaluronic acid	Ciprofloxacin	*S. typhi*	NA	NA	Antibacterial activity Antibiofilm activity	[73]
Alginate	Catechol	*Streptococcus mutans*	NA	NA	Antibacterial activity Antibiofilm activity	[74]

[a]The biofilm test for all pathogenic microorganisms was performed *in vitro*.
[b]Biofilm reduction concentration.
[c]Reduction compared with nontreatment.
[d]Not available.

compared with colistin sulfate treatment. Yuan and colleagues synthesized florfenicol-chitosan oligosaccharide conjugates as agents for biofilm treatment by leaving the active group of florfenicol unaffected [72]. Florfenicol-chitosan oligosaccharide conjugates efficiently inhibited *S. enterica* biofilms that showed strong resistance to florfenicol, suggesting that conjugation with chitosan oligosaccharides can suppress drug resistance. Arshad and colleagues synthesized ciprofloxacin-hyaluronic acid conjugates through an amide bond between the amino group of ciprofloxacin and the carboxyl group of hyaluronic acid to improve the solubility and permeability of ciprofloxacin [73]. Ciprofloxacin-hyaluronic acid conjugates showed higher antibiofilm activity against *S. typhi* biofilms than free ciprofloxacin. Wu and colleagues prepared a soft gel by mixing ferrous cations (Fe^{2+}) and alginate-catechol conjugates, which effectively inhibited *S. mutans* biofilm on hydroxyapatite [74]. The antibiofilm activity of hydroxyapatite-coated with alginate-catechol conjugates/Fe^{2+} was attributed to its highly hydrophilic properties (Figure 1Cb). Owing to the presence of numerous hydrophilic carboxyl and hydroxyl groups in the alginate-catechol conjugates, the surface of the hydroxyapatite materials that were coated with alginate-catechol conjugates/Fe^{2+} exhibited hydrophilic characteristics. Therefore, hydrophobic bacterial proteins will not interact with the hydrophilic group present on the surface of the hydroxyapatite; consequently, the bacteria will not be able to adhere to the surface.

Several marine polymers have been conjugated with antibiotics to improve their efficacy compared with conventional antibiotics. Accordingly, marine polymer-antibiotic conjugates exhibit stronger antibiofilm properties than conventional antibiotics [70,71,73]. These conjugates increase antibiotic susceptibility by destroying antibiotic-resistant biofilms or by not activating the pathogen drug pump systems [70,72].

Application of marine-derived materials

Coatings that can prevent microbial attachment are classified as antimicrobial or antifouling. Antimicrobial coatings can kill microorganisms that access the surface, whereas antifouling coatings prevent the buildup of organisms [75]. Accordingly, antibiofilm materials can be used as surface coating materials when designing antifouling surfaces against marine microbial pathogens or manufacturing medical devices (Figure 2). Marine polymers in various formulations have also been used as edible food packaging film to reduce microbial biofilm development on food products (Figure 2).

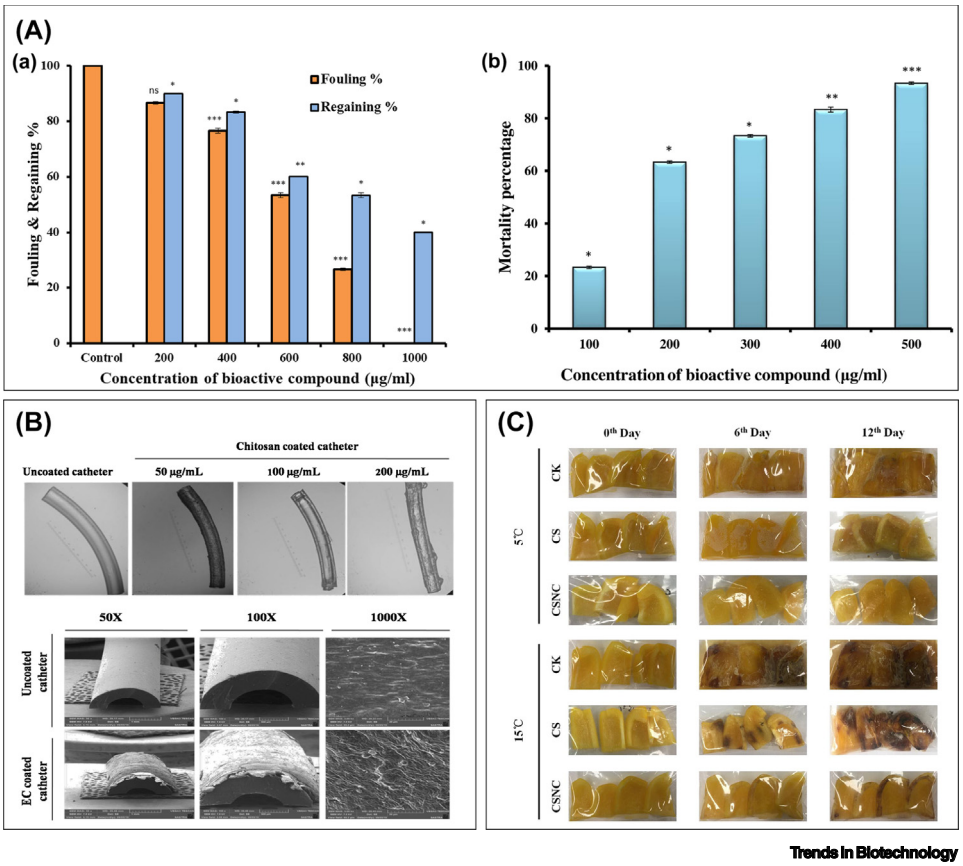

Figure 2. Application of marine-derived materials. (A) Antifouling activities of oxycyclopentadien. Oxycyclopentadien (a) inhibited the mollusk foot adherence and (b) was toxic to the marine crustacean *Artemia salina*. Reproduced from [79]. Copyright © 2022, Elsevier B.V. (B) Catheter coated with chitosan extracted from the shell of the marine crab *Portunus sanguinolentus*. Reproduced from [81]. Copyright © 2021, Elsevier B.V. (C) Preventing microbial contamination and extending the shelf life of fresh-cut bell pepper using chitosan nanocoating film with different treatment and storage conditions. Reprinted, with permission, from [85]. Copyright © 2020, Elsevier B.V. Abbreviations: CK, untreated group; CS, chitosan coating; CSNC, chitosan nanocoating; EC, extracted chitosan. ***$P < 0.0001$, **$P < 0.01$, *$P < 0.5$.

Marine antifouling properties

Marine-derived materials have been proposed as an environmentally friendly alternative to toxic biocides in antifouling paints [76]. Napyradiomycin isolated from the marine bacterium *S. aculeolatus* was found to inhibit the biofilm formation of marine bacteria and settlement of *Mytilus galloprovincialis* [77]. The antibiofilm and antifouling activities of napyradiomycin were attributed to the presence of bromine substitute present in C-16. 6-Pentyl-2*H*-pyrone-2-one, a metabolite of the marine fungus *Trichoderma reesei*, inhibited biofilm formation of the marine bacterium *Loktanella hongkongensis* and settlement of the barnacle *Amphibalanus amphitrite* [78]. Oxycyclopentadien, a bioactive compound from the marine bacterium *S. thermolineatus*, inhibited biofilm formation of the marine bacteria *Psychrobacter celer*, *Paracoccus alimentarius*, and *Kocuria rhizophila* and foot adherence by mollusks and exhibited anticrustacean activity with an LC_{50} value of 173.72 µg/ml (Figure 2A) [79].

Coating of medical devices to prevent microbial infection

Marine polymers have been applied to medical device coatings due to their biocompatibility, nontoxicity, and biodegradability. Chitosan modified with fatty acids has shown promise as a

biofunctional catheter coating material due to its self-organization and antibacterial properties [80]. Catheters coated with chitosan reduced preformed biofilms and QS-mediated virulence factors of *S. epidermidis* and *C. albicans* and exhibited reduced contact angles compared with untreated catheters, preventing pathogen attachment [81]. The uncoated catheter had a thickness of 2.05 mm, whereas the catheter coated with chitosan at a concentration of 200 μg/ml had a thickness of 2.17 mm with uniformity, as shown in the scanning electron microscope image (Figure 2B). These results suggest that chitosan can be used as a powerful antifouling material on medical implant surfaces because it can prevent attachment of pathogens and inhibit biofilm formation and QS-mediated virulence factors. Amoxicillin-doped hyaluronic acid/fucoidan multifunctional coatings exhibited antibiofilm activity against *S. aureus* and biocompatibility toward human osteoblasts, indicating their potential as coatings to enhance the bioactivity of steel-based orthopedic implants [82]. The aforementioned study showed that changing the ratio of hyaluronic acid to fucoidan layers allowed control over the release of amoxicillin from hyaluronic acid-amoxicillin-fucoidan multifunctional coatings. The greatest proportion of amoxicillin release was discovered in one layer each of hyaluronic acid, amoxicillin, and fucoidan, which exhibited significant antimicrobial activity. Replacing one hyaluronic acid layer with four layers of hyaluronic acid and three layers of amoxicillin with fucoidan boosted the release of amoxicillin by 5.5%–57.5%, whereas replacing two hyaluronic acid layers with fucoidan increased the release of amoxicillin by 18%–70%. These findings suggest that hyaluronic acid and fucoidan are effective in preventing biofilm infection on medical devices such as implants as they immediately release drugs.

Food packaging using marine biopolymer

The formation of biofilms on food surfaces can ultimately cause food poisoning in humans after consumption [83]. In particular, food matrix components such as carbohydrates, proteins, and lipids impact microbial adhesion, creating an environment conducive to biofilm production [84]. Therefore, a variety of marine biopolymers have been employed in food packaging as edible films. It has been shown that chitosan NPs prevented the development of biofilms by pathogens such as *Escherichia coli*, *S. enterica*, and *Listeria monocytogenes*, which are responsible for food poisoning [85]. Furthermore, using chitosan NPs as a nanocoating can potentially increase the shelf life of fresh-cut bell peppers (Figure 2C). The chitosan NP treatment proved successful in preventing the microbial infection of fresh-cut bell peppers while maintaining the edible qualities of the pepper. Edible films containing alginate and vanillin were reported to have antiadhesion capabilities by lowering the hydrophobicity and self-aggregation of foodborne pathogens (*E. coli*, *S. aureus*, *S. typhi*, *Shigella flexneri*, and *Bacillus cereus*) [86]. In particular, the alginate-vanillin film was able to extend the shelf life of lettuce by inhibiting the growth of bacteria while maintaining the physical properties of lettuce compared with commercial low density polyethylene film.

Cytotoxicity of marine-derived materials

Marine-derived materials should be tested for cytotoxicity before being used as antibiofilm treatments for human infections (Table S2 in the supplemental information online). In particular, NPs and conjugates should be proved to be nontoxic and biocompatible since their nanosize may induce toxicity by interacting with cellular components in biological systems [87]. Meleagrin inhibited intracellular fatty acid production, causing very strong cytotoxicity against human cervical cancer cell lines KB-3-1 and KB-V1, with IC_{50} values of 3.07 and 6.07 μM, respectively [88]. N-hexadecenoic acid was nontoxic to mouse embryonic fibroblasts at 100 mg/l, where cell survival was 97% [89]. Another study found that laminarin-AuNPs did not affect the viability of monkey kidney Vero cells at 100 μg/ml [59]. Phloroglucinol-chitosan NPs were shown not to have any cytotoxic effect on RAW264.7 macrophages [61]. Chitosan-AgNPs inhibited the proliferation of the human hepatocellular carcinoma cell line HepG-2 and the human colon carcinoma cell line Caco-2 in a concentration-dependent manner, which was attributed to the interaction of the positively charged amino group of

chitosan with the cancer cell membrane [58]. Oligosaccharide-colistin conjugates showed reduced cytotoxicity against the human kidney cell line HK-2 compared with colistin alone because the Dab residue that mediates colistin toxicity was used for amide conjugation with the oligosaccharide [71]. Ciprofloxacin-hyaluronic acid conjugates also showed reduced cytotoxicity against RAW264.7 macrophages compared with ciprofloxacin alone [73]. Alginate-catechol conjugates/Fe^{2+} were shown to promote cell proliferation without affecting the viability of human fibroblast HGF-1 [74]. Most marine-derived materials have been shown to have low cytotoxicity against normal cell lines, making them suitable for systemic application. Additionally, several of them showed potent anticancer activity against cancer cell lines, suggesting that they could be used as drugs for cancer treatment.

Concluding remarks and future perspectives

Antimicrobial resistance is a growing global problem caused by biofilm-forming microbial pathogens. This problem highlights the need for alternative strategies to combat the associated infections. A growing number of studies are being conducted on the use of bioactive compounds derived from marine organisms for various biological purposes, and considerable emphasis has been placed on the use of these compounds as antibiofilm drugs. Experiments conducted at the phenotypic level, as well as verification via the expression of QS signaling, biofilm formation, and virulence genes have primarily resulted in the identification of the mechanism of action. In addition, the *in vitro* activity was confirmed by *in silico* molecular docking of QS-regulating proteins against some MNCs. The loading of MNCs onto polymeric NPs or the use of MNCs as reducing and capping agents in the synthesis of metallic NPs have both been shown to reduce application limitations of MNCs, which are mainly antibiofilm and antivirulence. Several studies have been conducted on the application of conventional antibiotics conjugated with marine polymers. These studies aimed to produce a synergistic effect to reduce virulence characteristics and suppress biofilm formation. Polymers possess intrinsic antibacterial properties and thus have been used to encapsulate drugs, nanomaterials, or conjugated drugs, showing improved synergistic efficacy. In addition, several questions must be addressed to completely understand the mechanisms of biofilm inhibition and virulence attenuation by marine-derived materials (see Outstanding questions). Furthermore, the following future research perspectives are recommended to increase the antibiofilm and antivirulence capabilities of marine-derived materials and their diverse formulations.

Given the limited number of MNCs currently accessible for commercial use, greater emphasis must be placed on the process of isolating and purifying substances from marine species via the development of effective instruments. To reveal antibiofilm and antivirulence properties, repurposing of marine chemicals, which have previously been recognized as having anticancer, antidiabetic, antihelminthic, and antioxidant properties, is necessary. It would be more suitable to increase the stability and activity of marine-derived materials by synthesizing additional conjugates with previously identified antibiofilm and antivirulence compounds.

Natural products and marine organisms are both important sources of phenolic compounds. Phenolic compounds are sensitive to light, heat, and pH [90]. Most of them have an astringent and bitter flavor, challenging their use in oral treatments [91]. Furthermore, some MNCs are known to be the least soluble in water, limiting their application in oral care, cosmetic formulation, and topical ointment [92]. Due to these characteristics, MNCs may face difficulties developing agents for application in the medical field. As a result, while designing antimicrobial and antibiofilm agents using MNCs, it is essential to retain their effectiveness by encapsulating or coating them with polymeric nanomaterials [91].

The antibiofilm and antivirulence qualities of marine-derived materials may be more helpful if the *in vitro* experiments are performed under host-mimicking conditions, which resemble the natural

Outstanding questions

Do all reported MNCs, marine-bioinspired nanomaterials, or marine polymer conjugates have species-specific activity, or are they also effective against a wide spectrum of biofilm-forming microbial pathogens?

To avoid infection linked to medical implants and equipment, is it possible to use MNCs, marine-bioinspired nanomaterials, or marine polymer conjugates as coating materials? Is there any technical limitation associated with the coating of the medical device using these materials?

A small population of metabolically least active 'persister' cells responsible for persistent and recurring infections is also present in the biofilm environment. Therefore, can these marine-derived materials be designated as anti-persister agents? It is essential to gain a solid understanding of how these compounds react with persister cells.

There are various seaweed- and animal-derived food items. Is it conceivable to apply the polymeric nanomaterial as a packaging film in order to avoid the development of the biofilm?

The majority of marine-derived materials have been proved to exhibit little or no cytotoxicity against normal cell lines, whereas others have shown substantial anticancer activity against cancer cell lines. What signaling component or process might be responsible for selectively targeting normal or cancer cells by these MNCs?

environments of the host organisms. To fully understand the antibiofilm and antivirulence properties of these marine-derived materials, *in vivo* experiments using an array of animal model organisms are necessary. When these marine-derived materials are introduced into a host system, they may be encountered by polymicrobial species, thereby neutralizing their activity. Interactions between microbial species promote biofilm development, leading to enhanced resistance to antimicrobial treatments. Therefore, antibiofilm effectiveness should be investigated *in vitro* and *in vivo,* employing multiple microbes.

Since there is continuous secretion and release of body fluid in the mouth, lungs, stomach, intestines, and urine tract system, exposure to pathogenic microbes in these areas is more likely [93]. The microfluidic system is a new method for replicating the host environment to study how hydrodynamic parameters such as flow rate and shear forces affect microbial pathogens in fluid flow. Exploring how MNCs affect biofilm formation and eradication of the mature biofilm in the microfluidic system would be fascinating. Future research should use transcriptomic and proteomic approaches to identify additional genes and proteins that can be used as targets for *in silico* antibiofilm screening from the MNC database that can bind to and modify these targets [94]. Furthermore, future research should incorporate machine learning in which a new algorithm is frequently employed to predict the antibiofilm agent from the MNC database [94].

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplemental information

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ARTICLES FOR FACULTY MEMBERS

ELUCIDATION OF ANTIBIOFILM FORMATION MECHANISMS USING MARINE EXTRACTS AGAINST INFECTION AND ANTIMICROBIAL RESISTANCE (AMR)

Marine polysaccharides for antibiofilm application: A focus on biomedical fields / Jeong, G. J., Khan, F., Kim, D. K., Cho, K. J., Tabassum, N., Choudhury, A., Hassan, M. I., Jung, W. K., Kim, H. W., & Kim, Y. M.

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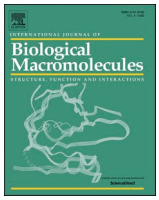

Review

Marine polysaccharides for antibiofilm application: A focus on biomedical fields

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ABSTRACT

Microbial pathogens such as bacteria and fungi form biofilms, which represent substantial hurdles in treating human illness owing to their adaptive resistance mechanism to conventional antibiotics. Biofilm may cause persistent infection in a variety of bodily areas, including wounds, oral cavity, and vaginal canal. Using invasive devices such as implants and catheters contributes significantly to developing healthcare-associated infections because they offer an ideal surface for biofilm formation. Marine organisms produce a variety of polysaccharides, which have recently attracted worldwide attention due to their biochemical features, various applications, and advantageous properties such as bioactivity, biodegradability, and biocompatibility. Because of their antimicrobial and antibiofilm features, several polysaccharides such as chitosan, fucoidan, carrageenan, alginate, and hyaluronic acid have been used to treat infected wounds as well as ophthalmic, oral, and vaginal infections. In addition, marine polysaccharides are currently employed as coatings on medical devices and implant materials, alone or in combination with other bioactive substances or nanomaterials, to protect the materials' undertones from microbial contamination. This review discussed the recent advancements in marine polysaccharides and their derivatives as a therapeutic potential against biofilm-associated diseases. The potential obstacles in the scalability of their production, clinical translation, and/or regulatory hurdles have also been discussed.

1. Introduction

Biofilms are structured communities of microorganisms that adhere to living or non-living surfaces and can consist of single or multiple microbial species [1]. Biofilm formation is a critical virulence strategy for numerous pathogenic microorganisms, including *Staphylococcus aureus* [2], *Pseudomonas aeruginosa* [3], *Streptococcus mutans* [4], and *Candida albicans* [5]. Biofilm development occurs in sequential phases, beginning with planktonic cells aggregating on a surface, followed by adhesion and the release of extracellular polymeric substances [6]. Extracellular polymeric substances, which include extracellular DNA, proteins, polysaccharides, and lipids, play a fundamental role in anchoring biofilm cells and maintaining structural integrity [7]. Moreover, extracellular polymeric substances promote biofilm stability through intensive interactions, such as cell-to-cell communication, genetic exchange, and the establishment of cooperative microenvironments [8]. Quorum sensing, a cell signaling mechanism, is essential for biofilm development and the regulation of virulence factors [9]. This

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quorum sensing system adapts gene expression to cell density changes [10]. Additionally, quorum sensing supports biofilm formation and provides resilience against external pressures, including immune responses and antibiotic treatments [11].

Biofilms pose substantial challenges in treating infections, including those associated with wounds [12], eye diseases [13], periodontal conditions [14], vaginosis [15], and medical devices like catheters [16] and implants [17]. Infections caused by biofilm-forming pathogens are persistent and difficult to eradicate, leading to chronic conditions and complications [18]. Biofilms protect bacteria from antibiotics and disinfectants, reducing the effectiveness of standard therapies and leading to prolonged patient suffering, higher healthcare costs, and increased risks of severe outcomes such as sepsis and organ failure [1]. Moreover, biofilm-related infections contribute to higher rates of device failure and the need for replacement surgeries [19]. The ability of biofilms to develop on both implanted devices and natural tissues complicates treatment strategies [20], underscoring the need for innovative antimicrobial coatings and methods to prevent and disrupt biofilms. Addressing biofilm-related challenges is essential for improving patient outcomes and reducing the burden of healthcare-associated infections, especially given the growing issue of antibiotic resistance and the limitations of current therapies.

Marine polysaccharides from organisms like algae, seaweeds, and marine bacteria have emerged as promising candidates for overcoming biofilm-related challenges. Marine polysaccharides—including chitosan, hyaluronic acid, alginate, carrageenan, and fucoidan—offer diverse advantages for antibiofilm applications. Chitosan, derived from chitin, effectively disrupts bacterial cell membranes with its positive charge, displaying antimicrobial and antibiofilm properties beneficial for wound care [21]. Its mucoadhesive properties [22], wettability [23], and ability to promote bone fusion [24] further enhance its therapeutic potential. Hyaluronic acid, found in connective tissues, is biocompatible and non-immunogenic [25]; its hydrophilic quality prevents pathogen adhesion, making it an excellent coating for medical devices [26–28]. Its moisture-retention properties also support ocular drug delivery and wound dressings by promoting hydration and healing [29,30]. Alginate, derived from brown algae, is highly absorbent, fostering a moist environment for wound healing and serving as a bioactive compound carrier [31]. Alginate hydrogels mimic the extracellular matrix, enabling controlled drug release and enhanced wound healing through exudate absorption [32,33]. Carrageenan, especially κ-carrageenan from red algae, acts as a potent hemostatic [34] and antibiofilm agent [35], proving valuable in wound dressings and drug delivery. Fucoidan from brown algae demonstrates antibacterial and antibiofilm effects by disrupting bacterial membranes, making it a valuable biomaterial component [36]. Together, these marine polysaccharides offer multifunctional benefits for wound healing, drug delivery, and infection control, positioning them as promising materials in medical applications. For instance, chitosan-based materials have been developed into nanoparticles, hydrogels, and films specifically targeting biofilm-forming pathogens [12,37–50]. Hyaluronic acid is utilized in wound dressings and ocular formulations for its protective and moisture-retentive properties [26,29,51,52], while alginate is employed in antimicrobial hydrogels and dressings, demonstrating its effectiveness in controlled drug release and infection prevention [31,32,53–57]. Carrageenan provides innovative wound care solutions with hemostatic and antimicrobial properties [58], while fucoidan exhibits antibiofilm activities, presenting promising opportunities for new biomaterial development [27].

This review aims to provide a comprehensive overview of the current research on marine polysaccharides for antibiofilm applications, emphasizing their biomedical potential. It details various aspects, including: (1) the types of marine polysaccharides as potential antibiofilm materials, (2) their biomedical applications in managing biofilm-related diseases, (3) their diverse formulations and uses in treating infections linked to biomedical devices, (4) potential obstacles in scalability of marine polysaccharide production, clinical translation, or/and regulatory hurdles, and (5) proposed future research directions to optimize polysaccharide-based therapies and address current limitations.

2. Marine polysaccharides and their applications in the field of antibiofilm

The ocean, covering over 70 % of Earth's surface, remains largely uncharted, holding the potential for untapped resources and valuable assets. With its extreme environmental conditions, including varying temperatures, high pressures, solar radiation, salinity, and pH levels, the ocean provides a complex habitat that supports a vast array of organisms, fostering remarkable biodiversity [59]. This diversity makes marine organisms a rich source of bioactive compounds that stem from their unique biological adaptations [60]. Marine polysaccharides, derived from diverse marine organisms, exhibit distinct structural features and biochemical properties that set them apart from terrestrial polysaccharides. They are primarily divided into three main types: those from marine plants, marine animals, and marine microorganisms. Prominent examples include fucoidan, alginate, chitosan, xanthan gum, and glucan, each possessing unique chemical properties that allow for significant biological activity and therapeutic potential in a range of applications [61]. Specifically, marine polysaccharides contain unique structural elements, such as sulfate and uronic acid groups, which impart antioxidant properties—traits that are seldom found in terrestrial polysaccharides [62]. In contrast to terrestrial polysaccharides, which mainly serve as structural components in plant cell walls and are predominantly made up of cellulose, marine polysaccharides are rich in sulfated forms like laminarin, alginate, fucoidan, agarose, and carrageenan [61,62]. These components, primarily derived from marine algae and diatoms, constitute over half of the dry biomass of these organisms [62]. Marine polysaccharides regenerate quickly, reducing environmental strain compared to terrestrial resources [63]. They also address concerns about resource allocation for non-food production [64]. As a result, marine polysaccharides present an alternative solution that helps alleviate issues associated with terrestrial resource scarcity, specific diseases, and religious considerations, positioning them as a valuable resource with promising nutritional and functional potential in both the food and pharmaceutical sectors [63–65].

Marine-derived polysaccharides are increasingly utilized for the treatment of biofilm-mediated infections (wound infection, keratitis, periodontitis, and vaginosis) and the prevention of infections associated with biomedical devices (catheters, implants, and other applications). Tables 1, 2 and 3 provide an in-depth review of research on antibiofilm applications in the biomedical field based on marine polysaccharides over the past five years, focusing exclusively on marine-derived polysaccharides and their derivatives. The key marine polysaccharides highlighted include chitosan, hyaluronic acid, alginate, carrageenan, and fucoidan, with subsequent sections focusing on their specific roles in antibiofilm applications (Fig. 1).

2.1. *Chitosan as an antibacterial, antibiofilm, drug-delivery, mucoadhesive, anticoagulant, and bone fusion-promoting agent*

Chitosan is a biopolymer composed of glucosamine (GlcN) and *N*-acetylglucosamine (GlcNAc), derived through the deacetylation of chitin, which is primarily found in the exoskeletons of crustaceans [66]. The key physicochemical properties of chitosan vary based on the ratio of the two monosaccharides, GlcN and GlcNAc, which in turn influences characteristics such as the degree of deacetylation, degree of substitution, molecular weight, and viscosity [66]. Notably, the degree of deacetylation, molecular weight, and degree of substitution of chitosan significantly affect its positive charge density, which is closely linked to its antimicrobial and antibiofilm activities [21]. The antimicrobial and antibiofilm mechanisms of chitosan are primarily attributed to the

Table 1
Application of marine polysaccharides for biofilm-related diseases.

Marine polysaccharides	Drugs	Name of materials	Target pathogens	Active concentrations[a]	Biofilm reduction[b]	Functions	References
Wound infection							
Chitosan	NA[c]	*N*-SuC NPs	*Staphylococcus aureus* *Escherichia coli*	0.5–8.0 mg/mL	NA	Antibacterial activity Antibiofilm activity *In vitro* and *in vivo* wound healing	[37]
Chitosan	Silver nanoparticle	*N*, *O*-CMC/AgNps hydrogel	*S. aureus* *E. coli* *Pseudomonas aeruginosa*	101 mg/mL (*S. aureus*) 103 mg/mL (*E. coli*) 55 mg/mL (*P. aeruginosa*)	75.07 ± 0.02 % (*S. aureus*) 68.86 ± 0.05 % (*E. coli*) 83.22 ± 0.01 % (*P. aeruginosa*)	Antibacterial activity Antibiofilm activity *In vitro* blood clotting	[39]
Chitosan	Octenidine dihydrochloride	Cs-Ocd bandage	*S. aureus* *Candida auris*	0.1 % octenidine dihydrochloride	45.08 ± 6.67 % (*S. aureus*) 65.53 ± 5.35 % (*C. auris*)	Antimicrobial activity Antibiofilm activity *In vitro* blood clotting	[38]
Chitosan	Chlorhexidine acetate	CSN	*P. aeruginosa*	6.25 μg/mL	59.1 %	Antibacterial activity Antibiofilm activity Anti-inflammatory activity *In vivo* wound healing	[12]
Chitosan	Zinc oxide quantum dot	CS-ZnO QDs	Methicillin-resistant *S. aureus*	NA	68.5–75.9 %	Antibacterial activity Antibiofilm activity Antioxidant activity Anti-inflammatory activity *In vivo* wound healing	[40]
Chitosan	Amikacin sulphate	Ch-nDE-AK	*S. aureus* *E. coli*	NA	67.4 % (*S. aureus*) 58.8 % (*E. coli*)	*In vitro* and *ex vivo* antibacterial activity Antibiofilm activity	[41]
Alginate	Silica-supported silver nanoparticle	Alginate films containing silica-supported silver nanoparticles	*S. aureus* *S. epidermidis* *P. aeruginosa* *C. albicans*	NA	NA	Antimicrobial activity Antibiofilm activity	[53]
Alginate	Gallic acid-functionalized silver nanoparticle	GA@AgNPs-SA	*S. aureus* Methicillin-resistant *S. aureus* *E. coli*	NA	NA	Antibacterial activity Antibiofilm activity *In vivo* wound healing	[31]
Alginate	Tannic acid	SA-PBA/TA	*S. aureus*	NA	>99 %	*In vitro* and *in vivo* antibacterial activity Antibiofilm activity Antioxidant activity *In vivo* anti-inflammatory activity	[32]
Alginate Chitosan	NA	CS-poly(MA-*co*-AA)SA hydrogel	*C. albicans*	100 μg/mL	50 %	Antifungal activity Antibiofilm activity *In vitro* wound healing	[54]
Alginate Chitosan	Tyrosol	Nio-Tyro@CS-AL	*S. aureus* *P. aeruginosa*	50–100 %	NA	Antibacterial activity Antibiofilm activity	[55]

(*continued on next page*)

Table 1 (*continued*)

Marine polysaccharides	Drugs	Name of materials	Target pathogens	Active concentrations[a]	Biofilm reduction[b]	Functions	References
						In vivo wound healing	
Alginate κ-carrageenan	*Lannea coromandelica* extract	κ-Carrageenan and sodium alginate hydrogel incorporated with CaO@SiO2 nanocomposite and L. *coromandelica extract*	*S. aureus* *P. aeruginosa* *E. coli* *Enterococcus faecalis*	100 μg/mL	60–86 % (*S. aureus*) 60–80 % (*P. aeruginosa*) 60–80 % (*E. coli*) 60–86 % (*E. faecalis*)	Antibacterial activity Antibiofilm activity *In vitro* and *in vivo* wound healing	[58]
Keratitis							
Chitosan	Fluconazole loaded MgO/CuO nanocomposite from an aqueous extract of *Talaromyces pupureogenus*	FLC-CS/MgO/CuO nanocomposites	*C. albicans*	10 μg/mL	88.1 %	Antifungal activity Antibiofilm activity Anticandidal activity on contact lenses and cases	[42]
Hyaluronic acid	$FeSO_4$	$FeSO_4$-loaded HA hydrogel	Methicillin-resistant *S. aureus*	16 μM	NA	Antibacterial activity Antibiofilm activity *In vivo* therapeutic effect	[29]
Periodontitis							
Chitosan	Ethanolic extracts of Malaysian propolis	Chitosan-propolis nanoparticles	*E. faecalis*	250 μg/mL propolis	>99 %	Antibacterial activity *Ex vivo* antibiofilm activity	[43]
Alginate	Glucose oxidase	FeAlg/GOD	Clinical oral plaque biofilm	2 mg/mL	NA	Antibacterial activity Antibiofilm activity *In vivo* therapeutic effect	[56]
Vaginosis							
Chitosan	Metronidazole	MCSNP	*E. coli*	NA	62 %	*In vitro* and *in vivo* antimicrobial activity Antibiofilm activity Anti-inflammatory activity	[45]
Chitosan	Metronidazole	MBCSNPs	*E. coli* *C. albicans*	4 μg/mL	72 %	*In vitro* and *in vivo* antimicrobial activity Antibiofilm activity	[44]

[a] Biofilm reduction concentration.
[b] Reduction compared with non-treatment.
[c] Not available.

ability of the positively charged amine groups in GlcNAc units to interact with negatively charged bacterial cell walls, membranes, and biofilm components [21,67]. These interactions disrupt cell membrane permeability, resulting in the leakage of intracellular contents and ultimately causing cell death [67]. Previous studies have shown that both high molecular weight and low molecular weight chitosan can inhibit planktonic and biofilm cells of methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) [Minimal biofilm inhibitory concentration (MBIC) values of 5–8 mg/mL] [68]. Additionally, chitosan has shown antifungal and antibiofilm properties against *C. albicans*, indicating its potential as an agent for controlling oral candidiasis (MBIC values of 0.5–1.5 mg/mL) [69]. In addition to its unique antibiofilm properties, chitosan has been widely utilized in the biomedical industry as a drug-delivery system in various forms, including nanoparticles, hydrogels, films, and coatings. In the field of wound healing, the positive charge of chitosan attracts plasma, promoting faster blood coagulation [38]. Its unique antimicrobial and antibiofilm properties can inhibit bacterial growth in the early stages of wound healing and suppress biofilm cells associated with chronic wound infections, making it a promising material for wound care [67,70]. Additionally, chitosan exhibits mucoadhesive properties, resulting from specific interactions with mucus through electrostatic attraction, hydrogen bonding, and hydrophobic effects [22]. These mucoadhesive properties facilitate strong interactions with the mucus on corneal and vaginal surfaces, positioning chitosan as a promising therapeutic option for delivering antimicrobial agents to these environments [45,71]. Additionally, chitosan enhances surface wettability and lubricity, reducing mechanical friction with body tissues when used as a catheter coating [23]. Its bone fusion-

Table 2
Application of marine polysaccharides for biomedical device-associated infections.

Marine polysaccharides	Antibiofilm agents	Name of materials	Target pathogens	Active concentrations[a]	Biofilm reduction[b]	Functions	References
Catheter							
Chitosan	NA[c]	EC	*Staphylococcus epidermidis* *Candida albicans*	100 μg/mL (*S. epidermidis*) 200 μg/mL (*C. albicans*)	68 % (*S. epidermidis*) 60 % (*C. albicans*)	Antimicrobial activity Antibiofilm activity Coated catheters	[46]
Chitosan	Silver nanoparticle	AgNPs@CS/SCS	*S. aureus* *Escherichia coli*	400 μg/mL	58 % (*S. aureus*) 80 % (*E. coli*)	Antibacterial activity Antibiofilm activity *In vivo* therapeutic effect on catheter biofilms	[47]
Alginate Chitosan	NA	ZW@CMC	*S. epidermidis* *E. coli*	NA	NA	*In vitro* and *in vivo* antibacterial activity Antibiofilm activity *In vitro* and *in vivo* antithrombotic activity Coated catheters	[57]
Implant							
Chitosan	Surfactant Amoxicillin	PESC	*E. coli* *S. aureus*	NA	86 % (*E. coli*) 43 % (*S. aureus*)	Antibiofilm activity Adsorption of serum proteins	[48]
Fucoidan Hyaluronic acid	Amoxicillin	HA/AX/FU multifunctional coating	*S. aureus*	NA	56–85 %	Antibacterial activity Antibiofilm activity Antioxidant activity Coated implants	[27]
Other application							
Chitosan	Copper oxide	CuO/CMC	*S. aureus* *E. coli*	NA	48.67 % (*S. aureus*) 60.14 % (*E. coli*)	Antibacterial activity Antibiofilm activity Applied as biomedical films	[49]
Chitosan Hyaluronic acid	Acylase	ACY/QCS	*Pseudomonas aeruginosa*	NA	71 ± 2 %	Antibacterial activity Antibiofilm activity Anti-quorum sensing activity Applied as biomedical films	[26]
Chitosan	Sophorolipid	SLs-chitosan hydrogel	*S. aureus*	NA	NA	Antibacterial activity Antibiofilm activity Applied as biomedical films	[50]

[a] Biofilm reduction concentration.
[b] Reduction compared with non-treatment.
[c] Not available.

promoting properties also make it a promising coating agent for creating bioactive surfaces on implants [24].

2.2. Hyaluronic acid as an antibacterial, antibiofilm, drug-delivery, viscoelastic, hygroscopic, and moisture-retention agent

Hyaluronic acid, a non-sulfated linear polysaccharide composed of glucuronic acid and GlcNAc, is abundantly found in the skin and connective tissues [51]. The hyaluronic acid polymer provides benefits such as biocompatibility, biodegradability, and non-immunogenicity, leading to its extensive use in various clinical applications, including dermatology, orthopedics, and ophthalmology [25]. In particular, hyaluronic acid exhibits concentration-dependent antibacterial, antibiofilm, and anti-adhesion effects against *S. aureus*, enhancing its role in the prevention of wound infections [51,52]. Bacteria exhibit reduced proliferation rates in the presence of excessive hyaluronic acid because bacterial enzymes cannot efficiently degrade it, hindering the bacteria's ability to maintain high permeability within the tissue [72,73]. Furthermore, the negative charges of hyaluronic acid induce electrostatic repulsion with negatively charged bacterial cell walls, resulting in strong antibiofilm and antifouling properties [73,74]. Due to these unique antibacterial and antibiofilm properties, it is used in the production of wound dressings, either alone or in combination with other polymers, by being manufactured into hydrogels and films [75,76]. Additionally, the hydrophilic nature of hyaluronic acid enables it to function as a protective coating on medical devices, significantly reducing pathogen adhesion [26–28]. In addition to these applications, the viscoelastic and hygroscopic properties of hyaluronic acid make it well-suited for use in ocular drug-delivery systems [29,30]. The carboxyl groups on the backbone of hyaluronic acid give it strong moisture-retaining properties, enabling it to retain moisture for extended periods and effectively lubricate the surface of the eye [77,78].

2.3. Alginate as a drug-delivery, exudate absorption, and moisture-retention agent

Alginate is an anionic linear polysaccharide derived from brown algae, consisting of varying ratios of β-D-mannuronic and α-L-guluronic acid units [79]. It is recognized as a hydrophilic, biocompatible, pH-sensitive, non-toxic, biodegradable, and non-immunogenic material [80]. Notably, alginate readily forms hydrogels in aqueous solutions when ionic crosslinkers such as Ca^{2+} are added [53]. This property makes alginate one of the most valuable polymers for wound dressing materials. Alginate dressings mimic the extracellular matrix, maintaining a moist environment at the wound site, absorbing exudates, and thus accelerating wound healing [31]. Additionally, alginate can be structured into forms such as films, hydrogels, foams, and gauzes, making it highly suitable for controlling the release of bioactive molecules in wound dressings [33]. Alginate dressings can facilitate localized drug release by encapsulating antimicrobial and antibiofilm active compounds, thereby minimizing the risk of wound infection by pathogens [32,33]. These promising properties enhance its suitability for wound

Table 3
Marine polysaccharide-based smart materials for biofilm control.

Antibiofilm agents	Name of materials	Trigger	Target pathogens	Functions	References
Chitosan					
Emodin	Emo-CS-NP	Light	*Streptococcus mutans*	Antibiofilm activity	[127]
Mesoporous TiO_2-Ag	DT-Ag-CS^+	Sono	*S. mutans*	Antibacterial activity Antibiofilm activity *In vivo* anti-periodontitis efficacy	[128]
Tanshinone IIA	TA@CS	pH	*S. mutans*	Antibacterial activity Antibiofilm activity	[129]
L-arginine Indocyanine green	CAI NP	Light	Methicillin-resistant *Staphylococcus aureus*	*In vitro* and *in vivo* antibiofilm activity	[7]
Hyaluronic acid					
Gentamicin Tannic acid Silver nanoparticles	MNPs@Ag@HA	Enzyme pH	*S. aureus* *Escherichia coli*	Antibacterial activity Antibiofilm activity	[130]
Ascorbic acid	AA@Ru@HA-MoS_2	Enzyme Light	*S. aureus* *Pseudomonas aeruginosa*	*In vitro* and *in vivo* Antibacterial activity Antibiofilm activity	[131]
Alginate					
3-Amino-7-chloro-2-nonylquinazolin-4(*3H*)-one	NA[a]	pH	*P. aeruginosa*	Antibacterial activity Antibiofilm activity	[132]
Nisin	PPC-NCS	pH	*S. epidermidis* *Enterococcus faecium* *E. faecalis*	Antibacterial activity Antibiofilm activity	[133]

[a] Not available.

healing applications [31,32,53].

2.4. Carrageenan as an antibacterial, antibiofilm, drug-delivery, and anticoagulant agent

Carrageenan is a sulfated linear polysaccharide derived from red algae, consisting of galactose and anhydrogalactose units linked by glycosidic bonds [81]. It exhibits structural diversity and is primarily found in the forms of ι-carrageenan, κ-carrageenan, and λ-carrageenan [81]. Among these, κ-carrageenan is particularly recognized for its exceptional gel-forming properties, biocompatibility, biodegradability, and drug-delivery capabilities, making it a popular choice for preparing hydrogels, either on its own or in combination with other natural polymers [58,82]. κ-Carrageenan also possesses superior properties as a hemostatic dressing material due to its ability to concentrate coagulation factors, thereby promoting rapid hemostasis [34]. Additionally, previous studies have shown that κ-carrageenan exhibits antibacterial and antibiofilm activity against clinical strains of *S. aureus* by interacting with glycoprotein receptors on the polysaccharide surface, thereby increasing cytoplasmic membrane permeability and leading to protein leakage [35]. These unique antimicrobial and antibiofilm characteristics indicate that carrageenan is a promising candidate for drug-delivery systems aimed at wound healing.

2.5. Fucoidan as an antibacterial, antibiofilm, and drug-delivery agent

Fucoidan is a sulfated anionic polysaccharide derived from brown algae, primarily composed of L-fucose monosaccharides [83]. This sulfated polysaccharide demonstrates various biological activities, including antibacterial and antibiofilm properties [84,85], which are influenced by its molecular weight, degree of sulfation, and molecular structure [83]. Fucoidan demonstrates antimicrobial activity through an apoptosis pathway that involves interaction with bacterial membrane proteins, leading to cell membrane disruption [36]. Fucoidan is employed in biomaterial fabrication alongside other polymers due to its capacity to assemble with cationic polysaccharides, its structural similarity to mammalian glycosaminoglycans, high biocompatibility, low toxicity, and inherent antimicrobial and antibiofilm properties [27,86].

3. Biomedical application of traditional materials based on marine polysaccharides for controlling biofilm-related diseases

3.1. Controlling biofilm related to wound infection

Wound infection is a complication of skin damage that leads to severe patient pain, presents a public health risk, and results in substantial financial costs [87]. The skin typically acts as the body's primary barrier against external microbes and plays a vital role in supporting the immune system. However, when the skin is damaged by severe trauma, it becomes vulnerable to microbial invasion, leading to wounds that heal more slowly [38,88]. If wounds infected by pathogenic microorganisms are not thoroughly treated, these microorganisms can form biofilms, which alter the wound microenvironment and delay the healing process [12]. Bacteria can form a mature biofilm on a wound site within 24 h, and once established, the biofilm becomes integrated into the wound area [89]. Biofilms have been reported to be involved in 78.2 % of wound infections [90], highlighting the clinical importance of biofilm control in wound healing. The issue of biofilms is particularly prominent in chronic wounds (60 %) compared to acute wounds (6 %) [91]. Biofilms in chronic wounds facilitate genetic exchange through microbial interactions, resulting in antimicrobial resistance and leading to symptoms such as necrosis, pain, excessive exudate, and foul odor [92]. Moreover, biofilms effectively shield pathogenic microorganisms from phagocytic attacks by macrophages and neutrophils, which are crucial components of the body's innate immune system [93]. In chronic wounds, biofilms are composed of multiple microbial groups. Pathogenic microorganisms such as *S. aureus*, *Enterococcus faecalis*, *P. aeruginosa*, *C. albicans*, and *Klebsiella pneumoniae* form polymicrobial biofilms in chronic wounds, leading to clinical complications [18,92,94]. Consequently, preventing biofilm formation on wound surfaces is effective in reducing infection rates and promoting faster healing. The antibiofilm wound dressing market was valued at $571 million in 2019 and is projected to grow at a compound annual rate of 9.1 % by 2027 [95], underscoring the clinical importance of biofilm control in treating wound infections. Therefore, it is crucial to develop new wound dressings or agents targeting wounds infected with biofilm-forming pathogens, especially by creating alternative therapeutic strategies to address the issue of antimicrobial resistance.

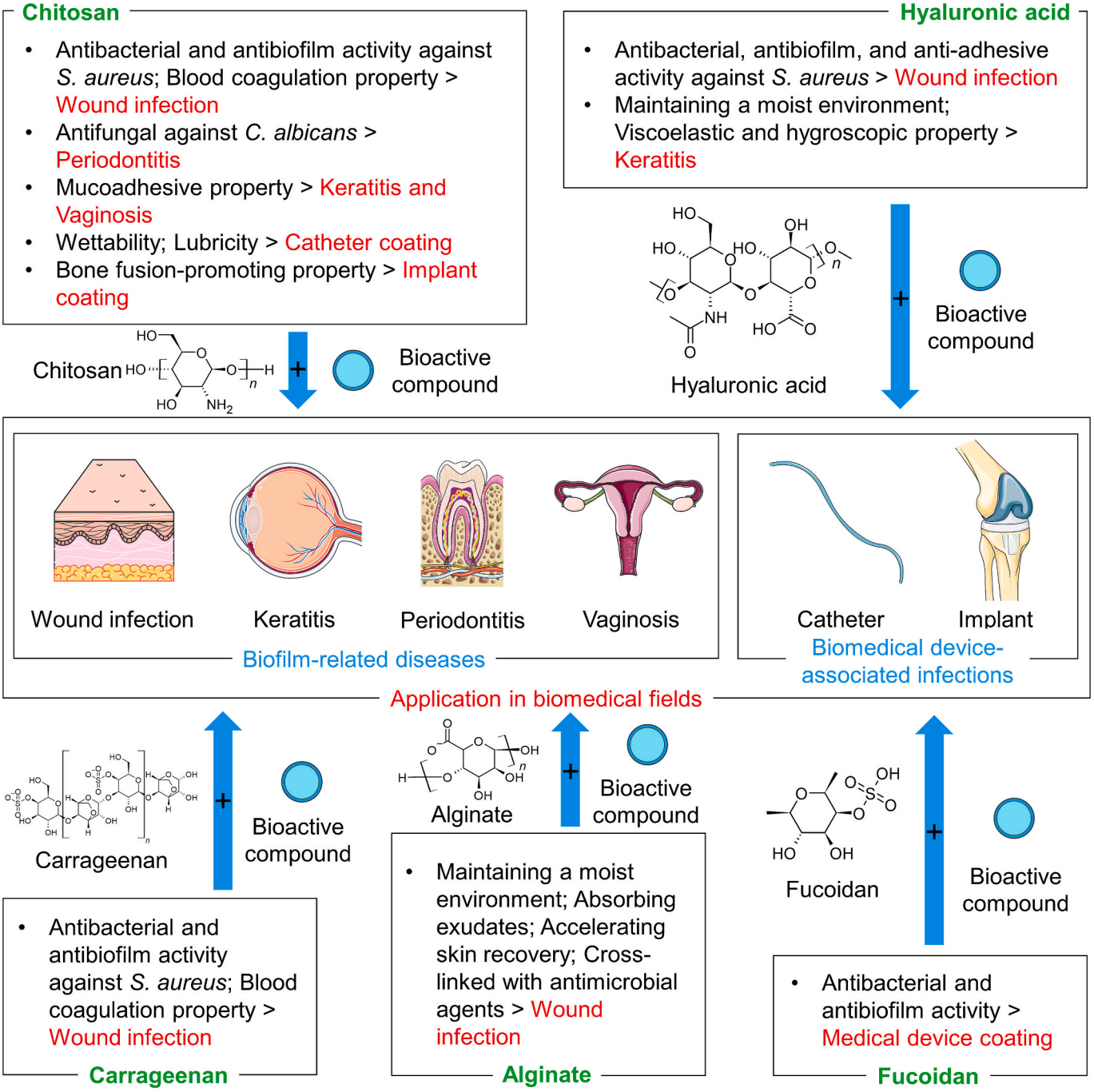

Fig. 1. Marine polysaccharides as antibiofilm materials. Marine-derived polysaccharides, including chitosan, hyaluronic acid, alginate, carrageenan, and fucoidan, are utilized as materials for nanomaterials in the biomedical field, specifically for antibiofilm applications. Owing to their inherent antibiofilm properties and diverse material characteristics, these polysaccharides are being applied in the treatment of biofilm-associated diseases—such as wound infections, keratitis, periodontitis, and vaginitis—as well as in the prevention of infections linked to biomedical devices, including catheters and implants.

Chitosan and its derivatives are some of the most extensively studied marine polysaccharides in wound healing, known for their ability to accelerate the healing process and their versatility in being processed into various forms, including hydrogels, membranes, nanoparticles, and scaffolds [96]. Additionally, chitosan and its derivatives possess intrinsic antibacterial and antibiofilm properties, which, in combination with loaded drugs, can prevent bacterial growth during the early stages of wound healing, ultimately reducing the risk of chronic infection [67]. Thao et al. synthesized *N*-succinyl chitosan nanoparticles (*N*-SuC NPs) using the ion gelation method and created an *N*-SuC NP film by employing the solution casting method with the synthesized nanoparticles [37]. The *N*-SuC NP film demonstrated concentration-dependent inhibition of biofilms formed by *S. aureus* and *Escherichia coli*, indicating its potential as a promising candidate for treating chronic wounds. The *in vivo* wound healing results showed that the *N*-SuC NP film significantly enhanced granulation tissue formation and epithelialization, thereby accelerating the wound healing process. These findings resulted from the introduction of the succinyl group into the GlcN unit at the *N*-terminus of chitosan, which disrupted both intermolecular and intramolecular hydrogen bonds, thereby exposing a greater number of carboxyl and amino groups. The increased abundance of carboxyl groups enhanced the film's moisture retention properties, promoting a

moist environment in the wound area. Additionally, the increased prevalence of amino groups enhanced its antibacterial and antibiofilm activities. Pandian et al. fabricated *in-situ* silver nanoparticles incorporated *N*, *O*-carboxymethyl chitosan based self-healing hydrogels (*N*, *O*-CMC/AgNps hydrogels) using an ethylenediaminetetraacetic acid-ferric ion complex [39]. The *N*, *O*-CMC/AgNps hydrogels effectively suppressed biofilm formation by *S. aureus*, *E. coli*, and *P. aeruginosa*, primarily due to the incorporation of silver nanoparticles within the hydrogel. The carboxyl group of *N*, *O*-carboxymethyl chitosan facilitated the reduction of $AgNO_3$ to Ag^+ ions, initiating the diffusion-driven growth of seed crystals and leading to the formation of silver nanoparticles through metal atom clustering. In its wound healing capacity, the chitosan within the *N*, *O*-CMC/AgNps hydrogel exhibited a strong affinity for plasma, interacting with blood and displaying hemostatic properties. Pandian et al. developed octenidine dihydrochloride incorporated chitosan-based flexible bandages (Cs-Ocd bandages) using a simple freeze-drying method [38]. Octenidine dihydrochloride in the chitosan bandages exhibited a burst release of 50 % on the first day, which was attributed to electrostatic repulsion between the cationic drug and the cationic chitosan. The Cs-Ocd bandage treatment reduced biofilm formation in *S. aureus* and *C. auris*, which was attributed to the strong affinity of octenidine dihydrochloride for the cell membrane, leading to membrane leakage. In *in vitro* blood coagulation experiments, the Cs-Ocd bandages demonstrated excellent hemostatic properties, attributed to the positively charged chitosan's strong attraction to plasma, which enhanced platelet adhesion. Additionally, the highly porous nature of the Cs-Ocd bandages contributed to improved swelling properties, a slow degradation profile, and controlled release of octenidine dihydrochloride. These characteristics suggest that Cs-Ocd bandages could serve as promising wound dressings with antibiofilm properties, effectively enhancing wound healing. Cai et al. designed a chitosan-modified lipophilic self-nano emulsifying system (CSN) using lipophilic chlorhexidine acetate through a low-energy emulsification method [12]. CSN effectively inhibited the biofilm formation of *P. aeruginosa* and eradicated mature biofilms by altering membrane permeability and disrupting cell membrane metabolism, thereby facilitating the entry of more drugs into the cytoplasm. The cationic components of chitosan and chlorhexidine acetate interacted with the negatively charged bacterial cell wall, increasing cell permeability and ultimately leading to cell death. In an *in vivo* infected wound model, CSN treatment significantly reduced the initial infection and promoted the healing of *P. aeruginosa*-infected skin wounds by lowering the levels of inflammatory cytokines (TGF-β, TNF-α, and IL-6). Shu et al. prepared a ZnO-quantum dots-chitosan biocomposite dressing (CS-ZnO QDs) by incorporating ZnO-quantum dots into chitosan (Fig. 2) [40]. The CS-ZnO QD treatment was more effective at removing MRSA biofilm compared to treatment with either chitosan or ZnO quantum dots alone. This effect was attributed to the interaction between chitosan and bacteria, which facilitated the penetration of ZnO quantum dots into the biofilm, leading to severe cell damage and oxidative stress. The *in vivo* wound healing ability results showed that CS-ZnO QDs alleviated inflammation by regulating the NFκB-p65 pathway, while also directly enhancing re-epithelialization and collagen deposition.

Mothilal et al. constructed an amikacin sulphate incorporated chitosan and diopside nanoparticles composite dressing (Ch-nDE-AK) (Fig. 3) [41]. The addition of diopside nanoparticles helped reduce the drug release rate of Ch-nDE-AK by compensating for the weak interaction between amikacin sulfate and chitosan. This effect is attributed to the interaction between the calcium ions of the diopside nanoparticles and the sulfate ions of amikacin sulfate. Ch-nDE-AK demonstrated *in vitro* antibacterial and antibiofilm activity against clinical isolates of *S. aureus* and *E. coli*. In an *ex vivo* antibacterial experiment, Ch-nDE-AK effectively inhibited the growth of *S. aureus* and *E. coli* infected on pig skin. The antibacterial and antibiofilm activities of Ch-nDE-AK stemmed from the disruption of protein synthesis in bacterial cells by amikacin, which is attributed to its polycationic nature. Specifically, the incorporation of diopside nanoparticles enhanced fibroblast cell migration compared to chitosan alone, indicating that Ch-nDE-AK may improve the wound healing process.

Alginate is utilized in the development of wound dressing materials due to its ability to enhance the hydrophilicity of biomaterials, absorb wound exudate, and accelerate skin recovery [97]. Additionally, alginate can be easily cross-linked with antimicrobial agents, making it an effective microbe-resistant wound dressing material for clinical

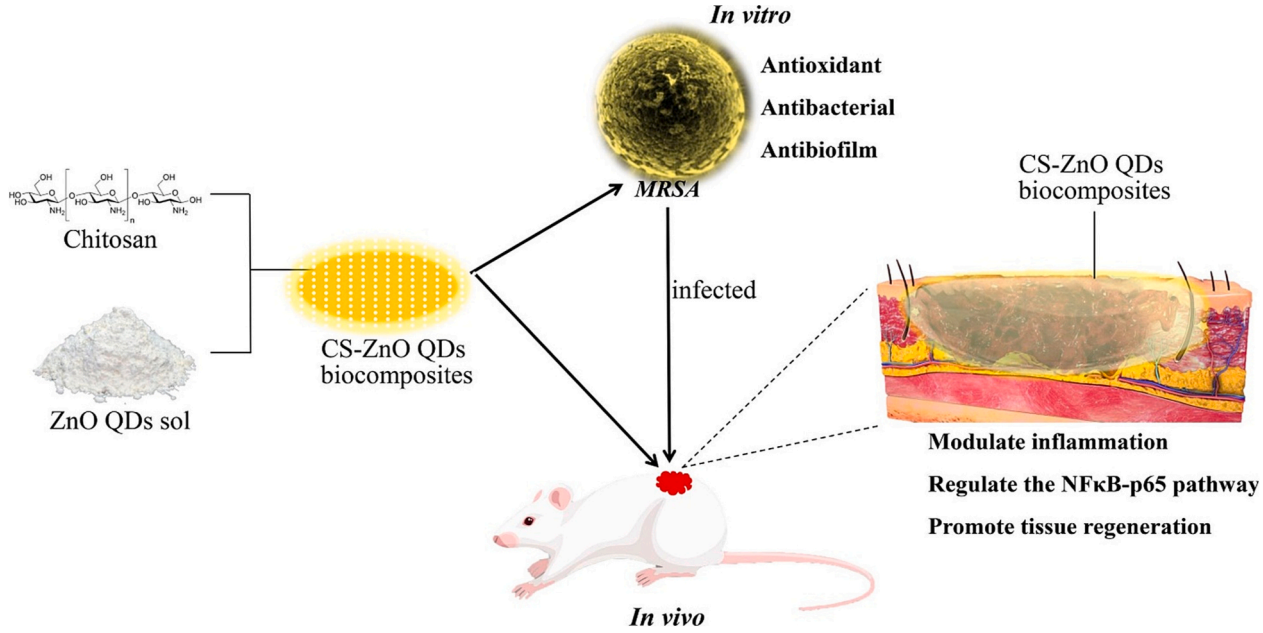

Fig. 2. Preparation and application of ZnO-quantum dots-chitosan biocomposite dressing (CS-ZnO QDs). CS-ZnO QDs were synthesized by incorporating ZnO QDs into chitosan, which demonstrated *in vitro* antibacterial and antibiofilm activity against methicillin-resistant *Staphylococcus aureus*. Furthermore, CS-ZnO QDs showed excellent wound healing properties by modulating the NFκB-p65 pathway, reducing inflammation, and promoting re-epithelialization.
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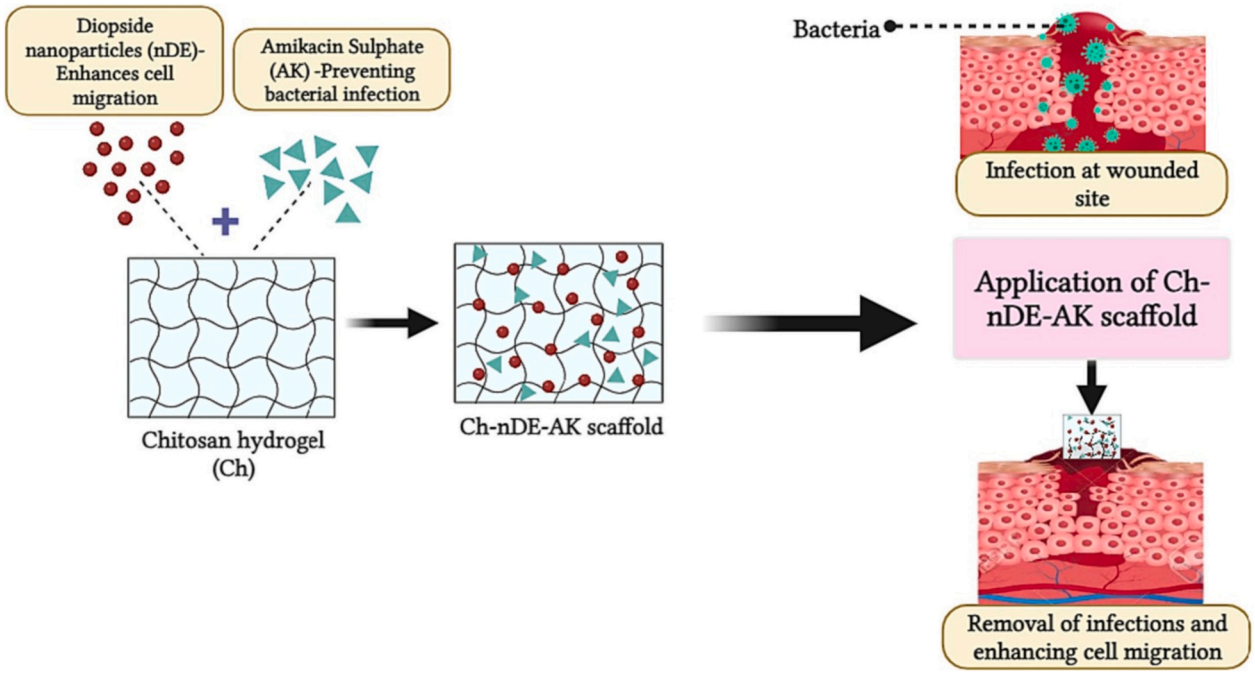

Fig. 3. Preparation of amikacin sulphate incorporated chitosan and diopside nanoparticles composite dressing (Ch-nDE-AK) and its wound healing mechanisms. Ch-nDE-AK exhibited enhanced wound-healing effects by controlling bacterial infection at the wound site and promoting cell migration.
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applications [33]. Ambrogi et al. synthesized silica-supported silver nanoparticles (CAB-O-SIL-Ag) *via* a solid-state sintering method without using solvents or reducing agents and subsequently utilized them in the production of CAB-O-SIL-Ag film [53]. The CAB-O-SIL-Ag film rapidly released 2 % of the total silver ions within the first 5 h, followed by a slower release of an additional 3 % over the next 24 h. The CAB-O-SIL-Ag film exhibited antimicrobial and antibiofilm effects against *S. aureus*, *P. aeruginosa*, *S. epidermidis*, and *C. albicans* in the contact area. The intrinsic antimicrobial and antibiofilm activities of the CAB-O-SIL-Ag film were attributed to its nanostructure and controlled silver ion release properties. The CAB-O-SIL-Ag film demonstrated biocompatibility by showing no cytotoxicity to human fibroblasts and keratinocytes, which was attributed to its slow silver ion release characteristics. Hu et al. developed an injectable hydrogel dressing by cross-linking sodium alginate molecular chains with gallic acid-functionalized silver nanoparticles using calcium ions [31]. This hydrogel demonstrated antibacterial and antibiofilm activity against *E. coli*, *S. aureus*, and MRSA by releasing silver ions in a controlled manner. *In vivo* wound healing results demonstrated that this hydrogel alleviated the inflammatory response by reducing the expression of IL-6 and TNF-α, while promoting angiogenesis by upregulating the expression of VEGF, CD31, and α-SMA, thereby facilitating wound healing. Zeng et al. fabricated an alginate-based antibacterial hydrogel (SPT hydrogel) by incorporating boronate ester bonds formed between tannic acid and phenylboronic acid (Fig. 4) [32]. The SPT hydrogel was designed to continuously release tannic acid by cleaving the boronate ester bonds when exposed to an acidic environment. *In vitro* antibacterial and antibiofilm experiments revealed that the released tannic acid inactivated *S. aureus* by destabilizing the cell membrane, increasing membrane permeability, and inhibiting enzyme activity. In an *in vivo* wound infection model, the SPT hydrogel significantly reduced the number of inflammatory cells and demonstrated excellent biocompatibility.

The use of a mixture of marine polysaccharides creates a framework with enhanced biological and mechanical properties, paving the way for new strategies in wound healing [54,55]. Raj et al. synthesized binary-grafted chitosan with acrylic acid and methacrylic acid using a free radical reaction, and then cross-linked it with alginate to prepare a self-healing grafted chitosan–sodium alginate-based hydrogel [CS-poly(MA-*co*-AA)SA] (Fig. 5) [54]. The cationic surface charge of the CS-poly(MA-*co*-AA)SA hydrogel attached to *C. albicans*, increased membrane permeability, and caused disruptions at the gene and protein levels. Additionally, treatment with CS-poly(MA-*co*-AA)SA hydrogel inhibited hyphal cell formation in *C. albicans* and reduced its ability to attach to abiotic surfaces. The results of the *in vitro* cell migration assay demonstrated that CS-poly(MA-*co*-AA)SA hydrogel effectively covered the free area within a specific time interval, indicating its potential for use in chronic wound treatment.

Beram et al. fabricated a tyrosol-loaded niosome integrated into chitosan–alginate scaffold (Nio-Tyro@CS-AL) using advanced electrospinning and 3D printing techniques [55]. The release of tyrosol-loaded niosomes from Nio-Tyro@CS-AL was attributed to deprotonation at pH levels above chitosan's isoelectric point and to the protonation of the amino group at acidic pH. The Nio-Tyro@CS-AL exhibited antibiofilm activity against *P. aeruginosa* and *S. aureus* by reducing the expression of the biofilm-related genes (*ndvB* in *P. aeruginosa* and *icaB* in *S. aureus*). The *in vivo* wound healing evaluation revealed that Nio-Tyro@CS-AL treatment accelerated the healing process by enhancing angiogenesis, tissue remodeling, and re-epithelialization. Haseef et al. developed a polymer hydrogel matrix composed of κ-carrageenan and alginate, incorporating a calcium silica nanocomposite and *Lannea coromandelica* bark extract [58]. This hydrogel prevented the biofilm formation of *E. coli*, *P. aeruginosa*, *S. aureus*, and *E. faecalis*. This effect was attributed to the O^{2-} ions released by the calcium silica nanocomposite, the aldehyde groups in κ-carrageenan, and the antibacterial substances (siloxanes and squalene) found in the *L. coromandelica* extract. *In vitro* and *in vivo* wound healing assays demonstrated that this hydrogel exhibited enhanced hemostatic efficiency through the release of calcium ions.

3.2. Controlling biofilm related to keratitis

Keratitis is a condition that occurs when external risk factors cause damage to the corneal epithelium, making the eye more vulnerable to bacterial and fungal infections [29]. External risk factors include corneal trauma, contact lens use, and the use of contaminated surgical

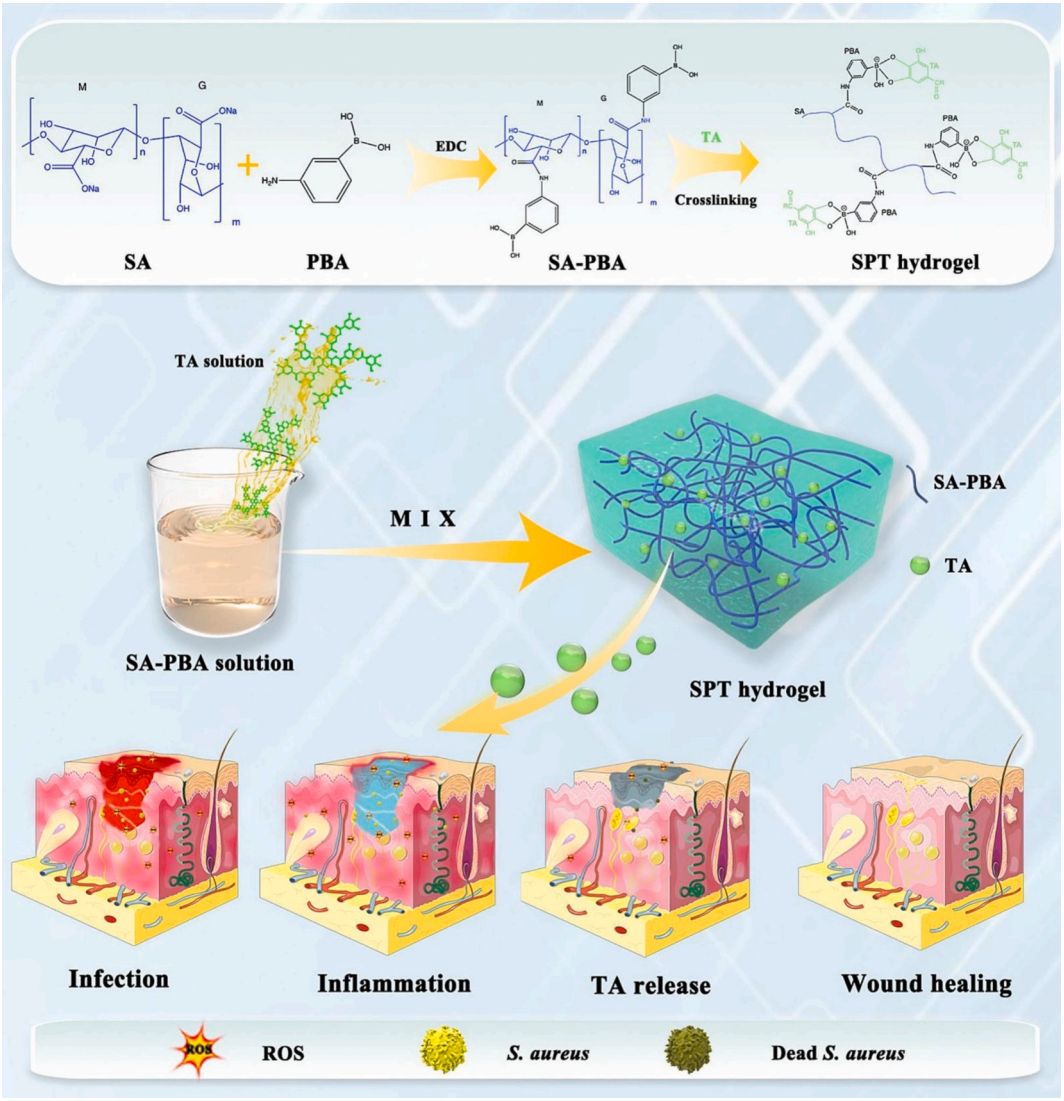

Fig. 4. Preparation and mechanism of alginate-based antibacterial hydrogel (SPT hydrogel) for wound healing. The SPT hydrogel was synthesized by forming boronate ester bonds between tannic acid and phenylboronic acid. This hydrogel released tannic acid, demonstrating antibacterial and anti-inflammatory properties, indicating its potential application as a wound dressing.
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instruments during eye surgery [98]. These factors weaken the cornea's defense mechanisms, allowing pathogens to invade [99]. When pathogens invade the corneal tissue, they initiate a severe inflammatory response, resulting in symptoms like ocular pain, tearing, redness, spasms, swelling, and visual impairment [99]. Bacterial keratitis is primarily caused by pathogenic bacteria such as *P. aeruginosa*, *S. aureus*, and *S. pneumoniae* [100]. These bacteria can develop resistance to various antibiotics and lead to additional complications, posing a significant threat to immunocompromised patients [100]. Fungal keratitis is primarily caused by *C. albicans*, *Fusarium* spp., and *Aspergillus* spp., which can grow on contact lenses and lens cases, potentially leading to keratitis when they come into contact with the eye's surface [101]. Pathogens responsible for keratitis are known to form biofilms, which serve as critical virulence factors in the disease [13]. Notably, bacteria and fungi often collaborate to form polymicrobial biofilms, contributing to co-infections. Frequent interactions between *Staphylococcus* spp. and *Aspergillus* spp. have been reported [102]. Ultimately, biofilms create a favorable environment for pathogens to survive on the host corneal epithelium, enabling them to persist and trigger a strong inflammatory response [102]. Therefore, controlling biofilms may be a promising strategy for treating keratitis infections caused by pathogens. This can be accomplished by inhibiting biofilm formation on ocular materials like contact lenses, cases, and surgical instruments or by removing biofilms that have already formed on the cornea.

Chitosan is an attractive biomaterial due to its mucoadhesive properties, which arise from the electrostatic attraction between chitosan and the mucin glycoproteins present in mucus on the mucosal surface [22,71]. Abbas et al. developed chitosan encapsulated fluconazole loaded MgO/CuO nanocomposite (FLC-CS/MgO/CuO nanocomposite) to control the colonization and biofilm development of *C. albicans* associated with keratomycosis (Fig. 6) [42]. FLC-CS/MgO/CuO nanocomposite demonstrated *in vitro* antibiofilm activity against *C. albicans* isolates from patients with keratomycosis. Additionally, treatment with the FLC-CS/MgO/CuO nanocomposite inhibited *C. albicans* biofilm formation on contact lens surfaces and within contact lens cases. Due to its positive charge, chitosan readily interacts with the negatively charged cell walls of pathogens, thereby demonstrating its capacity to inhibit biofilm formation. The hemolysis assay results demonstrated that the FLC-CS/MgO/CuO nanocomposite exhibits high hemocompatibility, suggesting that it could be an effective antibiofilm treatment option for

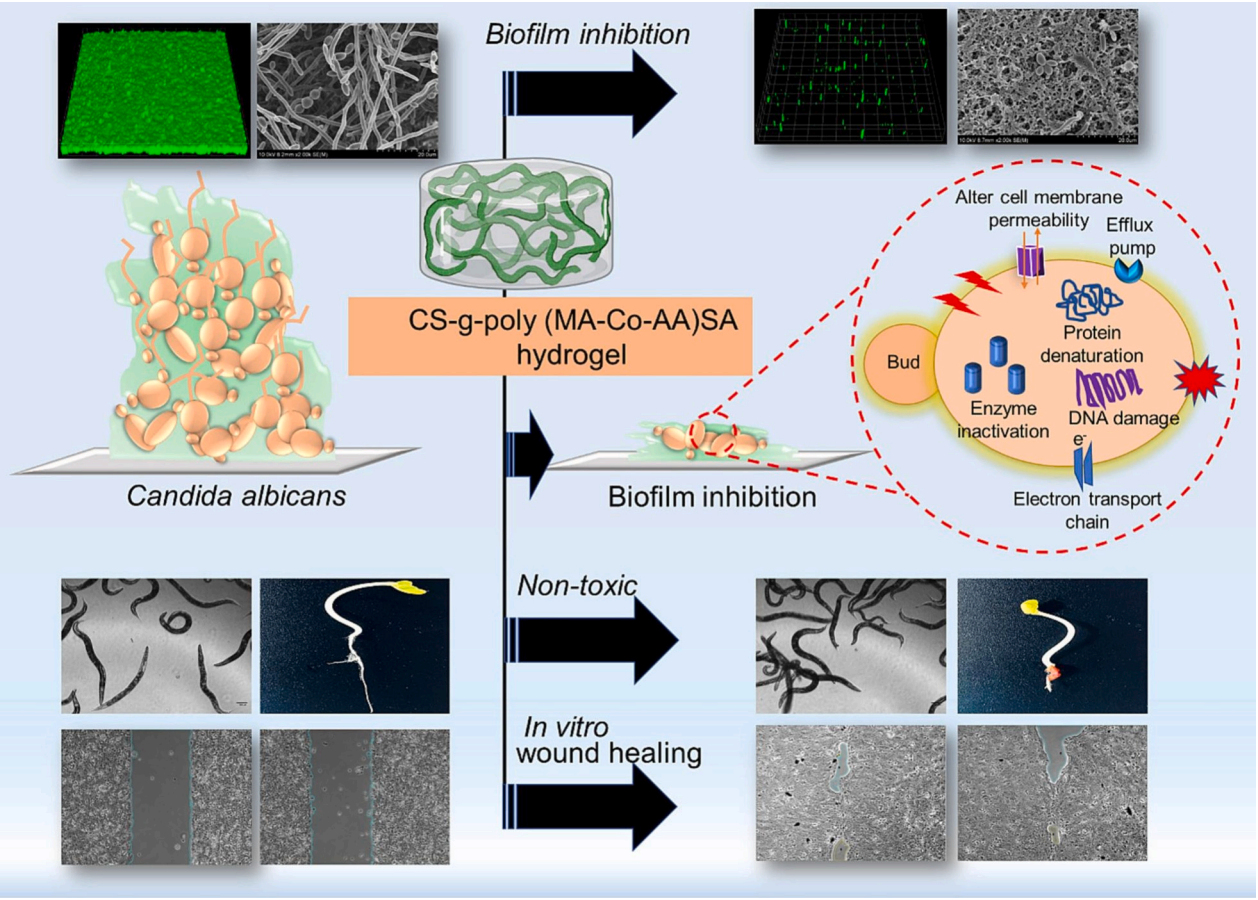

Fig. 5. Proposed mechanism of *Candida albicans* biofilm inhibition and wound healing by self-healing grafted chitosan–sodium alginate-based hydrogel [CS-poly (MA-*co*-AA)SA]. The CS-poly(MA-*co*-AA)SA hydrogel exhibited antibiofilm effects against *C. albicans* by disrupting gene and protein expression levels. The safety of the CS-poly(MA-*co*-AA)SA hydrogel was confirmed through tests conducted using *Caenorhabditis elegans* and *Raphanus raphanistrum* models.
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contact lens-related keratomycosis.

Hyaluronic acid is widely used in eye care for its ability to provide long-lasting hydration, maintain moisture, and effectively lubricate the ocular surface [30,77]. Wang et al. prepared ferrous sulfate-loaded hydrogels using hyaluronic acid and ascorbate (Fig. 7) [29]. The ferrous sulfate-hyaluronic acid hydrogel was created through electrostatic interaction between the positively charged Fe^{2+} ions of ferrous sulfate and the negatively charged carboxyl groups of hyaluronic acid. Ferrous sulfate inhibited *S. aureus* biofilm formation and eradicated preformed biofilm by inducing reactive oxygen species (ROS) production, causing lipid peroxidation, and promoting ferroptosis-like cell death. In a mouse keratitis model of *S. aureus* infection, treatment with the ferrous sulfate-hyaluronic acid hydrogel rapidly reversed keratitis, prevented *S. aureus* dissemination to the lungs, and alleviated systemic inflammation. Moreover, the ferrous sulfate-hyaluronic acid hydrogel demonstrated excellent cytocompatibility, hemocompatibility, and biosafety, suggesting its potential as a promising medical material for treating ocular infections caused by *S. aureus*.

3.3. Controlling biofilm related to periodontitis

Periodontitis is a widespread chronic disease affecting 10 % of the global population, marked by the formation of biofilm by pathogenic bacteria within dental plaque [14,103]. In a healthy oral environment, microorganisms within the periodontal biofilm exist in a symbiotic relationship, contributing to the maintenance of oral health [104]. However, when an imbalance occurs in the oral microbiome—resulting in a decrease in beneficial bacteria and an overgrowth of pathogenic bacteria—it triggers inflammation and destruction of periodontal tissues, ultimately contributing to periodontal disease [105]. Several bacterial species are associated with periodontitis, including *Porphyromonas gingivalis*, *Tannerella forsythensis*, *Treponema denticola*, *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans*, *E. faecalis*, and *Streptococcus* spp. [106,107]. Periodontal disease typically begins with gingivitis and progresses by damaging the supporting periodontal tissues, weakening the connection between the gums and teeth, and ultimately leading to tooth loss [56]. Additionally, pathogens within the periodontal biofilm can disseminate systemically through the adjacent gingival blood flow, potentially acting as risk factors for other systemic diseases, such as chronic liver disease and cardiovascular disease [14]. Treatment of periodontitis involves mechanical methods to remove the periodontal biofilm, along with strategies to restore the balance of the oral microbiome [105].

A chitosan-based drug-delivery system is being explored for the effective treatment of various pathological conditions associated with periodontal disease. This system provides prolonged retention in the oral cavity, facilitates effective drug penetration, and exhibits antibacterial activity against oral pathogens [108]. Parolia et al. synthesized chitosan-propolis nanoparticles (CPN) as an agent against *E. faecalis* biofilm in root canals [43]. CPN treatment was more effective than chitosan or propolis alone in eradicating *E. faecalis* biofilms formed on extracted human teeth. In particular, the antibiofilm activity of CPN was confirmed against *E. faecalis* isolated from patients with failed root canal treatments, suggesting CPN as a potential endodontic agent for future use.

Alginate has also been utilized as a drug-delivery polymer to manage

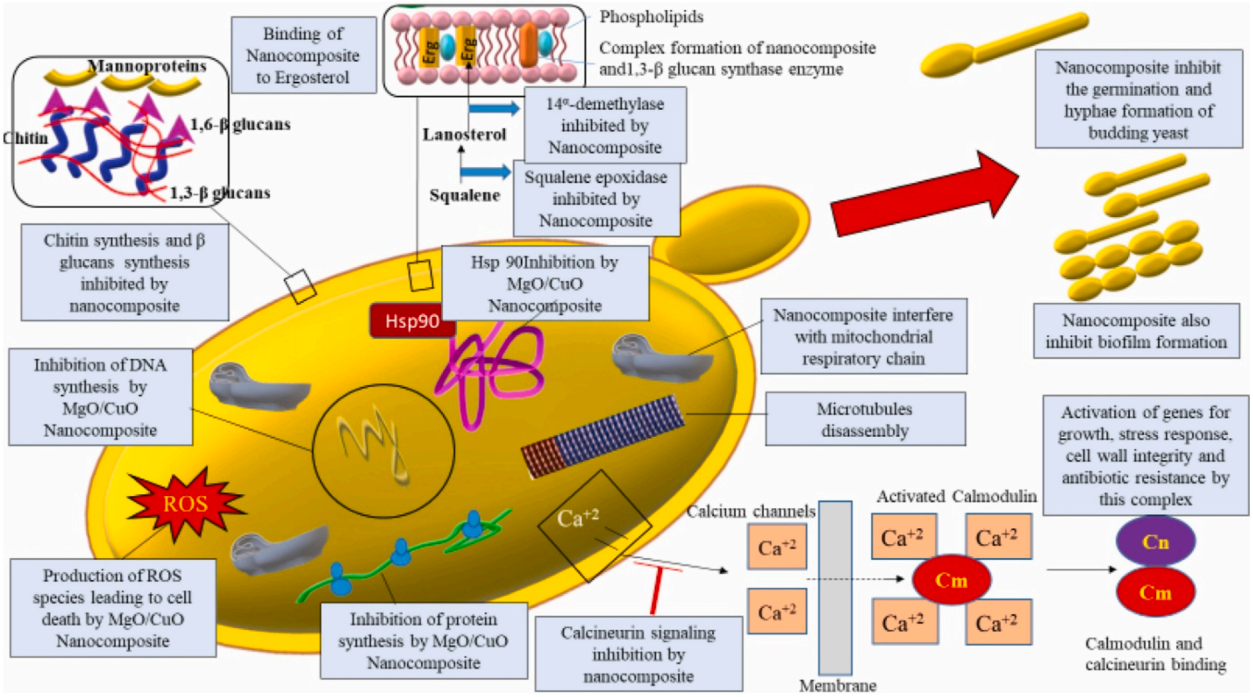

Fig. 6. Proposed antifungal mechanism of chitosan encapsulated fluconazole-loaded MgO/CuO nanocomposite (FLC-CS/MgO/CuO nanocomposite) against *Candida albicans*. The FLC-CS/MgO/CuO nanocomposite is proposed to exhibit anticandidal activity by damaging the cell wall of *C. albicans*. This nanocomposite reduces glucan levels in the cell wall, leading to the depletion of Hsp90 protein, which in turn inhibits biofilm formation. Additionally, the FLC-CS/MgO/CuO nanocomposite may exert its anticandidal effect by inhibiting calcineurin signaling.
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oral pathogens effectively. Its properties make it a valuable tool in targeting and controlling infections in the oral cavity. Wang et al. fabricated glucose oxidase loaded upon iron alginate (FeAlg/GOD) using a straightforward one-step cross-linking process [56]. The periodontitis therapy strategy using FeAlg/GOD involved glucose oxidase catalyzing the decomposition of glucose into gluconic acid and hydrogen peroxide after FeAlg/GOD penetrated deep into periodontal pockets. Subsequently, Fe^{3+} was converted to Fe^{2+}, triggering the Fenton reaction and generating numerous hydroxyl radicals. The FeAlg/GOD inhibited plaque biofilm formation on tooth slices, owing to the ROS generated from FeAlg/GOD, which compromised the structural integrity of the biofilm. In an experimental model of periodontitis in rats, FeAlg/GOD treatment was shown to reduce bone loss, decrease inflammatory responses, and promote healthier gum tissue.

3.4. Controlling biofilm related to vaginosis

Bacterial vaginosis is a prevalent vaginal condition, affecting an estimated 23–29 % of women of reproductive age [109]. Bacterial vaginosis typically arises when the microbial community within the vaginal area is disrupted [45]. The normal vaginal epithelium is predominantly composed of lactobacilli, which produce hydrogen peroxide and lactic acid [110]. These substances acidify the vaginal environment, thereby limiting the adhesion and proliferation of major pathogens [110]. However, when the vaginal microbial community is disrupted, the population of lactobacilli diminishes, creating an opportunity for anaerobic pathogens such as *Gardnerella vaginalis* to proliferate, ultimately leading to bacterial vaginosis [111]. Bacterial vaginosis is characterized by a polymicrobial biofilm predominantly composed of *G. vaginalis*, which plays a significant role not only in the pathogenesis of the condition but also in treatment failure and recurrence [15]. The *G. vaginalis* biofilm shows significant resistance to the hydrogen peroxide and lactic acid produced by lactobacilli, enabling it to evade the defensive mechanisms of the normal vaginal microbiota and persist long-term within the host [15]. Additionally, once *G. vaginalis* adheres to and colonizes the vaginal epithelium, it serves as a scaffold for other species (*e.g.*, *Atopobium vaginae*, *P. bivia*, *Bacteroides* spp., and *Prevotella* spp.) to attach, thereby playing a central role in the development of bacterial vaginosis [111]. Therefore, targeting vaginal biofilms may represent a promising strategy for treating bacterial vaginosis.

Polymeric drug-delivery systems with mucoadhesive properties, such as chitosan, provide an effective solution for intravaginal antimicrobial delivery [112]. Nayak et al. prepared metronidazole-loaded chitosan nanoparticles (MCSNP) using phytic acid as a cross-linker (Fig. 8) [45]. MCSNP exhibited high mucoadhesive properties by interacting with negatively charged mucus, attributed to their positive zeta potential and size range of 100–200 nm. MCSNP treatment reduced *E. coli* biofilm formation, attributed to the unique antibacterial properties of chitosan and the metal-chelating ability of phytic acid. The *in vivo* antibacterial evaluation using a mouse vaginal infection model demonstrated that MCSNP effectively inhibited bacterial biofilm formation and exhibited lower cytotoxicity compared to metronidazole alone. Additionally, histopathological analysis revealed that MCSNP treatment protected the vaginal epithelium from bacterial vaginosis by preventing hyperemia, hyperplasia, and lymphocyte infiltration.

Furthermore, Nayak et al. developed MCSNP using borax as an antibacterial cross-linker [44]. Borax cross-linker-based MCSNPs demonstrated high mucoadhesion efficiency due to their positive zeta potential and small size. Treatment with borax cross-linker-based MCSNPs showed higher antibiofilm efficacy against *E. coli* compared to metronidazole alone. Additionally, *in vivo* antibacterial results demonstrated that borax cross-linker-based MCSNPs significantly reduced bacterial counts in vaginally infected mice compared to commercial formulations and were safe for vaginal use. These results suggested that chitosan nanoparticles could serve as a potential topical alternative for the treatment of bacterial vaginosis.

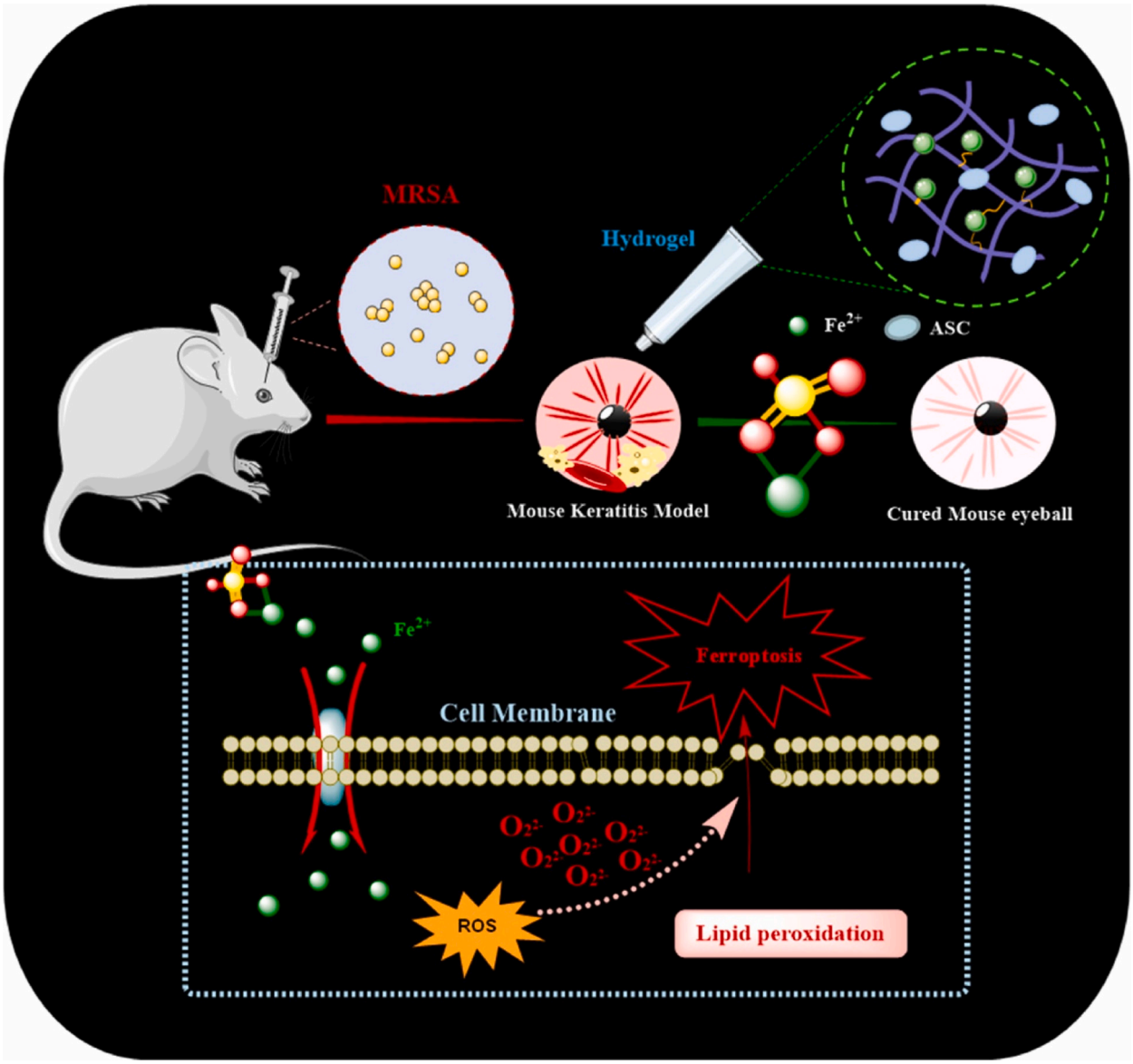

Fig. 7. Therapeutic process of ferrous sulfate-loaded hydrogels in methicillin-resistant *Staphylococcus aureus*-induced keratitis in mice. The ferrous sulfate-loaded hydrogels treated keratitis by inducing reactive oxygen species production, lipid peroxidation, and ferroptosis-like cell death.
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4. Application of traditional materials based on marine polysaccharides in controlling biomedical device-related infections

4.1. Antibiofilm efficacy on the catheters

Invasive medical devices play a vital role in modern medical practice, significantly enhancing clinical outcomes and improving patients' quality of life. However, during surgery or treatment, invasive devices can lead to biofilm-associated infections caused by bacteria, accounting for approximately 70 % of healthcare-associated infections [16]. The most commonly used invasive devices, such as urinary and vascular catheters, are the leading sources of biofilm-related infections. In fact, 98 % of urinary tract infections and 37 % of bloodstream infections in intensive care units are associated with catheter use [113]. Pathogens responsible for catheter-related infections include *S. aureus*, *P. aeruginosa*, *E. coli*, *Candida* spp., *Enterococcus* spp., and *Streptococcus* spp., which adhere to the catheter and form biofilms, leading to persistent bacterial infections and severe complications [16]. Strategies to prevent catheter-related biofilm infections have primarily focused on inhibiting biofilm formation through the use of novel coatings. Modifying, impregnating, or coating catheters with natural substances, synthetic compounds, or nanomaterials has proven effective in suppressing biofilm formation [1,114].

Chitosan is regarded as a promising material for catheter coatings due to its excellent antimicrobial properties, biodegradability, and capacity to reduce mechanical friction within the vascular lumen by enhancing surface wettability and lubricity [23]. Rubini et al. extracted chitosan from the shell of the marine crab *Portunus sanguinolentus* and applied it as a coating on the surface of urinary catheters to evaluate its antibiofilm efficacy against *S. epidermidis* and *C. albicans* [46]. The extracted chitosan effectively prevented the formation of single-species biofilms and dispersed preformed single-species and mixed-species biofilms. Transcriptome analysis showed that the extracted chitosan downregulated quorum sensing-related genes (*agrAC*) and biofilm-related genes (*bhp*) in *S. epidermidis*, as well as reduced the expression of hyphal formation-related genes, such as *ume6* and *hyr1*, in *C. albicans*, thereby supporting its antibiofilm activity. The chitosan-coated

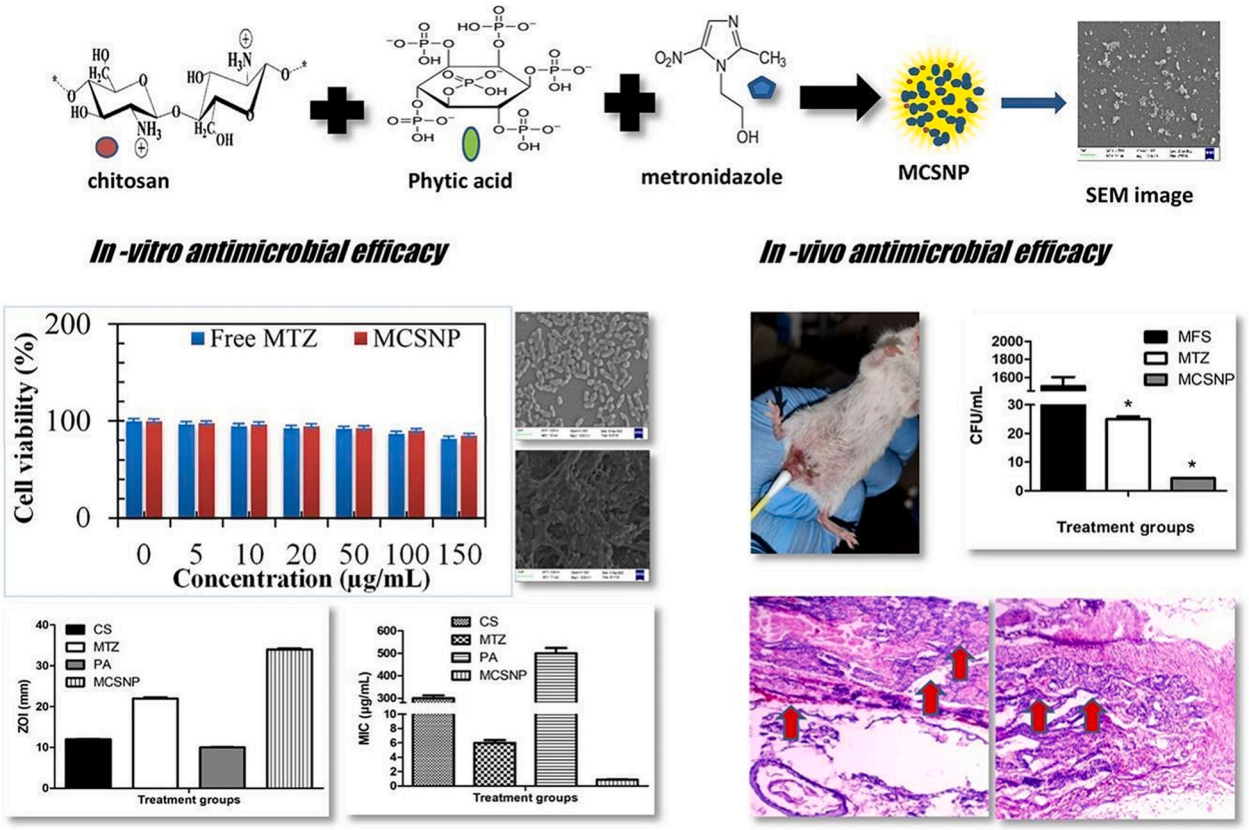

Fig. 8. Preparation and application of metronidazole-loaded chitosan nanoparticles (MCSNP). The MCSNP was synthesized by incorporating metronidazole into chitosan and using phytic acid as a cross-linking agent, specifically for the treatment of bacterial vaginosis. These nanoparticles exhibited excellent antibacterial activity against *Escherichia coli* in both *in vitro* and *in vivo* studies, underscoring their potential as a treatment for bacterial vaginosis.
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catheters additionally eradicated preformed mixed-species biofilms in a dose-dependent manner while demonstrating low cytotoxicity at effective concentrations. Fan et al. developed AgNPs@cs/SCS by first using sulfonated chitosan to adsorb Ag^+ ions, followed by the creation of Ag^+-loaded nanogels using chitosan, and subsequently generating silver nanoparticles *in situ* within the sulfonic acid and chitosan matrix (Fig. 9) [47]. The sulfonation of chitosan enhanced the electrostatic adsorption of Ag^+ ions without altering its fundamental properties, leading to a higher loading capacity of silver nanoparticles. This modification endowed the material with notable antibacterial activities and biofilm removal capabilities. AgNPs@cs/SCS released silver nanoparticles that disrupted the cell membranes of *S. aureus* and *E. coli*, causing cytoplasmic leakage and inducing oxidative stress, thereby exhibiting antibacterial effects. *In vivo* treatment using a catheter implantation model demonstrated that AgNPs@CS/SCS rapidly eradicated *S. aureus* biofilms on the catheter, significantly reduced inflammation at the implantation site, and promoted wound healing.

Lee et al. developed a hydrogel composed of sodium alginate and zwitterionic carboxymethyl chitosan for use as a catheter coating [57]. The zwitterionic-functionalized carboxymethyl chitosan was modified by introducing additional negatively charged sulfonic groups to the existing carboxyl groups, thereby increasing the overall negative charge density. This modification enhanced electrostatic repulsion and facilitated the formation of a hydration layer, resulting in excellent prevention of blood clotting factor attachment and biofilm formation. The hydrogel coating effectively inhibited the formation of biofilms by *E. coli*, *S. epidermidis*, MSSA, and MRSA on catheters. Additionally, in an MRSA-induced *in vivo* model, the coated catheter reduced bacterial load and demonstrated antithrombotic effects, suggesting that this hydrogel coating could be suitable for use in blood-contacting devices.

4.2. Antibiofilm efficacy on the implants

Implants are widely used and essential devices in both orthopedic and dental fields for diagnostic and therapeutic applications. However, microbial adhesion and biofilm formation can result in peri-implantitis and peri-mucositis, prolonging healing times and leading to additional infections [17,115]. In fact, around 6 % of orthopedic implant replacement surgeries require revision within five years, with 25 % of these revisions attributed to biofilm-related infections [17,116]. Implant infections are caused by various pathogens, which vary depending on the type of implant and its anatomical site [20]. These pathogens quickly adhere to the surface of biomaterials and survive within the host environment [20]. Moreover, biofilms formed on implant surfaces not only sustain infections but also shield pathogens from antibiotic treatment [17]. Therefore, developing surfaces that prevent the initial adhesion of pathogens is crucial for reducing implant-related biofilm infections. Considering that medical implants are made from various materials, such as titanium, polyethylene, alloys, and silicone, to achieve the necessary mechanical properties for the treatment site, it is crucial to apply coating materials to implant surfaces with these factors in mind [17,117].

Natural polysaccharides can be coated onto the surfaces of implants made from silicone, titanium alloys, and stainless steel to provide antimicrobial and antibiofilm properties [118]. Chitosan, in particular, is regarded as a promising material for creating biologically active surfaces on implants that come into direct contact with bone, as it promotes osseointegration [24]. Ajdnik et al. developed a crosslinker-free polyelectrolyte-surfactant complex (PESC) composed of chitosan, a lysin-based anionic surfactant (77KS), and amoxicillin, which was introduced onto the surface of polydimethylsiloxane (PDMS) [117]. The

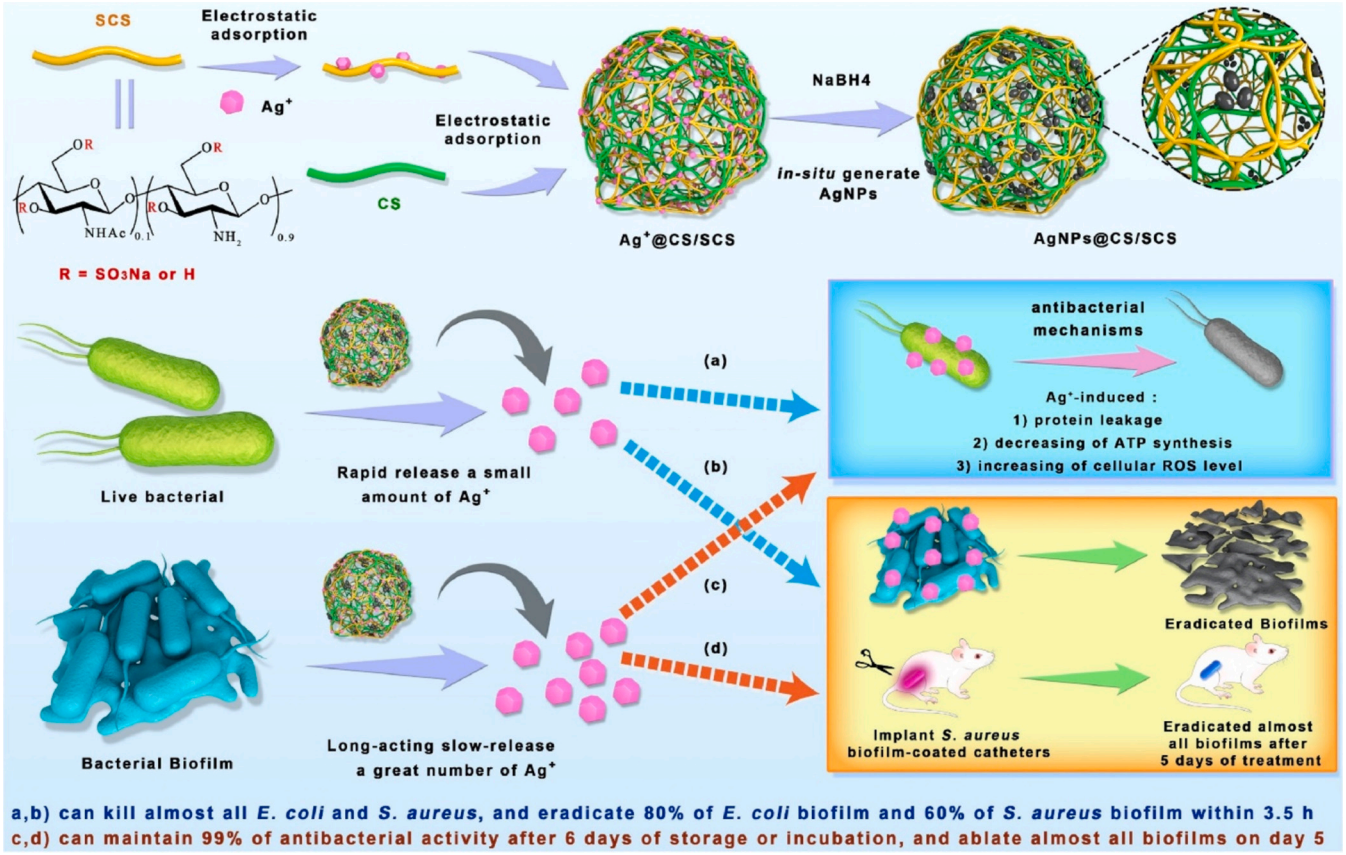

Fig. 9. Preparation of AgNPs@CS/SCS and its antibacterial and antibiofilm mechanisms. The AgNPs@CS/SCS was designed by first adsorbing Ag^+ ions onto sulfonated chitosan, followed by incorporating the Ag^+ into a nanogel using chitosan, and subsequently generating silver nanoparticles *in situ* within the nanogel matrix. AgNPs@CS/SCS exhibited strong antibacterial and antibiofilm effects against *Staphylococcus aureus* by releasing Ag^+ ions and showed remarkable efficacy in treating implant infections.
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PESC-coated PDMS significantly inhibited biofilm formation by *E. coli* and *S. aureus* compared to the pure PDMS surface, a result attributed to the interaction between the cell surface and both the cationic chitosan and the anionic, hydrophilic 77KS. Additionally, the PESC-coated PDMS surface demonstrated the ability to prevent the adsorption of blood proteins such as fibrinogen, bovine serum albumin, and γ-globulin,

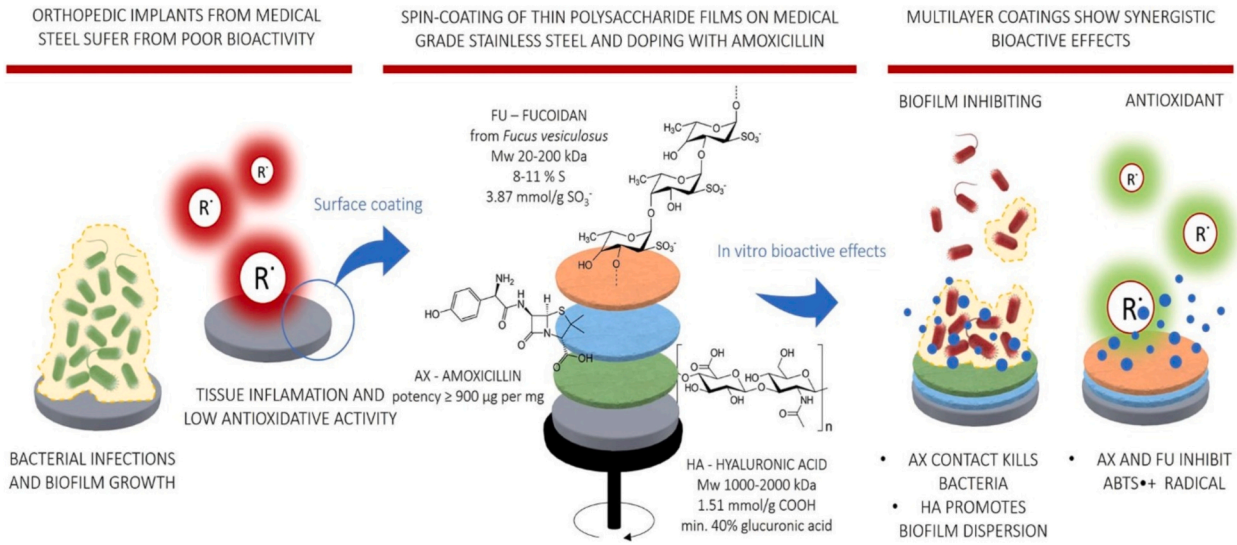

Fig. 10. Application of amoxicillin-doped hyaluronic acid/fucoidan multifunctional coatings on medical grade stainless steel. The multifunctional coating exhibited antibiofilm activity against *Staphylococcus aureus* due to the synergistic effect between hyaluronic acid and amoxicillin, suggesting its potential use as a medical-grade stainless steel implant material.
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indicating its potential to address issues related to nonspecific protein adsorption on implant surfaces after *in vivo* implantation. Bračič et al. developed a multifunctional coating doped with amoxicillin and composed of hyaluronic acid/fucoidan, intended for application on steel-based orthopedic implants (Fig. 10) [27]. The multifunctional coating surface reduced *S. aureus* biofilm formation more effectively than amoxicillin, hyaluronic acid, fucoidan alone, or pure medical stainless steel. The synergistic effect of amoxicillin, hyaluronic acid, and fucoidan contributed to the antibiofilm activity of the multifunctional coating. Furthermore, the multifunctional coating was found to be non-toxic to human osteoblasts, suggesting its potential as a promising material for medical stainless steel implants.

4.3. Other applications of marine polysaccharides in controlling biofilms

Peter et al. synthesized copper oxide nanoparticles (CuO/CMC) *via* chemical precipitation using carboxymethyl chitosan as a capping agent [49]. Carboxymethyl chitosan was employed to stabilize the CuO/CMC, which are otherwise unstable in solution due to the formation of copper oxide clusters. The CuO/CMC showed antibacterial activity against *S. aureus* and *E. coli*, attributed to copper oxide adhering to the bacterial cell walls and interacting with proteins and lipopolysaccharides, while carboxymethyl chitosan penetrated the cells and bound to DNA, disrupting its structure. Additionally, rubber films coated with CuO/CMC reduced biofilm formation by *S. aureus* and *E. coli* compared to uncoated films, which was attributed to the strong adsorption of CuO/CMC onto the rubber surface, enhancing the coating's hydrophilicity and surface uniformity. The CuO/CMC-coated films demonstrated potential as promising biomaterials for manufacturing rubber latex-based medical films by imparting antibacterial and antibiofilm activity to the surface. Zou et al. developed a multifunctional coating consisting of three natural materials: hyaluronic acid, quaternized chitosan, and acylase (Fig. 11) [26]. The multifunctional coating exhibited antibiofilm activity against *P. aeruginosa* through the following mechanisms: the outermost hyaluronic acid layer prevented initial bacterial adhesion, the quaternized chitosan in the second layer provided bactericidal properties, and the acylase in the third layer disrupted the quorum sensing detection system. This design minimized potential conflicts between different functions—such as anti-adhesion, bacterial killing, and anti-quorum sensing—compared to single or dual-function coatings, resulting in enhanced antibiofilm activity against *P. aeruginosa* for at least three days. Moreover, the multifunctional coating demonstrated broad applicability to various substrates such as glass, PDMS, stainless steel, and gold, with minimal *in vitro* cytotoxicity, showing great potential for biomedical applications.

Narciso et al. developed a 3D-printed antimicrobial coating composed of the biosurfactant sophorolipid and chitosan hydrogel [50]. The sophorolipid-impregnated chitosan hydrogel coating inhibited both planktonic and biofilm cells of *S. aureus*, a result attributed to the inherent properties of chitosan and the controlled release of sophorolipid. Additionally, this coating exhibited suitable printability on silicone substrates and showed no adverse effects on human skin fibroblasts, indicating its potential as an antimicrobial coating for silicone-based medical devices.

5. Marine polysaccharide-based smart biomaterials for antibiofilm applications

Drug-delivery systems with modulated physicochemical properties hold immense potential for treating infectious diseases. Effective therapy requires high local concentrations of antimicrobials at infection sites, and a timely release is essential, especially for combating biofilm

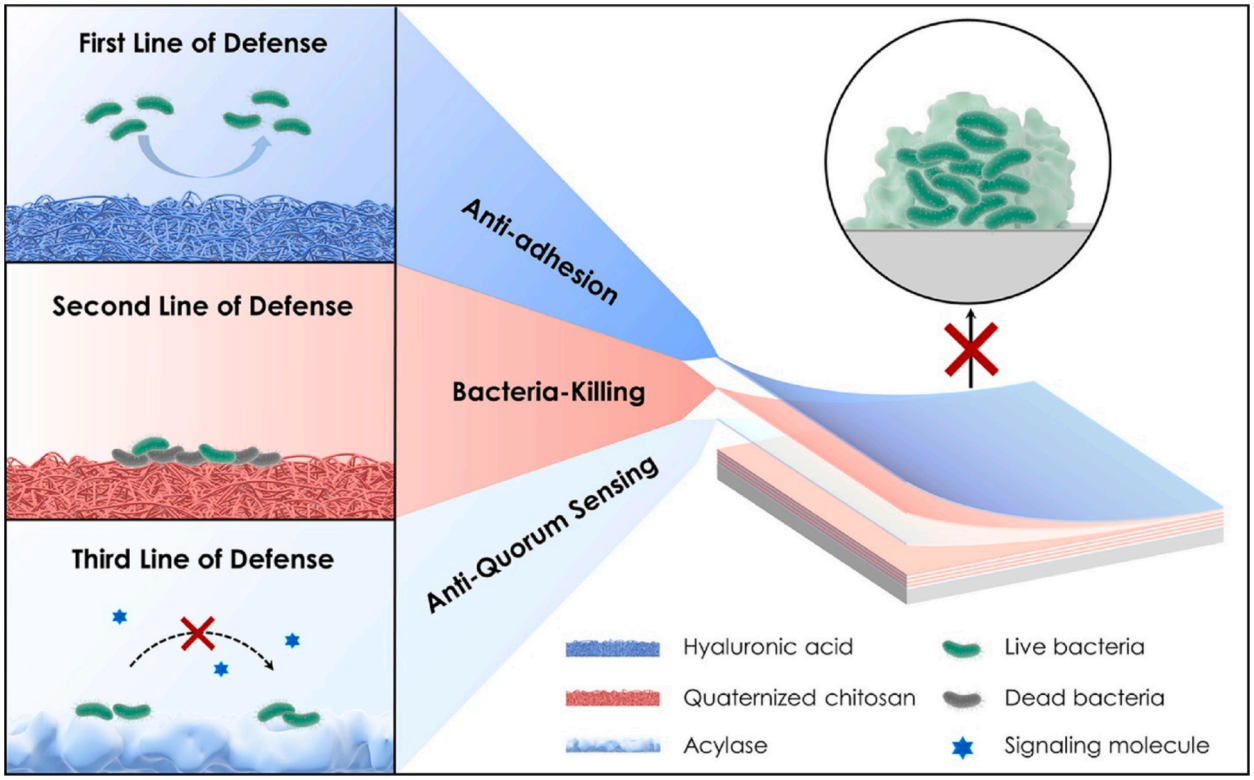

Fig. 11. Application of hyaluronic acid/quaternized chitosan/acylase multifunctional coating on biomedical applications. The outermost hyaluronic acid layer inhibited initial bacterial adhesion, the quaternized chitosan in the second layer imparted bactericidal properties, and the acylase in the third layer disrupted the quorum sensing detection system. This design showed great potential in the biomedical field by significantly enhancing antibiofilm activity against *Pseudomonas aeruginosa* compared to a single-layer coating.
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cells [119]. Smart drug-delivery systems can sustain the necessary antimicrobial concentration gradient at these sites [120]. These intelligent materials guide nanoparticles precisely to targeted areas, enhancing bioavailability [121]. Designed to respond to unique bacterial microenvironments, stimuli-responsive materials release drugs in response to changes in pH, ionic strength, and other specific conditions [122]. Additionally, external triggers like light, electric, or magnetic fields can further improve targeting capabilities [123]. Stimuli-responsive systems have been shown to increase drug penetration and efficacy against resilient biofilm structures [119].

Marine polysaccharides, such as chitosan, hyaluronic acid, and alginate, offer a unique combination of biocompatibility, biodegradability, and bioactivity, making them ideal for responsive drug delivery applications. Their inherent physicochemical properties allow for the creation of systems that respond to both internal stimuli (*e.g.*, pH, enzymes, ionic strength) and external stimuli (*e.g.*, temperature, light, magnetic fields) [124–126]. This responsiveness enables precise drug release control, which is particularly valuable for targeting challenging environments like biofilms and infection sites [119]. Marine polysaccharides can be engineered into nano-carrier systems, enhancing drug retention and facilitating targeted delivery while minimizing side effects (Table 3). Pourhajibagher et al. developed emodin-chitosan nanoparticles (Emo-CS-NPs) and utilized them to control *S. mutans* biofilms on enamel surfaces through blue laser light activation [127]. Upon blue laser irradiation, Emo-CS-NPs generated ROS, which altered cell membrane permeability, induced osmotic imbalance, and ultimately inhibited *S. mutans* biofilm formation. Notably, during photodynamic treatment, Emo-CS-NPs not only downregulated the expression of the *gtfB* gene associated with *S. mutans* biofilm formation but also reduced metabolic activity. In conclusion, Emo-CS-NPs effectively controlled *S. mutans* biofilms under photodynamic irradiation, highlighting their potential as an adjunctive therapy for dental caries. Xin et al. prepared a novel nano-sonosensitizer for robust ROS generation by first growing TiO_2 on dendritic large-pore mesoporous silica nanoparticles, followed by Ag deposition, and final modification with quaternary ammonium chitosan (DT-Ag-CS^+) [128]. With Ag deposition and the high positive charge of chitosan, DT-Ag-CS^+ showed enhanced bacterial cell penetration, demonstrating excellent *in vitro* and *in vivo* therapeutic efficacy. Furthermore, under ultrasound irradiation, DT-Ag-CS^+ induced ROS generation within *P. gingivalis* cells, a periodontal pathogen, exhibiting potent antibiofilm effects. These findings suggest that DT-Ag-CS^+ is a promising nanomaterial for non-invasive ultrasound sonodynamic therapy. Wang et al. designed a chitosan-based nanogel (TA@CS) by encapsulating Tanshinone IIA within chitosan (Fig. 12) [129]. TA@CS demonstrated high efficacy in acidic environments conducive to biofilm development, owing to its ability to selectively release Tanshinone IIA at low pH. Furthermore, TA@CS exhibited strong antibiofilm activity by efficiently penetrating the negatively charged *S. mutans* biofilm surface, effectively targeting it with its positive charge.

Zou et al. developed a near-infrared light-triggered nanoplatform (CAI NPs) by integrating the natural nitric oxide donor L-arginine and the phototherapy agent indocyanine green into chitosan for the purpose of biofilm eradication [7]. Upon NIR light activation, CAI NPs dispersed MRSA biofilms and generated nitric oxide and peroxynitrite, effectively killing MRSA and *P. aeruginosa* cells. In an *in vivo* wound infection model, CAI NPs successfully treated MRSA biofilms and promoted the healing of infected wounds under NIR irradiation without any observed side effects.

Wang et al. constructed a high-efficiency nanoplatform (MNPs@Ag@HA) for biofilm disruption by fabricating a multilayer film composed of tannic acid, gentamicin, and silver nanoparticles, which was coated onto magnetic nanoparticles through electrostatic interactions, with hyaluronic acid applied as a reactive outer shell [130]. MNPs@Ag@HA demonstrated on-demand release of gentamicin and Ag^+ ions in response to the acidic pH and elevated levels of hyaluronidase present at bacterial infection sites, effectively inhibiting *S. aureus* cells. Additionally, the superparamagnetic properties of the magnetic nanoparticles facilitated MNPs@Ag@HA penetration into the deep layers of *S. aureus* biofilms. Importantly, ROS generated by Ag^+ ions contributed to strong antibiofilm efficacy by breaking down extracellular polymeric substances. Liu et al. fabricated an enzyme-responsive nanoplatform with photothermal properties (AA@Ru@HA-MoS_2) by utilizing mesoporous ruthenium nanoparticles as nanocarriers loaded with ascorbic acid, encapsulated with hyaluronic acid, and combined with ciprofloxacin-coated molybdenum disulfide [131]. At infection sites, bacterial Hyal secretion triggered the degradation of the hyaluronic acid capping,

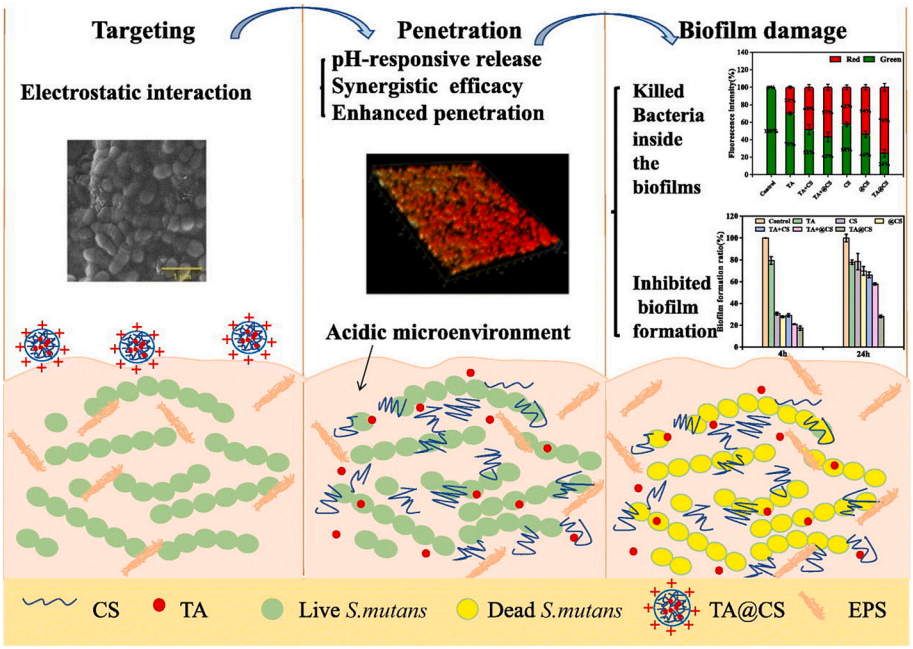

Fig. 12. Antibiofilm effects of chitosan-based nano-gel encapsulating Tanshinone IIA (TA@CS). TA@CS selectively released Tanshinone IIA in the presence of acidic pH found in biofilm environments, effectively inhibiting both cell activity and biofilm formation of *Streptococcus mutans*.
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leading to the release of ascorbic acid and the generation of hydroxyl radicals catalyzed by molybdenum disulfide. Furthermore, the photothermal properties of mesoporous ruthenium nanoparticles effectively inhibited *S. aureus* and *P. aeruginosa* and prevented biofilm formation. In an *in vivo* bacterial infection model, AA@Ru@HA-MoS_2 exhibited strong antibacterial effects and accelerated wound healing, highlighting its promise as an enzyme-responsive composite system for managing bacterial infections.

Singh et al. developed dual-action release nanoparticles by incorporating a pH-responsive linker between an alginate backbone and the quorum-sensing inhibitor 3-amino-7-chloro-2-nonylquinazolin-4(*3H*)-one, along with encapsulating positively charged ciprofloxacin through interactions with the carboxyl residues of alginate [132]. These dual-action release nanoparticles responded to the acidic environment of biofilms, demonstrating high antibiofilm efficacy against *P. aeruginosa*. In 2D keratinocyte and 3D *in vitro* skin infection models, the nanoparticles effectively prevented biofilm formation and eradicated mature *P. aeruginosa* biofilms, highlighting their potential as promising materials for nanoparticle-based combination therapy. Niaz et al. synthesized a nanocarrier system (PPC-NCS) by utilizing pH-responsive proteins, sodium caseinate, and sodium alginate to encapsulate the antimicrobial peptide nisin (Fig. 13) [133]. PPC-NCS effectively penetrated biofilms of oral pathogens *E. faecium*, *E. faecalis*, and *S. epidermidis*, responding to the oral cavity's pH and releasing nisin through Fickian diffusion, thereby exhibiting high antibiofilm efficacy. Furthermore, the mucoadhesive properties of alginate enabled PPC-NCS to adhere to oral surfaces such as teeth, tongue, and buccal mucosa, presenting a promising preventive and therapeutic approach for managing biofilm-associated oral infections.

6. Potential obstacles in scalability of marine polysaccharide production, clinical translation, or/and regulatory hurdles

Marine polysaccharides, such as chitosan, hyaluronic acid, alginate, carrageenan, and fucoidan, have garnered significant interest across various industries due to their biocompatibility, biodegradability, and potential applications in food, biotechnology, cosmetics, pharmaceuticals, and biomedicine [134,135]. However, despite the numerous potential applications, several challenges must be addressed before marine-derived polysaccharides can be effectively scaled up for healthcare and industrial production.

6.1. Scalability of marine polysaccharide production

Chitosan, a versatile polysaccharide derived from chitin, has gained prominence in biomedical and pharmaceutical applications due to its biocompatibility and biodegradability [136]. However, its limited solubility in neutral and alkaline environments restricts its applications [137]. Modifying its chemical structure holds promise for enhancing its functional properties and expanding its utility [136]. Chitosan oligosaccharide, a breakdown product of chitosan, offers several advantages, including lower molecular weight, higher degree of deacetylation, and improved water solubility, resulting in enhanced biological activity and cellular absorption [138]. Production of chitosan faces challenges similar to those in fine chemicals and pharmaceuticals, where scale-dependent physical parameters and phenomena can affect performance during scale-up [139]. To address these issues, researchers are exploring a range of chemical modifications and developing novel applications, including drug delivery, environmentally friendly nanoparticle production, and biocatalyst support [137].

Hyaluronic acid, a naturally occurring polysaccharide, is gaining popularity in bioengineering and biomedicine due to its biocompatibility and biodegradability [140]. Its applications in drug delivery, immunomodulation, and tissue engineering have been extensively studied [141,142]. The molecular weight of hyaluronic acid influences its structural, physical, and physicochemical properties, directly affecting its therapeutic benefits [140]. Recent advancements have enabled the development of hyaluronic acid-based nanocarriers for targeted cancer therapies, combination treatments, and theranostics [142].

Alginate, a polysaccharide derived from algae, has a wide range of applications in the food, pharmaceutical, and biomedical sectors due to

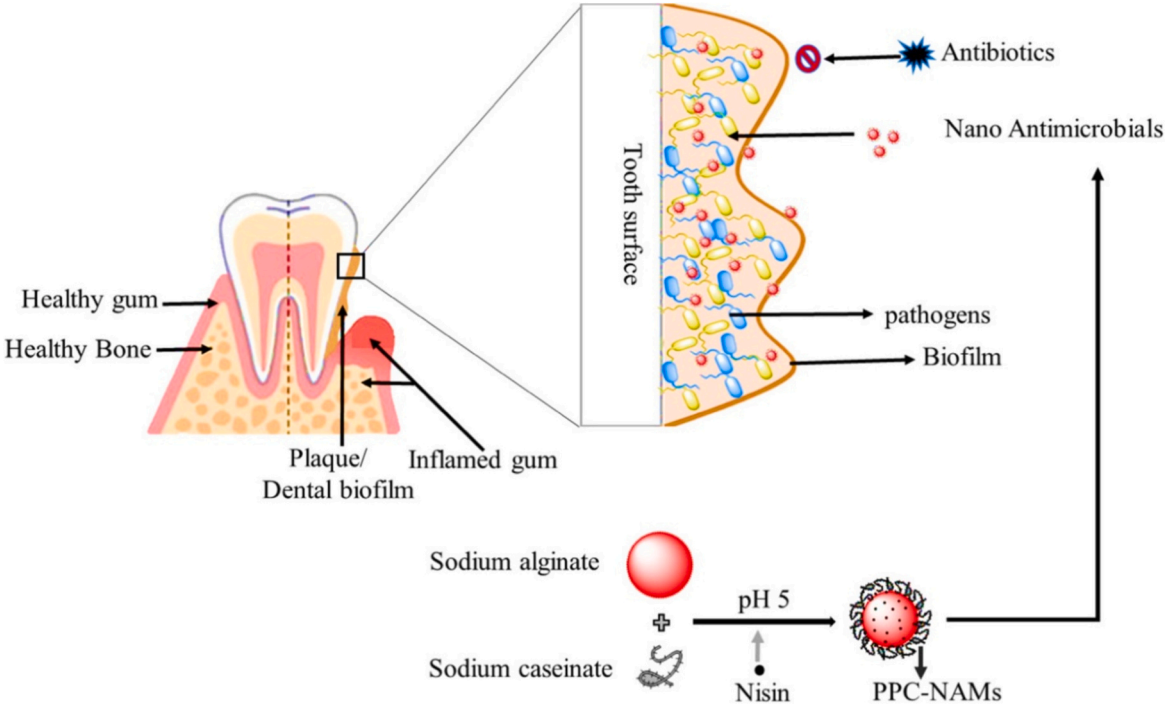

Fig. 13. Preparation, antimicrobial, and antibiofilm mechanism of nisin-loaded alginate-sodium caseinate-based nano-carrier system (PPC-NCS). PPC-NCS responded to the oral pH to release nisin, demonstrating high antibiofilm efficiency against oral biofilm pathogens.
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its unique properties [143,144]. However, scaling up its production poses challenges. The performance of algae-based alginates in advanced applications can be limited by inconsistencies in molecular weight and composition [145]. Additionally, its limited water solubility and high viscosity at large concentrations restrict its potential, especially in the food industry [146]. To address these issues, researchers are exploring microbial synthesis of alginate, which allows for the customization of alginate molecules with consistent properties [145]. Another approach is the production of alginate oligosaccharides, which offer greater water solubility and unique bioactivities [146]. Despite these advancements, high manufacturing costs continue to hinder the commercialization of microbial alginates, underscoring the need for research into more cost-effective production methods [145].

Carrageenan, a sulfated polysaccharide extracted from red seaweeds, has gained attention for its versatile applications in drug delivery, food, pharmaceuticals, and cosmetics [135,147]. Its unique structural and physicochemical properties, such as thixotropic and shear-thinning behaviors, make it a highly adaptable functional material [147]. Carrageenan shows promise in various biomedical fields, including targeted drug delivery systems, tissue engineering, and 3D bioprinting. However, its large molecular size, low solubility, and hydrocolloid nature pose challenges for efficient utilization [148]. To address these limitations, researchers are exploring carbohydrate-active enzymes and investigating metabolic pathways of algal polysaccharides [148].

Similarly, fucoidan, a sulfated polysaccharide derived from brown seaweeds, has garnered interest for its potential bioactive properties [149]. However, several challenges limit its scalability and acceptance. Obtaining FDA approval requires overcoming substantial obstacles, including fucoidan structure heterogeneity, co-extracted contaminants, and environmental concerns [150]. The bioactivity of fucoidan is influenced by its chemical composition, such as molecular weight, monosaccharide profile, and sulfate content—parameters that vary with seaweed type and extraction methods [151]. Effective extraction and purification processes are crucial for preserving fucoidan's structural integrity, essential for its biological functions [152]. Researchers are exploring innovative approaches like enhanced extraction and purification techniques, tissue culture methods, and enzymatic synthesis to address these issues [150]. Additionally, novel extraction techniques—such as ultrasonic, microwave, and enzyme-assisted extractions—are being studied to improve fucoidan production [150].

For polysaccharides like chitin/chitosan and hyaluronic acid, eco-friendly processes integrating microbial, chemical, enzymatic, and membrane-based strategies have been proposed to enhance production efficiency [153].

6.2. Obstacles in clinical translation

Although marine polysaccharides like chitosan, hyaluronic acid, alginate, carrageenan, and fucoidan have shown significant therapeutic potential in preclinical studies [154], translating these compounds from the laboratory to clinical applications presents several challenges.

6.2.1. Bioavailability and delivery

Marine polysaccharides, such as chitosan and alginate, have shown promise as drug delivery systems due to their biocompatibility and biodegradability [155]. However, their large molecular size and hydrophilic nature present challenges for oral administration, limiting their bioavailability and effectiveness [156]. To address these barriers, researchers have developed various strategies, including chemical conjugation, absorption enhancers, and nanoparticle-based systems [156–160]. Approaches such as nanoparticles, liposomes, and microspheres are also being explored to enhance the bioavailability and efficacy of marine polysaccharides [161].

Polysaccharide-based nanocarriers have garnered significant interest for oral protein and peptide delivery, as they can protect these macromolecules from enzymatic degradation in the gastrointestinal tract while enhancing transepithelial transport [157,162]. These delivery systems improve oral absorption, control release, and target specific tissues [161]. Recent advancements in polysaccharide-based nanocarriers have focused on enhancing their muco-adhesiveness, pH responsiveness, and resistance to enzymatic degradation, offering promising strategies to increase the bioavailability of orally administered macromolecules [162]. However, challenges remain in designing and manufacturing these carriers while preserving the bioactive properties of the polysaccharides [163]. Careful management of the interaction between the polysaccharide and the delivery vehicle is essential to prevent degradation or loss of functionality [163].

6.2.2. Toxicity and safety concerns

These biopolymers are generally considered non-toxic and safe for use in various biomedical applications [134]. However, recent studies have raised some toxicity concerns. For instance, chitosan nanoparticles have been shown to cause decreased hatching rates, increased mortality, and malformations in zebrafish embryos at higher concentrations [164]. Conversely, studies in rats and mice indicate low toxicity; oral administration of chitosan derived from lobster shells did not result in lethality or significant behavioral changes in rats [165]. Similarly, acute and subacute toxicity studies in mice showed no significant toxicological effects at doses up to 5000 mg/kg body weight for low molecular weight chitosan and self-assembled chitosan microparticles [166]. These varying results suggest that the toxicity of chitosan-based materials may depend on factors such as particle size, molecular weight, and the specific organism tested, highlighting the need for further investigation to ensure safe application.

Hyaluronic acid injections are generally considered safe for treating knee osteoarthritis. Multiple studies have shown that hyaluronic acid products have a safety profile similar to intra-articular placebo injections, with no significant differences in overall or serious adverse events [167,168]. The most common adverse events are transient local reactions, such as pain, swelling, and arthralgia, which typically resolve quickly [167,169]. Repeated courses of hyaluronic acid injections continue to provide or improve pain relief without increasing safety risks [169]. The metabolism of 1,4-butanediol diglycidyl ether-crosslinked hyaluronic acid fillers results in harmless byproducts or substances naturally present in the skin [170]. While local adverse events are slightly more frequent with hyaluronic acid than with saline injections, they are generally mild and resolve within a few days [168]. Overall, hyaluronic acid injections have a favorable long-term safety profile for knee osteoarthritis treatment.

Sodium and potassium alginates are considered safe for use in animal feed, with a maximum dose of 40000 mg/kg deemed safe across various animal species [171]. In a human study evaluating alginate as a dietary iron chelator, a daily intake of 3 g of alginate was well-tolerated, causing minimal side effects and no adverse changes to hematological parameters or the intestinal microbiome [172].

Research indicates that ingested carrageenan is minimally absorbed, metabolized, or degraded in the gastrointestinal tract [173]. Chronic animal studies have shown no significant toxicological effects at dietary doses up to 5 %, apart from mild softening of stools [173]. *In vitro* studies on oral cavity cells and tissues suggest that carrageenan is safe for use in mouthwashes [174]. Additionally, intranasal application of iota-carrageenan has been found safe, with no evidence of systemic absorption or local toxicity [175]. The European Food Safety Authority has set a temporary acceptable daily intake of 75 mg/kg body weight per day for carrageenan and processed *Eucheuma* seaweed but noted some uncertainties in the current data, recommending further research [176].

Multiple studies have demonstrated the safety of fucoidans in rodent models. In rats, oral administration of fucoidan at doses up to 1350 mg/kg body weight per day for 4 weeks did not result in significant toxicological effects [177]. Similarly, fucoidan consumption at doses up to 1000 mg/kg body weight per day for 28 days was found to be safe in rodents [178]. Low molecular weight fucoidan showed no mutagenic

effects at 5000 μg/mL and exhibited no toxicological signs at 2000 mg/kg body weight per day [179].

6.3. Obstacles in regulatory hurdles

The regulatory landscape for marine polysaccharides presents a significant barrier to their widespread adoption in both clinical and commercial applications. Since these compounds are derived from marine organisms, they are subject to regulations governing natural products and biotechnological processes.

Notably, chitosan and alginate have shown potential in wastewater treatment, effectively removing various pollutants when used as hydrogels or films [180]. Their efficacy is attributed to unique physicochemical properties and reactive functional groups [181]. These natural polysaccharides can be modified to enhance performance and tailored to specific applications [182]. Their use aligns with sustainability objectives, as they are renewable, eco-friendly, and may help reduce the environmental impact of human activities [180,182].

However, rising challenges in extraction, purification, and intellectual property protection hinder the commercialization of marine polysaccharides. Innovative extraction methods, such as ultrasonic, microwave, and enzyme-assisted techniques, are being explored to increase yields while preserving polysaccharide integrity [151]. Biotechnology also enables the controlled production of specific polysaccharides in bioreactors [183]. Chemical and physical modifications can help achieve desired properties, while enzymatic modifications hold promise for creating more defined molecules suited for medicinal applications [184]. Despite their potential, few marine polysaccharides have reached commercialization, highlighting the need for further research and development to overcome regulatory and manufacturing challenges [184,185].

7. Conclusion and future perspectives

Biofilms, generated by microbial pathogens on host tissues and medical device surfaces, pose significant challenges in healthcare and medical device management. Marine polysaccharides have emerged as promising agents for biofilm control across various medical applications, including wound healing, ophthalmic, oral, and vaginal infections, as well as medical device coatings. Chitosan, hyaluronic acid, alginate, carrageenan, and fucoidan are among the marine polysaccharides known to have antibiofilm capabilities. Their unique structural and biochemical properties offer distinct advantages in addressing biofilm-related diseases, which often resist traditional antibiotic therapies.

In wound healing, marine polysaccharides enhance tissue regeneration due to their ability to create an environment conducive to cell growth. Their antibacterial properties are crucial in preventing and treating infections in chronic wounds, while their capacity to form hydrogels aids in moisture retention and establishes a protective barrier against pathogens, thereby accelerating the healing process. Ongoing research into optimizing their application techniques and formulations continues to highlight their potential for integration into advanced wound dressings and treatment systems. Marine polysaccharides also offer innovative solutions for managing biofilm development on contact lenses and other ocular devices. Their adhesive properties enable the development of microbial-resistant coatings, while their inherent antibacterial abilities prevent biofilm formation—an essential factor in reducing the risk of contact lens-associated infections that can lead to chronic conditions. In oral health, marine polysaccharide-based treatments can disrupt tooth biofilms linked to periodontal disease and tooth caries by reducing bacterial adhesion and providing antibacterial effects. These therapies hold the potential to revolutionize oral hygiene products and treatments. Similarly, marine polysaccharides show promise in addressing biofilm-related diseases such as bacterial vaginosis. Their ability to disrupt biofilm structures and restore microbial balance offers a novel therapeutic option for managing recurrent infections.

Marine polysaccharides are being investigated for use in medical device applications as coatings on implants and catheters to reduce biofilm formation, a common source of device-related infections. Marine polysaccharide-based coatings, such as those incorporating copper oxide nanoparticles stabilized with carboxymethyl chitosan, multifunctional coatings with hyaluronic acid, quaternized chitosan, and acylase, as well as 3D-printed antimicrobial coatings with sophorolipid-impregnated chitosan hydrogel, have demonstrated effectiveness in reducing bacterial adherence and prolonging the functional lifespan of medical devices. Although these marine polysaccharides have sparked great interest in a variety of sectors owing to their biocompatibility and biodegradability, there are many challenges, including limitations in the scalability of clinical translation production and regulatory barriers. The present review has also addressed potential challenges in the application of these marine polysaccharides in many fields, such as food, biotechnology, cosmetics, medicines, and biomedicine.

Future research will focus on elucidating the mechanisms by which marine polysaccharides and their formulations control microbial biofilms in host systems and on medical devices. Gaining deeper mechanistic insights will be essential for developing novel therapeutic strategies and improving outcomes in managing biofilm-associated infections.

1. Scalable and cost-effective production, along with efficient extraction, purification, and characterization techniques for marine polysaccharides and their derivatives, are crucial for successful commercialization.
2. Future research should focus on developing multifunctional biomaterials that combine marine polysaccharides with therapeutic agents, such as antibiotics, anti-inflammatory drugs, or growth factors. This approach could simultaneously improve infection control and promote wound healing.
3. Advancing marine polysaccharide delivery technologies, such as controlled-release formulations or targeted delivery mechanisms, could enhance the effectiveness of biofilm treatments. Research into nanoparticle carriers, microneedle patches, and injectable hydrogels with improved drug-delivery capabilities may offer better therapeutic options for chronic infections and biofilm-related diseases.
4. Developing combination therapies that integrate polysaccharides with novel or repurposed antibiotics/antimicrobial agents or utilize polysaccharide-derived compounds with unique mechanisms of action, could be key in combating the growing resistance of biofilm-forming microbial pathogens.
5. Investigating the synergistic effects of marine polysaccharides combined with other biomaterials, such as hydrogels or nanoparticles, could enhance the efficacy and durability of wound care products. These synergistic interactions may also offer effective, broad-spectrum solutions for preventing biofilm formation on a wide range of biomedical devices.
6. Comprehensive preclinical and clinical studies are essential to ensure the safe and effective use of marine-derived polysaccharide materials. These studies should address potential adverse effects, interactions with other treatments, and long-term outcomes.
7. Integrating marine polysaccharide-based drugs with advanced technologies such as bioinformatics, artificial intelligence, and smart materials could drive innovation and expand therapeutic possibilities.
8. Additional *in vivo* research is necessary to assess the effects of marine polysaccharide coatings on tissue integration, immune response, and patient safety.
9. Advanced manufacturing techniques, such as 3D printing and nanotechnology, enable the creation of multifunctional coatings

with precise control over marine polysaccharide release and microbial interactions, allowing for customized biomedical coatings.

10. Future research could explore the integration of smart technology with antimicrobial coatings. Incorporating sensors that detect biofilm formation or infections could enable real-time adjustments to the coating's antimicrobial activity, enhancing its effectiveness.
11. Long-term clinical studies and real-world testing are essential to validate the efficacy and safety of new coatings. These investigations will assess the performance of these coatings across diverse patient demographics and therapeutic settings, demonstrating their practicality and reliability.

Abbreviation

GlcN	glucosamine
GlcNAc	*N*-acetylglucosamine
MRSA	methicillin-resistant *Staphylococcus aureus*
MSSA	methicillin-sensitive *S. aureus*
PDMS	polydimethylsiloxane
ROS	reactive oxygen species

CRediT authorship contribution statement

Geum-Jae Jeong: Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Conceptualization. **Fazlurrahman Khan:** Writing – review & editing, Writing – original draft, Validation, Supervision, Investigation, Funding acquisition. **Do-Kyun Kim:** Writing – review & editing, Investigation. **Kyung-Jin Cho:** Writing – review & editing, Investigation. **Nazia Tabassum:** Writing – review & editing, Investigation. **Arunabh Choudhury:** Writing – review & editing, Investigation. **Md. Imtaiyaz Hassan:** Writing – review & editing, Investigation. **Won-Kyo Jung:** Writing – review & editing, Investigation, Funding acquisition. **Hyun-Woo Kim:** Writing – review & editing, Investigation. **Young-Mog Kim:** Writing – review & editing, Writing – original draft, Validation, Supervision, Investigation, Funding acquisition.

Consent for publication

Not applicable.

Ethics approval and consent to participate

This review paper does not contain any studies with human participants or animals.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

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Data availability

No data was used for the research described in the article. All data have been included in this manuscript.

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ELUCIDATION OF ANTIBIOFILM FORMATION MECHANISMS USING MARINE EXTRACTS AGAINST INFECTION AND ANTIMICROBIAL RESISTANCE (AMR)

Recent advances in marine-derived compounds as potent antibacterial and antifungal agents: A comprehensive review / Bharathi, D., & Lee, J.

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Review

Recent Advances in Marine-Derived Compounds as Potent Antibacterial and Antifungal Agents: A Comprehensive Review

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Abstract: The increase in antimicrobial resistance (AMR) in microorganisms is a significant global health concern. Various factors contribute to AMR, including alterations in cell membrane permeability, increased efflux pump activity, enzymatic modification or inactivation of antibiotics, target site changes, alternative metabolic pathways, and biofilm formation. Marine environments, with their extensive biodiversity, provide a valuable source of natural products with a wide range of biological activities. Marine-derived antimicrobial compounds show significant potential against drug-resistant bacteria and fungi. This review discusses the current knowledge on marine natural products such as microorganisms, sponges, tunicates and mollusks with antibacterial and antifungal properties effective against drug-resistant microorganisms and their ecological roles. These natural products are classified based on their chemical structures, such as alkaloids, amino acids, peptides, polyketides, naphthoquinones, terpenoids, and polysaccharides. Although still in preclinical studies, these agents demonstrate promising in vivo efficacy, suggesting that marine sources could be pivotal in developing new drugs to combat AMR, thereby fulfilling an essential medical need. This review highlights the ongoing importance of marine biodiversity exploration for discovering potential antimicrobial agents.

Keywords: antibacterial activity; antifungal activity; bioactive compounds; marine-derived drugs; therapeutic applications

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1. Introduction

Antimicrobial resistance (AMR) is an escalating global health issue, significantly undermining the efficacy of current antibiotics and increasing the risk of untreatable infections [1–3]. This problem stems from the widespread overuse and misuse of antibiotics in both medical and agricultural settings, leading to the emergence of multidrug-resistant (MDR) pathogens [4,5]. The World Health Organization (WHO) projects that, without intervention, AMR could cause up to 10 million deaths annually by 2050 [6]. Bacteria can develop resistance through several mechanisms, including changes in cell membrane permeability, activation of efflux pumps, enzymatic degradation of antibiotics, modifications to target sites, alternative metabolic pathways, and biofilm formation [7–9]. The antibiotic resistance mechanism of currently available antibiotics against microbial pathogens is shown in Figure 1a. These strategies collectively reduce the effectiveness of traditional antimicrobial treatments.

The vast and largely untapped biodiversity of marine environments offers a promising avenue for discovering new antimicrobial agents [10]. Marine organisms, especially microorganisms, have evolved unique biochemical pathways to survive the extreme conditions of their habitats. These environments include high pressure, low light, varying temperatures, and high salinity, which have driven marine life to develop novel adaptations [11,12]. As a result, these organisms produce a wide array of bioactive compounds not typically found in terrestrial environments. Marine microorganisms, in particular, have become a focal point of research due to their ability to synthesize secondary metabolites

with remarkable biological activities [13,14]. These secondary metabolites are not directly involved in the normal growth, development, or reproduction of the organism. Instead, they often assist ecological functions, such as defense mechanisms against predators, competition with other microorganisms, or communication within their communities [15]. The unique properties of these compounds have garnered significant interest for their potential therapeutic applications. Among the various bioactive compounds produced by marine microorganisms, several classes stand out for their antimicrobial properties [16]. For example, peptides are short chains of amino acids that can disrupt microbial cell membranes, leading to cell death [17,18]. Polyketides are another class of compounds known for their structural diversity and potent biological activities [19]. Alkaloids, which contain nitrogen atoms, have been found to possess a range of pharmacological effects, including antimicrobial activity [20]. Terpenoids, derived from isoprene units, and polysaccharides are long carbohydrate molecules, which also contribute to the arsenal of bioactive compounds with potential antimicrobial applications (Figure 1b) [21,22].

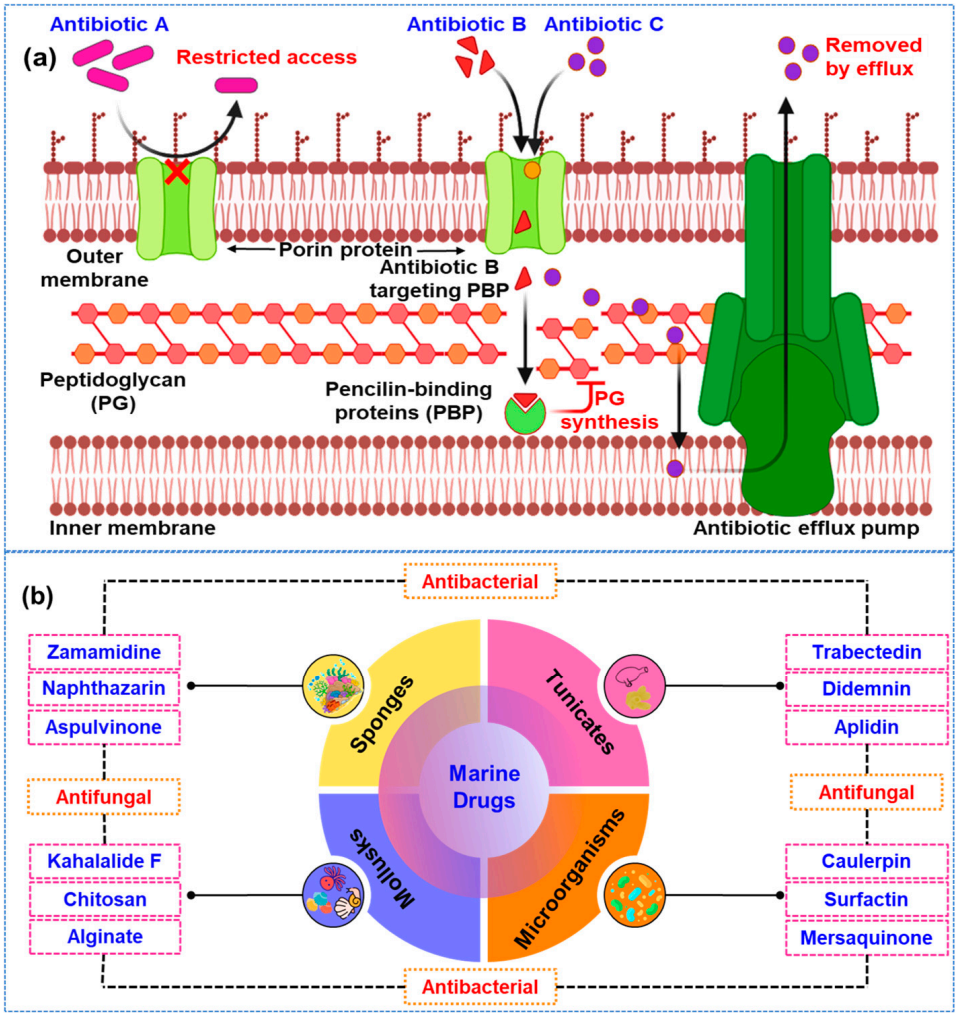

Figure 1. (**a**) The antibiotic resistance mechanisms of currently available antibiotics against pathogenic microorganisms. The image, created with BioRender.com (accessed on 25 May 2024), illustrates various mechanisms by which pathogens develop resistance, including alteration of antibiotic targets, enzymatic degradation of antibiotics, and efflux pump activation. Each mechanism is depicted with representative examples of affected antibiotics and the corresponding microbial adaptations, (**b**) Different sources of marine drugs with their potential compounds for antibacterial and antifungal properties. These marine-derived compounds play a crucial role in the development of new therapeutic agents to combat microbial infections.

Recent research has increasingly turned to marine microbial compounds for potential antibiotics. This shift is driven by the unique, high-stress environments of marine habitats, which promote the production of metabolites distinct from those in terrestrial organisms, and advancements in bioprospecting technologies that enable the collection of samples from previously inaccessible areas like deep-sea vents and polar regions [23–25]. For example, secondary metabolites from marine-derived *Streptomyces* sp. have shown significant activity against drug-resistant strains of *Enterococcus faecium*, *Staphylococcus aureus*, and *Mycobacterium tuberculosis* [26,27]. In the fight against AMR, exploring marine natural products is particularly critical due to the urgent need for new antimicrobial agents with unique mechanisms of action [28]. Compounds such as nocardiopsistins, stremycins, and chlororesistoflavins from marine sources have demonstrated effectiveness against methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant Enterococci (VRE), and multidrug-resistant *M. tuberculosis* (MDR-TB) [26,27,29]. These marine-derived compounds not only offer new mechanisms of action but also possess structural features that are absent in conventional antibiotics, making them less likely to encounter cross-resistance [30,31]. The exploration and characterization of such compounds are essential for expanding our target of antimicrobial agents and providing unique solutions to combat AMR.

Marine compounds contain unusual ring systems, halogenated compounds, highly branched molecules, and sulfated polysaccharides, which offer different modes of action compared to traditional antibiotics and also it can reduce the likelihood of cross-resistance [30]. Halogen atoms are able to establish non-covalent interactions with biomolecules, ensuring binding, specificity and enhanced drug efficacy against AMR pathogens [1]. Some marine-derived compounds can also enhance the efficacy of existing antibiotics by inhibiting bacterial efflux pumps, a common resistance mechanism. This synergistic effect not only restores the potency of conventional antibiotics but also broadens their spectrum of activity. For instance, an antibiotic that was previously ineffective against a particular strain of bacteria due to resistance mechanisms might regain its effectiveness when used in combination with a marine-derived efflux pump inhibitor [32,33].

While previous reviews address the isolation of marine-derived compounds for antibacterial and antifungal activities [12–16,21,24–26], this review stands out by focusing on recent research articles highlighting the extraction or isolation of marine-derived compounds with potential antimicrobial activity against multidrug-resistant (MDR) pathogens. Unlike previous reviews that focus on a single type of compound from specific marine sources, this review provides a detailed summary of compounds derived from various marine sources. It spans recent advancements and research articles from approximately 2010 to 2024, highlighting significant progress in marine-derived compounds as potent antibacterial and antifungal agents. This paper emphasizes the vast potential of marine biodiversity, showcasing marine-derived compounds with unique structures and mechanisms of action against resistant pathogens. It categorizes these compounds based on their chemical structures, including alkaloids, amino acids, peptides, polyketides, naphthoquinones, terpenoids, and polysaccharides, each with distinct mechanisms of action. Additionally, this review details how the unique chemical structures of marine-derived compounds result in different modes of action compared to traditional antibiotics. Furthermore, this review explores the connection between the antimicrobial properties of these compounds and their ecological roles, providing insights into their evolutionary significance and potential applications. Also, this review highlights significant progress and discoveries in marine-derived compounds as potent antibacterial and antifungal agents.

2. Marine-Derived Alkaloids

Marine organisms, such as sponges, tunicates, mollusks, algae, and microorganisms like bacteria and fungi, generate a diverse range of alkaloids [34–38]. These compounds have evolved as part of the organisms' defense mechanisms, resulting in the unique structures and potent biological activities. Sponges are a prolific source of bioactive compounds, most notably alkaloids, which have garnered significant attention for their potent antibac-

terial and antifungal activities [39,40]. These sessile invertebrates inhabit diverse marine environments, from shallow reefs to the deep sea, where they have evolved complex chemical defense mechanisms to survive against a myriad of microbial threats. The unique structural diversity of sponge-derived alkaloids underpins their broad-spectrum bioactivity, making them promising candidates for developing new antimicrobial agents [41]. The chemical structure of various marine-derived alkaloids is shown in Figure 2.

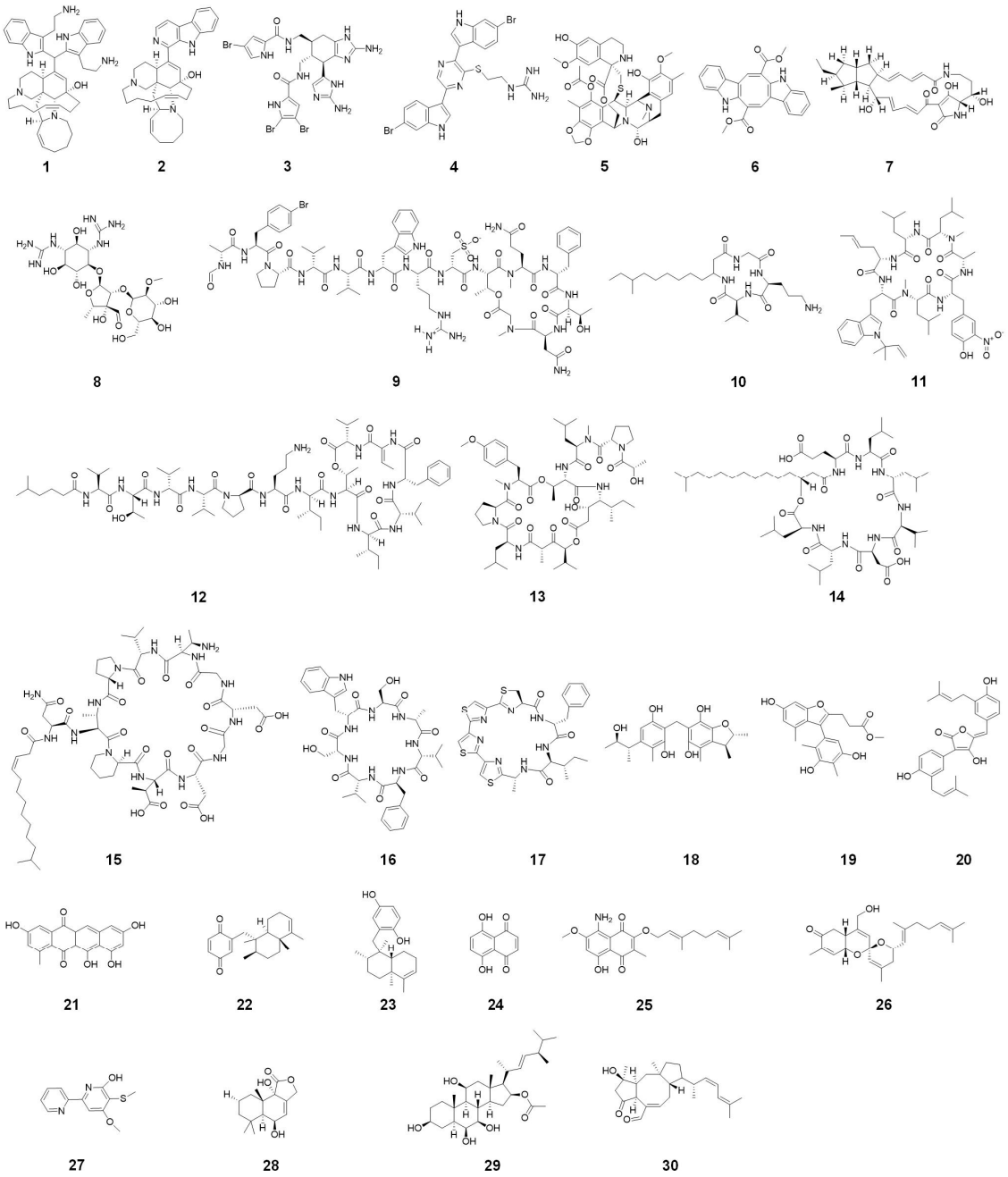

Figure 2. Chemical structures of zamamidine D (**1**), manzamine A (**2**), bromoageliferin (**3**), dragmacidin G (**4**), trabectedin (**5**), caulerpin (**6**), alteramide A (**7**), streptomycin (**8**), halicylindramide A (**9**), rhodopeptins C1 (**10**), ilamycin B1 (**11**), kahalalide F (**12**), didemnin B (**13**), surfactin (**14**), friulimicin B (**15**), asperversiamide A (**16**), marthiapeptide A (**17**), dicitrinone E (**18**), talarominine A (**19**), aspulvinones H (**20**), mersaquinone (**21**), avarone (**22**), avarol (**23**), 5,8-Dihydroxy-1,4-naphthoquinone (**24**), flaviogeranin B (**25**), phorbaketal A (**26**), collismycin C (**27**), strobilactone A (**28**), penicisteroid A (**29**), and ophiobolin K (**30**).

Takaaki Kubota et al. [42] demonstrated the isolation and analysis of zamamidine D (**1**). Compound **1** was obtained at a yield of 1.7 mg from 0.68 kg of wet sponge material, which corresponds to 0.00025% of the wet weight. The antimicrobial assays showed that zamamidine D is highly potent, with an MIC of 0.032 mg/mL for *E. coli*, and 0.008 mg/mL for *S. aureus*, *B. subtilis*, and *M. luteus*, and IC_{50} values of 0.016 mg/mL for *C. albicans* and *A. niger*, and 0.002 mg/mL for *C. neoformans*. These results underscore the compound's significant antimicrobial potential. Other notable sponge-derived alkaloids include manzamine A (**2**) [43], which has shown promising antibacterial activity against *M. tuberculosis*. Research by Rateb et al. [44] underscores the potential of manzamine A as a lead compound for developing new anti-TB drugs. Alkaloids isolated from marine sponges exhibit a wide range of chemical structures, including pyrrole, quinoline, isoquinoline, and indole derivatives, each contributing to their potent biological activities [45,46]. These compounds are often synthesized as secondary metabolites, serving as chemical defenses to protect the sponges from pathogenic microorganisms and predators. This evolutionary pressure has resulted in the production of alkaloids with highly specialized and effective antimicrobial properties [47,48].

Recently, Pech-Puch et al. [49] isolated eight alkaloids from the sponge *Agelas dilatata*, exhibiting potent antibacterial activity. Bromoageliferin (**3**) notably exhibited strong antibacterial effects against *P. aeruginosa*. Quantitative analyses showed that the MICs for bromoageliferin against different strains of *P. aeruginosa* ranged from 0.008 to 0.032 mg/mL, highlighting its potency.These results indicate that bromoageliferin holds promise as a lead compound for developing new antibacterial treatments targeting multidrug-resistant pathogens.

The pharmaceutical potential of sponge-derived alkaloids extends beyond their antimicrobial properties. The unique chemical structures of these compounds provide a scaffold for the progress of novel drugs with better efficacy and reduced resistance. For instance, researchers have been exploring the synthetic modification of sponge alkaloids to enhance their stability, bioavailability, and selectivity for microbial targets, paving the way for new therapeutic applications. A study by Hong et al. [50] discusses the potential for synthetic chemistry to unlock new derivatives of sponge alkaloids with enhanced pharmacological properties.

Moreover, the ecological role of alkaloids in marine sponges underscores their importance in maintaining marine biodiversity and health. By producing bioactive compounds, sponges contribute to the regulation of microbial populations in their habitats, preventing the overgrowth of harmful pathogens and promoting a balanced ecosystem. This ecological function highlights the potential benefits of preserving marine biodiversity, as the loss of sponge species could mean the loss of valuable bioactive compounds with significant medical applications [51]. Leal et al. [52] emphasize the ecological significance of sponge-derived alkaloids and the need for conservation efforts to protect these valuable marine resources.

Recent advancements in marine biotechnology have facilitated the sustainable extraction and synthesis of sponge alkaloids, reducing the environmental impact of bioprospecting. Advances in aquaculture and microbial fermentation techniques are enabling the large-scale production of these compounds, making them more accessible for pharmaceutical research and development. A review by Li et al. [53] discusses the potential of microbial symbionts in sponges as an alternative source of bioactive alkaloids, highlighting innovative approaches to sustainable drug discovery. Compound **4** shown in Figure 2 is dragmacidin G. Dragmacidin G isolated from a deep-water sponge of the genus *Spongosorties* exhibited broad-spectrum antibacterial activity against MRSA and *M. tuberculosis* [54].

Marine tunicates, commonly known as sea squirts, are a notable source of bioactive alkaloids with significant antibacterial and antifungal properties [55,56]. Prominent tunicate-derived alkaloids include trabectedin (**5**), which has been noted for its antifungal activity, highlighting its potential for new antifungal therapeutics [57]. Additionally, Blunt et al. [40] discussed the ecological importance of these alkaloids and their role in the chemical defense of tunicates, reinforcing the value of marine biodiversity in discovering new bioactive compounds. Moreover, researchers explored the biosynthetic pathways of

tunicate alkaloids, providing insights into their complex structures and potential applications in synthetic biology. Recently, several researchers demonstrated the use of microbial fermentation techniques to sustainably produce marine-derived alkaloids, which reduces the environmental impact typically associated with traditional bioprospecting. Their study highlights the potential of using engineered microorganisms to replicate the biosynthesis pathways of these marine compounds, thus enabling large-scale production without the need to harvest marine organisms directly [58,59].

Additionally, Xiong et al. [60] reviewed the potential of genome mining to identify new alkaloid-producing genes, highlighting innovative strategies for drug discovery. These advancements not only improve access to these compounds for pharmaceutical research but also contribute to marine ecosystem conservation [61]. Continued exploration of marine alkaloids holds great promise for developing novel antibacterial and antifungal agents, addressing the urgent need for new treatments in combating infectious diseases.

Marine microorganisms, including algae, bacteria, and fungi, are prolific producers of bioactive alkaloids with significant antibacterial and antifungal properties. Algae-derived alkaloids, particularly from green algae like *Caulerpa* sp., have demonstrated remarkable antibacterial activity against several pathogens, including multi-drug resistant strains. For instance, caulerpin (**6**), an indole alkaloid derived from *Caulerpa*, has demonstrated effectiveness against *E. coli*, *S. aureus*, *Streptococcus* sp., and *Salmonella* sp. The antimicrobial activity of caulerpin was assessed, revealing a MIC of 5.25 mg/mL for *E. coli*, *S. aureus*, and *Salmonella* sp. However, the MIC value against *Streptococcus* sp. was higher, at 15.50 mg/mL [62].

Alteramides are a general term for a class of compounds, and compound **7** shown in Figure 2 is alteramide A [63]. Additionally, marine bacteria, particularly those from the genera *Streptomyces* produce alkaloids like streptomycin (**8**), which exhibit extensive antibacterial and antifungal activities [64]. For example, four indole alkaloids, designated streptoindoles A–D, were obtained from *Streptomyces* sp. ZZ1118, which was cultured on a rice solid medium derived from a gut sample of marine shrimp (*Penaeus* sp.). Streptoindole C showed strong inhibition against *E. coli* and *C. albicans*, with a MIC of 0.007 mg/mL. Streptoindole D displayed weak activity solely against MRSA with an MIC of 0.025 mg/mL. Streptoindoles A and B were effective against all three pathogens, with MIC values ranging from 0.007 to 0.025 mg/mL [65]. These compounds often disrupt essential cellular processes in pathogens, such as protein synthesis, and DNA replication.

Marine-derived fungi also contribute significantly to the arsenal of bioactive alkaloids with antimicrobial properties. Species from marine environments, such as *Penicillium* and *Aspergillus*, produce various alkaloids which have shown strong antifungal activities against *C. albicans* and *A. niger* [66,67]. The ecological and biochemical diversity of marine organisms presents a valuable resource for developing novel antibacterial and antifungal agents, addressing the growing challenge of antimicrobial resistance. Their unique properties and bioactivities, derived from the challenging marine environments, make them promising candidates for addressing antibiotic resistance and other infectious diseases.

3. Marine-Derived Amino Acids

Marine-derived amino acids represent a rapidly growing area of research in the quest for new antimicrobial agents [68]. These amino acids are produced by a variety of marine organisms, including bacteria, fungi, and algae, and have shown significant antibacterial and antifungal activities [51]. Recent studies have demonstrated the effectiveness of these compounds in combating resistant microbial strains. Halicylindramides are a general term for a class of compounds, and compound **9** shown in Figure 2 is halicylindramide A [69]. Halicylindramides isolated from the Japanese marine sponge *H. cylindruta* exhibited in vitro antifungal activity against *M. ramanniana* at a concentration of 0.0075 mg/disk. Notably, the macrocyclic structure of compounds is crucial for their antifungal properties [70]. Rhodopeptins are a general term for a class of compounds, and compound **10** shown in Figure 2 is Rhodopeptins C1 [71]. Recently, Rhodopeptins C1, C2 C3, C4, and B5 were

isolated from *Rhodococcus* sp. These antifungal cyclic lipotetrapeptides consist of a β-amino acid and three standard α-amino acids. The Rhodopeptin exhibited in vitro antifungal activity against *C. albicans* with MIC between 0.00125 and 0.005 mg/mL, and against *C. neoformans* with an MIC values ranging from 0.00063 to 0.00125 mg/mL [72].

In addition to their antifungal properties, marine-derived amino acids have demonstrated significant antibacterial activities. Four cyclic heptapeptides, identified as L-156,373 and its derivatives, were obtained from a marine *Streptomyces* sp. culture. These heptapeptides showed notable activity against pathogens including *S. aureus*, MRSA, *B. Calmette-Guérin*, and *B. subtilis*, with MIC values ranging from 0.00025 to 0.00125 mg/mL [73]. The continued exploration of marine-derived amino acids and their conjugates could lead to the identification of novel antimicrobial agents with specific activities against resistant bacterial and fungal pathogens. Furthermore, advancements in cultivation and chemical profiling techniques, as demonstrated by the integrated strategy used in these studies, are crucial for the effective discovery and optimization of these bioactive compounds.

4. Marine-Derived Peptides

Marine-derived peptides are emerging as potent candidates in combating bacterial and fungal infections, especially given the increasing challenge of AMR [74]. Ilamycins are a general term for a class of compounds, and the name of compound **11** is ilamycin B1 [75]. Cyclic oligopeptides such as ilamycins, sourced from marine bacteria like *Streptomyces atratus*, have shown considerable effectiveness against drug-resistant pathogens including *M. tuberculosis* [76]. Notable examples include kahalalide F (**12**), which is effective against *C. albicans*, and surfactin, which targets MRSA [77].

Cyclic oligopeptides are a class of peptides consisting of 2–20 amino acids arranged in a cyclic structure. These peptides are primarily synthesized by non-ribosomal peptide synthetases, which contribute to their structural diversity and biological activity. One notable example is the ilamycin family, which includes ilamycin B1–F and ilamycins G–R. These peptides are produced by the marine-derived bacterium *S. atratus* and have shown potent activity against *M. tuberculosis*. The antimicrobial activity of ilamycins is ascribed to their ability to disrupt bacterial cell walls, making them effective against drug-resistant strains of *M. tuberculosis* [78]. Recent research by Wang et al. [24] has highlighted the structural and functional diversity of cyclic oligopeptides derived from marine microorganisms. The study discusses how modifications in the side chains of amino acids can significantly influence the antimicrobial potency of these peptides. For instance, the presence of specific side chain modifications in ilamycins have been shown to enhance their activity against *M. tuberculosis*, indicating the potential for structural optimization to improve therapeutic efficacy [79].

Cyclic depsipeptides are another important class of marine-derived peptides known for their potent antimicrobial properties [80,81]. These peptides contain ester bonds in addition to amide bonds, which contribute to their unique structural characteristics. Compound **13** shown in Figure 2 is didemnin B [82]. Compounds **12** and **13** has demonstrated significant antifungal activity against *C. albicans* [83,84]. The mechanism of action involves disrupting the fungal cell membrane, leading to cell lysis and death.

Cyclic lipopeptides are characterized by the presence of a lipid tail attached to a cyclic peptide core. These compounds exhibit strong surfactant properties, which enhance their ability to disrupt microbial cell membranes [24]. Marine-derived cyclic lipopeptides such as surfactin (**14**) produced by *Bacillus* sp., have shown remarkable antibacterial and antifungal activities. Surfactin has been effective against a range of Gram-positive bacteria, including MRSA. The antimicrobial action of surfactin involves the insertion of the lipid tail into the bacterial cell membrane, leading to increased membrane permeability and cell death [85]. Friulimicin B (**15**) is a natural cyclic lipopeptide composed of eleven amino acids, synthesized by the *Actinoplanes friuliensis*. It exhibits antibacterial activity against a broad range of Gram-positive bacteria, including antibiotic-resistant pathogens [86]. Asperversiamides are cyclic heptapeptides and the name of compound **16** is Asperversiamide A.

Asperversiamide A, B and C extracted from a coral-derived fungal strain *Aspergillus versicolor* CHNSCLM-0063, exhibiting strong anti-*M. marinum* activity, with asperversiamide B also showing moderate anti-TB activity [87,88]. Asperheptatides A–B are structurally closest to asperversiamide B and also shows moderate anti-TB activity [89]. By using asperversiamide A as a core structure, various cinnamic acid groups were introduced onto the hydroxyl group of the serine side chain, resulting in cinnamic acid derivatives that exhibited an eight-fold increase in anti-TB action. This process also confirmed the anti-TB efficacy of the cinnamic acid structure [88].

The cyclic peptide marthiapeptide A (**17**) isolated from the deep-sea-derived *Marinactinospora thermotolerans* SCSIO 00652 showed antibacterial MICs of 0.004, 0.002, 0.002, and 0.008 mg/mL for *B. subtilis*, *M. luteus*, *B. thuringiensis*, and *S. aureus*, respectively, and showed no activity against Gram-negative bacteria like *E. coli* [90,91]. Marine-derived peptides often exhibit synergistic effects when combined with conventional antibiotics and antifungals. This synergy can enhance the overall antimicrobial efficacy and reduce the likelihood of resistance development. Some peptides inhibit bacterial efflux pumps, which are responsible for expelling antibiotics from bacterial cells. By blocking these pumps, peptides can restore the potency of existing antibiotics and expand their spectrum of activity. Additionally, marine peptides may target multiple bacterial pathways simultaneously, reducing the chances of resistance [92].

Understanding the structure-activity relationships (SARs) of marine-derived peptides is crucial for optimizing their antimicrobial properties. Structural modifications, such as altering amino acid residues or adding functional groups, can significantly impact the activity and stability of these peptides [93]. For instance, modifications to the side chains of amino acids in ilamycins have been shown to enhance their activity against *M. tuberculosis*. Similarly, altering the lipid tail length in cyclic lipopeptides can improve their ability to penetrate microbial membranes. A wide-ranging review by Wang et al. [24] discusses the SAR of various marine-derived peptides, emphasizing the importance of specific structural features in determining their antimicrobial efficacy. Their review highlights that fine-tuning the peptide structure can lead to the development of more potent antimicrobial properties.

5. Marine-Derived Polyketide

Marine-derived polyketides represent a promising frontier in the search for new antimicrobial agents [92]. These compounds, isolated from marine organisms such as bacteria, fungi, and algae, have shown significant potential due to their distinctive characteristics and various ways of combating pathogens. Unlike many terrestrial antibiotics, marine-derived polyketides often possess distinctive chemical features that reduce the likelihood of cross-resistance with existing drugs. Marine-derived polyketides including macrocyclic lactones, polyenes, and polyethers contribute to their potent biological activities and their ability to target pathogens in novel ways [92]. The antimicrobial mechanisms of marine-derived polyketides are varied and can include the inhibition of cell wall synthesis, disruption of membrane integrity, interference with protein and nucleic acid synthesis, and inhibition of critical metabolic pathways. This diversity in modes of action not only enhances their effectiveness but also reduces the risk of resistance development.

Dicitrinones are a general term for a class of compounds, and the compound **18** in Figure 2 is dicitrinone E [94]. Recently, dicitrinones were extracted from the starfish-associated *Penicillium* sp. GGF 16-1-2. These compounds demonstrated potent antifungal properties against *Colletotrichum gloeosporioides*, with LD_{50} values between 0.00958 mg/mL and 0.01614 mg/mL [95]. Five new polyketides (two chromones, two phenyl derivatives, and one tandyukusin derivative) were isolated alongside five known ones. Among these, few showed significant antifungal activities against *Penicillium italicum*. The study underscores the potential of mangrove-derived fungi in yielding bioactive compounds with significant antimicrobial properties [96]. A study by Song et al. [97] focused on aromatic polyketides from the endozoic fungus *Talaromyces minioluteus*, associated with deep-sea cold-seep mussels. Researchers identified five new aromatic polyketides, includ-

ing a unique benzofuran derivative and four chromone analogs. Compound **19** shown in Figure 2 is talarominine A [97]. Talarominine A and its derivatives demonstrated notable antibacterial activity, particularly against MRSA and *P. aeruginosa*. The study emphasized the distinctive structures of these polyketides, which are essential for developing new antibacterial agents aimed at combating MRSA. Van Anh et al. [98] explored rifamycin-related polyketides from the marine-derived bacterium *Salinispora arenicola*, identifying eight polyketides, including three new derivatives. The rifamycin-related compounds exhibited moderate cytotoxic activity against various cancer cell lines, suggesting potential antibacterial applications due to their ability to inhibit bacterial RNA synthesis.

Aspulvinones are a general term for a class of compounds, and the compound **20** should be aspulvinones H [99]. Recently, four antimicrobial compounds named aspulvinones B′, H, R, and S were isolated from *A. flavus* KUFA1152, sourced from the marine sponge *Mycale* sp. These compounds demonstrate significant antibacterial properties against MREF ATCC 29212 and MRSA *S. aureus* ATCC 29213. In addition to their antibacterial effects, these compounds also prevent biofilm formation by these strains. The MIC values for aspulvinones B′, H, R, and S range between 0.004 and 0.064 mg/mL [100]. A study by Koch et al. [101] isolated 2-carboxymethyl-3-hexylmaleic acid anhydride from the endozoic fungus *Aspergillus tubingensis* OY907, found in the Mediterranean marine sponge *Ircinia variabilis*. This compound demonstrated inhibitory activity against *Neurospora crassa*. Tan et al. [102] emphasized the importance of marine-derived polyketides by identifying novel compounds from the marine-derived fungus *Penicillium* species, showing potent antifungal and antibacterial activities. These compounds were particularly effective against *C. albicans* and *S. aureus*, demonstrating broad-spectrum antimicrobial potential. The study suggested these polyketides could be developed into therapeutic agents for treating bacterial and fungal infections. In addition to their antibacterial properties, marine-derived polyketides show promise in disrupting biofilms, which protect bacterial communities from antibiotics, making infections difficult to treat. Wibowo et al. [26] highlighted the potential of marine-derived bacterial secondary metabolites including polyketides in antibiotic and antibiofilm applications. Their review underscores the importance of marine bacteria as a valuable resource for discovering new antimicrobial agents.

6. Marine-Derived Naphthoquinones

Marine-derived naphthoquinones are characterized by their naphthalene ring structure with two ketone groups. Isolated from various marine organisms, including sponges, algae, and bacteria, these compounds exhibit a wide range of biological activities [103]. Marine-derived naphthoquinones demonstrate a wide range of antimicrobial activities. They often disrupt the electron transport chain in microbial cells, hindering ATP production and causing cell death. Some naphthoquinones also intercalate into DNA, blocking replication and transcription, which prevents microorganisms from reproducing and functioning properly. Additionally, these compounds can induce the formation of ROS within microbial cells, leading to oxidative damage to proteins, lipids, and DNA, and resulting in cellular dysfunction and death [51,104,105].

Mersaquinone (**21**), a newly discovered derivative of tetracene, was isolated from *Streptomyces* sp. EG1, obtained from sediment off Egypt's North Mediterranean coast. The chemical structure of mersaquinone was meticulously identified using HRESIMS, IR spectroscopy, and both one-dimensional and two-dimensional NMR spectroscopy. This substance demonstrated the ability to suppress the growth of the MRSA strain TCH1516, showing a MIC of 0.00336 mg/mL [106]. Marine sponges are rich sources of naphthoquinones. Compounds such as avarone (**22**) and avarol (**23**), isolated from *Dysidea avara*, have demonstrated potent antibacterial and antifungal activities. These compounds have shown effectiveness against marine bacteria (*C. marina*, *M. stanieri*, *V. fischeri*, and *P. haloplanktis*) and marine fungi (*H. mediosetigera*, *A. cruciatus*, *L. uniseptate*, and *M. pelagica*) [107]. Naphthazarins are a general term for a class of compounds, and compound **24** shown in Figure 2 is 5,8-Dihydroxy-1,4-naphthoquinone [108]. These compounds are mostly effective

against a range of bacterial and fungal pathogens. Recent studies have demonstrated the significant antibacterial potential of marine-derived naphthoquinones, such as those isolated from *Streptomyces* and *Talaromyces* sp. For example, Park et al. [109] identified two new naphterpin derivatives from *Streptomyces* sp. CNQ-509, which exhibited potent antibacterial activity against several pathogens. The study highlighted the role of the naphthoquinone moiety in the antibacterial efficacy of these compounds. Flaviogeranins are a general term for a class of compounds, and compound **25** shown in Figure 2 is flaviogeranin B. Shen et al. [110] reported the isolation of a group of naphthoquinone-containing compounds, specifically seven flaviogeranin (**25**) congeners, including three novel compounds, from *Streptomyces* sp. B9173. Their structures were determined by a combination of spectroscopic techniques, including 1D and 2D NMR, and high-resolution mass spectrometry. The compounds flaviogeranin B and flaviogeranin C2 exhibited potent inhibitory activity against *S. aureus* and *M. smegmatis*, with MIC values ranging from 0.005 to 0.009 mg/mL, comparable to the MIC of the positive control, erythromycin.

Phorbaketals are a general term for a class of compounds, and compound **26** shown in Figure 2 is phorbaketals A. Kim et al. [111] explored the antibiofilm properties of phorbaketals (**26**) extracted from the Korean marine sponge *Phorbas* sp. against *S. aureus*, including MRSA. The study isolated six different phorbaketals (phorbaketal A, A acetate, B, B acetate, C, and C acetate) and tested for their ability to prevent biofilm formation. Specifically, compounds B and C demonstrated notable antibiofilm activity without bactericidal effects, thereby minimizing the potential for resistance. These compounds also suppressed the production of staphyloxanthin, a virulence factor aiding *S. aureus* in evading the immune system. The antibiofilm action was attributed to the downregulation of crucial genes associated with biofilm formation and virulence, including α-hemolysin (*hla*) and nuclease (*nuc1*). Consequently, phorbaketals B and C show promise as potential antibiofilm agents for treating infections caused by drug-resistant bacteria. Another recent study by Lee et al. [112] demonstrated the antibiofilm activity of collismycin C (**27**), a compound isolated from the marine bacterium *Streptomyces* sp. MC025, against MRSA. The study involved screening 79 Micronesian marine microorganisms for their ability to inhibit *S. aureus* biofilm formation. Among the isolated compounds, collismycin C was the most effective, inhibiting MRSA biofilm development by over 90% at 0.05 mg/mL. The biofilm suppression activity of collismycin C was attributed to its iron-chelating properties, which interfere with the biofilm formation process. In addition to their antibacterial properties, marine-derived naphthoquinones also exhibit strong antifungal activities, addressing the critical need for new antifungal agents amidst rising drug resistance. Liu et al. [113] studied naphthoquinone derivatives from the mangrove-derived endophytic fungus *Talaromyces* sp., identifying twelve 1,4-naphthoquinone derivatives, including two new compounds. These derivatives showed significant antifungal effects against *C. albicans* and *A. niger*, with some compounds demonstrating higher potency than standard antifungal agents. The study emphasized the dual functionality of these naphthoquinones in addressing both bacterial and fungal infections.

7. Marine-Derived Terpenoids

Marine-derived terpenoids are characterized by their diverse and complex structures, often consisting of multiple isoprene units. Marine-derived terpenoids frequently display unique modifications such as halogenation, glycosylation, and unusual ring systems. These modifications arise from the distinctive biosynthetic pathways found in marine environments, contributing to the enhanced biological activities of these compounds [114]. Marine-derived terpenoid derivatives are increasingly recognized for their potent antibacterial and antifungal activities, making them a promising frontier in the development of new antimicrobial agents. Their unique bioactivities and mechanisms of action make them valuable candidates for novel therapies to combat resistant microbial strains and biofilm-associated infections. Marine-derived terpenoids can disrupt microbial cell membranes, prevent cell wall synthesis, restrict protein and nucleic acid synthesis, and inhibit critical

metabolic pathways. Additionally, some terpenoids can modulate the immune response, enhancing the host's ability to fight off infections. For instance, compounds like squalene and its derivatives have been shown to disrupt microbial membranes, leading to cell lysis and death.

Terpenoid derivatives isolated from marine sponges have demonstrated strong antimicrobial properties. Compound **28** shown in Figure 2 is strobilactone A [115]. Four drimane-type sesquiterpenoid lactones were extracted from the fungus *A. insuetus* (OY-207), which was sourced from the Mediterranean sponge *Psammocinia* sp. These compounds were identified as derivatives of strobilactone A, including a new compound identified as (E)-6-(4′-hydroxy-2′-butenoyl)-strobilactone A. In addition, the known derivatives strobilactone A and (E,E)-6-(6′,7′-dihydroxy-2′,4′-octadienoyl)-strobilactone A, along with 2α,9α,11-trihydroxy-6-oxodrim-7-ene, were also isolated. The antifungal properties of these compounds were tested, revealing that strobilactone A and (E,E)-6-(6′,7′-dihydroxy-2′,4′-octadienoyl)-strobilactone A exhibited mild antifungal activity against the fungus *Neurospora crassa* [116].

Terpenoids from marine fungi and algae have revealed considerable effectiveness against fungal pathogens like *C. albicans* and *A. niger*. Research on the endophytic fungus *P. chrysogenum* QEN-24s, isolated from the marine red alga *Laurenica* sp., resulted in the discovery of two new polyoxygenated sterols, penicisteroid A and B, along with the previously known steroid, anicequol. These compounds were evaluated for their antifungal properties. The findings revealed that penicisteroid A (**29**) exhibited antifungal activity against both *A. niger* and *Alternaria brassicae*, while anicequol was active only against *A. brassicae*. In contrast, penicisteroid B showed no antifungal activity against either of the two fungi [117]. Generally, terpenoid derivatives often disrupt fungal cell membranes or inhibit ergosterol synthesis, a critical component of fungal cell membranes, leading to fungal cell death. This dual functionality underscores the versatility and broad-spectrum potential of marine-derived terpenoids in antimicrobial therapy.

Marine-derived terpenoid derivatives have shown promise in addressing biofilm-associated infections. Terpenoid derivatives have demonstrated the ability to disrupt biofilm formation and maintenance. By targeting the biofilm matrix and preventing the development of new biofilms, these compounds offer a viable solution for treating biofilm-related infections. This characteristic is particularly valuable in medical settings, where biofilm formation on medical devices and implants poses significant challenges. Three novel sesterterpenes belonging to the ophiobolin class were isolated from the marine-derived fungus *Emericella variecolor* strain GF-10, which was sourced from sediment collected at a depth of 70 meters in the Gokasyo Gulf, Japan. These compounds were identified as ophiobolin K, 6-epi-ophiobolin K, and 6-epi-ophiobolin G. They were found to inhibit the biofilm formation of *M. smegmatis*, with ophiobolin K (**30**) demonstrating the highest activity, showing a MIC of 1.84 mg/mL [118].

8. Marine-Derived Polysaccharides

Marine-derived polysaccharides are complex carbohydrates that consist of long chains of monosaccharide units. These polysaccharides often display unique structural features such as sulfation, branching, and acetylation, which contribute to their diverse biological activities. Marine-derived polysaccharides have garnered significant interest for their antibacterial and antifungal properties, leading to their exploration in various industrial applications, from pharmaceuticals to food packaging. Marine-derived polysaccharides can inhibit microbial adhesion and biofilm formation, disrupt cell membranes, and modulate immune responses. Additionally, some marine polysaccharides have been shown to enhance the efficacy of existing antibiotics, potentially reducing the required dosage and minimizing side effects. Recent studies have highlighted the effectiveness of key marine polysaccharides such as chitin, chitosan, cellulose, fucoidan, and alginate. Figure 3 schematically illustrates an overview of the key marine polysaccharides and their sources from marine organisms.

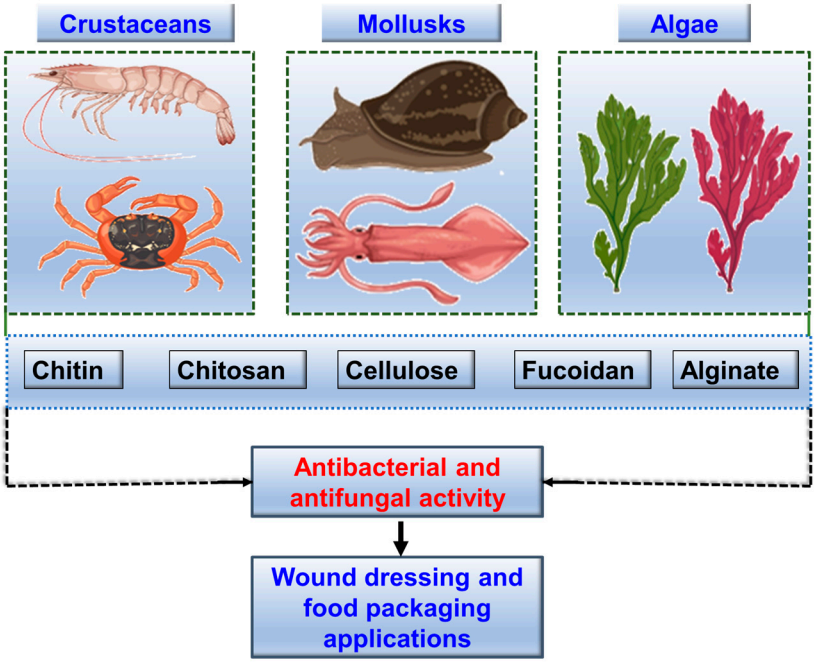

Figure 3. The sources of selected key biopolymers and their applications. Crustaceans and mollusks provide chitin, chitosan and cellulose. Algae (such as green and red algae) provide cellulose, fucoidan and alginate. These biopolymers exhibit antibacterial and antifungal activities, making them suitable for use in wound dressing and food packaging applications.

Chitin is a polymer consisting of (1→4)-β-linked N-acetyl-D-glucosamine units. It is the most abundant amino-polysaccharide polymer found in nature and ranks as the second most abundant polysaccharide after cellulose. Marine ecosystems, particularly crustacean shells from crabs, shrimp, lobsters, and krill, are considered the primary sources of chitin [119]. A study by Abdel-Rahman isolated highly pure chitin from Brazilian Atlantic Coast shrimp shells by the chemical treatment method. The isolated chitin exhibited antibacterial activity against *E. coli* by the chemo-luminescence technique. Chitosan, derived from the deacetylation of chitin found in the exoskeletons of crustaceans, has been extensively studied for its antimicrobial properties [120,121]. Its polycationic nature allows chitosan to interact with negatively charged bacterial membranes, causing cell leakage and death. Recent research has demonstrated the effectiveness of chitosan in food packaging and biomedical applications. A recent study by Mohammadi et al. [122] extracted chitosan from shrimp waste using different methods: conventional extraction and microwave-assisted extraction. The antibacterial action of the extracted chitosan was evaluated using an agar disc diffusion assay. The chitosan obtained through conventional extraction displayed the highest antibacterial zone of inhibition (mm) against *Listeria monocytogenes* (9.48), *E. coli* (8.79), and *S. Typhimurium* (8.57). On the other hand, chitosan produced via microwave-assisted extraction showed superior activity against *S. aureus* (8.05) and *E. coli* (8.37) with a similar antibacterial property against *L. monocytogenes* (6.52) and *S. Typhimurium* (7.34). A recent study by Verma et al. [123] demonstrated a process of extracting chitosan from the shells of horse mussels, a common waste product from fisheries. The extracted chitosan displayed a degree of acetylation of 57.43%, making it a suitable biopolymer for biomedical applications. The research highlights the antimicrobial efficacy of the chitosan, particularly against *E. coli* and *B. subtilis*, demonstrating its potential as a valuable material in antimicrobial applications.

Marine-derived cellulose and nanocellulose from algae such as *Ulva lactuca* shows significant antibacterial properties [124]. This cellulose is a promising antibacterial agent against *K. pneumonia* (ST627), *S. aureus* (ATCC6538), *E. coli* (ATCC25922) and coagulase-negative *staphylococci*. It disrupts bacterial membranes and adsorbs onto cells, effectively

inhibiting growth. Its biocompatibility and antibacterial nature also make it suitable for wound dressings and food packaging, promoting sterility and extending shelf life [125–127]. Ongoing research aims to enhance these properties, making marine-derived cellulose a sustainable antibacterial material. Fucoidan is a type of sulfated polysaccharide found mainly in various species of brown seaweed and some marine invertebrates [128,129]. Fucoidan is primarily composed of fucose, a type of sugar, along with sulfate groups. It may also contain other monosaccharides like glucose, galactose, and mannose. Recent studies have shown its effectiveness against various bacterial pathogens, including *S. aureus*, *E. coli*, *L. monocytogenes*, and *Salmonella enterica serovar Typhimurium*. The antibacterial activity of fucoidan is attributed to its ability to disrupt bacterial cell walls and membranes, inhibit bacterial adhesion and biofilm formation, and interfere with bacterial metabolism. Its sulfate groups interfere with the replication of viruses and the growth of bacteria. Additionally, fucoidan has shown potential against fungal pathogens such as *C. albicans* by impairing cell wall integrity and preventing adhesion and biofilm formation [127,129]. Carrageenan, extracted from algae, is known for its antibacterial and antifungal properties [130]. It forms protective barriers on surfaces, inhibiting the adherence and penetration of pathogens. This polysaccharide is widely used in the food industry as a natural preservative to enhance product shelf life by preventing microbial growth [130].

Alginate is a naturally occurring biopolymer derived primarily from the cell walls of brown seaweed and contain linear copolymers of α-L-guluronic acid and β-D-mannuronic acid. Alginate forms gels in the presence of calcium ions and exhibits notable antimicrobial properties [131]. Alginate-based wound dressings are highly absorbent and maintain a moist atmosphere favorable to healing while protecting against microbial infections. In the food industry, alginate is used as a thickener and stabilizer, with its antimicrobial properties enhancing food safety and quality [132]. Recent developments in composite films combining marine-derived polysaccharides have shown enhanced antimicrobial efficacy. For instance, *O*-carboxymethyl and pectin films, when combined with neem extracts, demonstrated significant antimicrobial activity and improved tensile properties [133]. Such composite films are biodegradable and offer a sustainable solution for food packaging and other applications requiring antimicrobial properties.

Overall, a variety of marine-derived substances have demonstrated potential as antibacterial and antifungal agents. Table 1 provides a comprehensive overview of various marine-derived compounds, including their type, sources, properties, and modes of action.

Table 1. List of marine-based drugs, outlining their characteristics and methods of action.

Type	Compounds	Marine-Sources	Properties	Mode of Action
Marine-derived alkaloids	Zamamidine D	*Amphimedon* sp.	Antibacterial and antifungal activity [42]	Inhibition of topoisomerase IV and bacterial DNA gyrase and membrane disruption, inhibition of ergosterol synthesis, and disruption of fungal cell wall.
	Manzamine	*Acanthostrongylophora* sp.	Antibacterial activity [134]	Inhibition of bacterial cell wall synthesis.
	Bromoageliferin	*Agelas dilatata*	Antibacterial activity [49]	Inhibition of bacterial protein synthesis, disruption of membrane integrity and preventing biofilm formation.
	Caulerpin	*Caulerpa* sp.	Antibacterial activity [62]	Disruption of bacterial cell membranes, and inhibition of enzymatic activity.
	Streptoindoles	*Streptomyces* sp.	Antibacterial activity [65]	Inhibition of protein synthesis and interruption of membrane integrity, DNA binding and interference.
Marine-derived amino acids	Halicylindramides	*Halichondria cylindruta*	Antifungal activity [70]	Alteration in membrane integrity, reserve enzymatic activity, and induction of oxidative stress.
	Rhodopeptins	*Rhodococcus* sp.	Antifungal activity [72]	Disruption of cell membrane integrity, inhibition of enzymatic activity, and induction of oxidative stress.
Marine-derived peptides	Ilamycins	*Streptomyces atratus*	Antibacterial activity [76]	Disruption of protein synthesis and inhibition of RNA synthesis.
	Kahalalide F	*Elysia rufescens*	Antifungal activity [83]	Destabilization of cell membrane, induction of apoptosis, and inhibition of enzymatic activity.
	Didemnins	*Tridemnum* sp	Antibacterial activity [55]	Inhibition of DNA synthesis, interference with protein synthesis, and induction of apoptosis in bacteria.
	Friulimicin	*Actinoplanes friuliensis*	Antibacterial activity [86]	Inhibtion of cell wall synthesis and interaction with cell membranes.
	Asperversiamides	*Aspergillus versicolor*	Antibacterial activity [87,88]	Damage to membrane integrity, suppression of protein synthesis, and inhibition of cell wall synthesis.
	Marthiapeptide	*Marinactinospora thermotolerans*	Antibacterial activity [91]	Damage to membrane integrity, inhibition of protein synthesis, and inhibition of cell wall synthesis.
Marine-derived polyketides	Dicitrinones	*Penicillium* sp.	Antifungal activity [95]	Disruption of cell membrane integrity, inhibition of enzymatic activity, and induction of oxidative stress.
	Talarominine	*Talaromyces minioluteus*	Antibacterial activity [97]	Inhibition of protein synthesis, damage to cell membrane integrity, and inhibition of nucleic acid synthesis.
	Aspulvinones	*Aspergillus flavus*	Antibacterial activity [100]	Disruption of cell membrane integrity, inhibition of enzymatic activity, and induction of oxidative stress.

Table 1. *Cont.*

Type	Compounds	Marine-Sources	Properties	Mode of Action
Marine-derived naphthoquinones	Mersaquinone	*Streptomyces* sp.	Antibacterial activity [106]	Inhibition of protein synthesis, disruption of cell membrane integrity, and inhibition of DNA synthesis.
	Avarone	*Dysidea avara*	Antibacterial and antifungal activity [107]	Disruption of cell membrane integrity, prevention of enzymatic activity, and induction of oxidative stress.
	Naphterpin	*Streptomyces* sp.	Antibacterial activity [109]	Disruption of cell membrane integrity, and disruption of cell wall synthesis.
Marine-derived terpenoids	Penicisteroid	*Penicillium chrysogenum*	Antifungal activity [117]	Disruption of cell membrane integrity, inhibition of enzymatic activity, and induction of oxidative stress.
	Ophiobolin K	*Emericella variecolor*	Antibacterial activity [104]	Disruption of cell membrane, inhibition of proteins, enzymes and DNA, and generation of ROSs.
Marine-derived polysaccharides	Chitin	Shrimp shell	Antibacterial activity [121]	Compromise of membrane integrity, and disruption of cell wall synthesis.
	Chitosan	Crustaceans	Antibacterial and antifungal activity [119,121]	Cell membrane disruption, suppression of protein and DNA synthesis, and chelation of essential nutrients
	Cellulose	*Ulva lactuca*	Antibacterial activity [124]	Compromise of cell membrane integrity, generation of ROSs and inhibition of enzymatic activity.
	Fucoidan	Brown algae	Antibacterial and antifungal activity [128,129]	Disruption of cell membrane integrity, inhibition of enzymatic activity, and binding to bacterial surface structures.
	Alginate	Seaweed	Antibacterial activity [131,132]	Disruption of cell membrane integrity, inhibition of enzymatic activity, and induction of oxidative stress.

9. Conclusions

In conclusion, the rising threat of antimicrobial resistance (AMR) necessitates the discovery of novel antimicrobial agents. Our review highlights the untapped potential of marine environments, which harbor a diverse array of bioactive compounds. Marine-derived alkaloids, peptides, polyketides, naphthoquinones, terpenoids, and polysaccharides exhibit significant antibacterial and antifungal properties against resistant pathogens. Notable compounds such as zamamidine D, manzamine A, ilamycins, and kahalalide F show particular promise. The unique mechanisms of action and potential for synergistic effects with existing antibiotics make natural marine products a valuable resource in the fight against AMR. Future research should focus on the exploration and sustainable utilization of these marine-derived compounds to develop new, effective antimicrobial therapies.

10. Future Prospectives

The future of marine-derived antimicrobial research lies in several key areas:

(a) Advanced Bioprospecting and Sustainable Harvesting

Advancements in bioprospecting technologies, including accessing previously unexplored marine habitats such as deep-sea vents and polar regions, can lead to the discovery of novel bioactive compounds. Emphasizing sustainable harvesting methods, including microbial fermentation and aquaculture, will minimize environmental impact and ensure a continuous supply of these valuable compounds.

(b) Synthetic Biology and Genome Mining

Synthetic biology offers the potential to enhance the production and structural diversity of marine-derived compounds. Genome mining techniques can identify new biosynthetic pathways and gene clusters responsible for producing bioactive metabolites, leading to the discovery of new compounds and the optimization of existing ones for improved antimicrobial efficacy.

(c) Structural Optimization and Drug Development

Understanding the SAR of marine-derived compounds is crucial for optimizing their antimicrobial properties. Structural modifications, such as altering amino acid residues or functional groups, can enhance the potency, stability, and bioavailability of these compounds. Collaborative efforts between chemists, biologists, and pharmacologists are essential to translate these compounds into clinically viable drugs.

(d) Combination Therapies

Exploring the synergistic effects of marine-derived compounds with conventional antibiotics and antifungals can enhance overall antimicrobial efficacy and reduce resistance development due to different targets of drugs. Combination therapies could be particularly effective against multidrug-resistant pathogens and biofilm-associated infections.

(e) Ecological and Conservation Considerations

Preserving marine biodiversity is paramount for maintaining the source of these bioactive compounds. Conservation efforts should focus on protecting marine ecosystems and mitigating the impacts of climate change, pollution, and overfishing. Additionally, understanding the ecological roles of these compounds can provide insights into their evolutionary significance and potential applications.

(f) Clinical Trials and Regulatory Approval

Rigorous preclinical and clinical trials are necessary to evaluate the safety, efficacy, and pharmacokinetics of marine-derived compounds. Establishing regulatory frameworks will facilitate the approval and commercialization of new marine-based antimicrobial agents.

(g) Toxicity Issues

Marine-derived compounds hold therapeutic potential but pose toxicity risks such as cytotoxicity, neurotoxicity, hepatotoxicity, and immunotoxicity. Future strategies to

mitigate these issues include advanced bioprospecting to identify safer compounds, synthetic biology for producing less toxic analogs, and structural optimization to improve selectivity. Rigorous preclinical and clinical testing, combination therapies to reduce doses, and comprehensive regulatory frameworks are essential.

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Thank you.

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