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Saponins from sea cucumber disrupt *Aeromonas hydrophila* quorum sensing to mitigate pathogenicity

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Abstract

Aeromonas hydrophila, a Gram-negative bacterium, poses significant threats to aquaculture, leading to substantial economic losses. Its pathogenicity is primarily driven by a sophisticated quorum sensing (QS) system that regulates virulence factors. This study investigates saponins extracted from the sea cucumber *Holothuria leucospilota* as potential natural inhibitors of QS in *A. hydrophila*, offering a novel disease management strategy for aquaculture. Specimens of *H. leucospilota* were collected, and saponins were extracted from their Cuvierian tubules through a process of homogenization, solvent extraction, and purification. The saponin extract's minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) against *A. hydrophila* were found to be 80 µg/mL and 30 µg/mL, respectively. Hemolysin, lipase, and protease are examples of QS-regulated virulence factors whose activities were found to be significantly reduced by sub-MIC levels of saponins. Additionally, swarming motility and biofilm formation were notably inhibited. A significant downregulation of the QS genes *ahyI* and *ahyR* was observed, indicating an effective disruption of the QS system.These findings suggest that saponins from *H. leucospilota* can inhibit the QS system in *A. hydrophila*, thereby reducing its pathogenicity. This disruption offers a promising method for controlling bacterial infections without directly killing the bacteria, potentially mitigating antibiotic resistance. The study highlights the potential of marine-derived compounds as natural QS inhibitors, contributing to environmentally friendly aquaculture practices. Overall, it provides evidence that sea cucumber saponins could serve as a novel class of anti-QS agents, presenting a new perspective on disease management in aquaculture and other bacterial infection contexts.

Keywords *Aeromonas hydrophila*, Quorum sensing, Saponins, Sea cucumber, Virulence factors

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Introduction

Aeromonas hydrophila, a Gram-negative opportunistic pathogen, thrives in varied aquatic environments and is implicated in several human diseases, including skin infections and gastrointestinal disorders (Qu and Liu 2024). The global aquaculture industry suffers large financial losses as a result of the serious illnesses, it causes in fish and aquatic animals, including hemorrhagic septicemia and motile aeromonad septicemia (Derome et al. 2016). Numerous bacterial virulence factors, such as adhesins, toxins, hemolysins, proteases, motility, and biofilm formation, are associated with the pathogenicity of *A. hydrophila* (Rasmussen-Ivey et al. 2016). A bacterial communication system called quorum sensing (QS) uses cell density to control behavior and physiological reactions. This system, first identified in the *Vibrio fischeri* lux operon, involves homologous genes across many species, notably luxI and luxR. In order for the QS to function, the LuxI protein must produce the autoinducer molecule N-acyl-homoserine lactone (AHL), which is then picked up by the membrane-bound LuxR receptor (Garg et al. 2014). In *Aeromonas hydrophila,* the QS system primarily relies on *ahyI* and *ahyR* genes, analogous to *luxI* and *luxR*, respectively. Here, AHL synthase encoded by ahyI produces signal molecules that, at sufficient cell densities, bind to the receptor synthesized by ahyR, forming an AHL-AhyR complex. This complex modulates the transcription of target genes, either activating or inhibiting them. The primary signaling molecule is butanoyl-homoserine lactone (C4-AHL), with hexanoyl-homoserine lactone (C6-AHL) produced in lower concentrations (dos Reis Ponce-Rossi et al. 2016). Numerous bacterial virulence factors, such as the production of exoprotease, the secretion of type 6 secretion system effectors, bacterial adhesion, motility, and biofilm formation, are positively regulated by the *ahyI/R* quorum sensing (QS) system (Qin et al. 2023).

Because bacterial pathogenesis depends on quorum sensing (QS), there is increasing interest in quorum sensing inhibitors (QSIs) derived from natural sources. To stop bacterial infections and the spread of disease, these QSIs are being investigated for use either by themselves or in conjunction with antibiotics (Asfour 2018). Bioactive compounds extracted from marine organisms are of particular interest due to the vast biological diversity and unique properties of these compounds (Peng et al. 2024). Recent research indicates that species within the Holothuroidea class, often found in extreme environments, produce a variety of secondary metabolites with beneficial effects, including antifouling, antibacterial, antipredation, and UV-protective activities (Pangestuti and Arifin 2018). Sea cucumbers are known to produce various triterpene glycosides, commonly referred to as saponins (Kamyab et al. 2020b). These saponins, composed of a hydrophilic glycone and a hydrophobic aglycone, are found in the Cuverian tubules, body wall, and viscera of holothuroids (Van Dyck et al. 2010a). Due to their membranolytic properties, saponins exhibit a wide range of bioactivities, including antibacterial, antifungal, antiviral, anti-inflammatory, and ichthyotoxic effects (Kamyab et al. 2020a, 2020b; Popov 2002). Nevertheless, it has not yet been investigated how saponins affect the expression of QS genes and QS-regulated virulence in pathogenic bacteria. This study aims to isolate and prepare saponin extracts from *Holothuria leucospilota* and investigate their impact on the expression of QS genes, specifically *ahyI/R*, as well as certain QS-associated traits in the fish pathogen *Aeromonas hydrophila*.

Material and methods

Sampling

Holothuria leucospilota specimens were collected using scuba diving techniques at depths of 10–30 m along the Qeshm coast, Bandar Abbas, Iran. Marine biology experts at the Faculty of Marine Biology, University of Hormozgan,Iran, identified the collected species. After collection, the specimens were transported to the laboratory and maintained in marine aquaria with circulating seawater (28 °C, salinity 31‰). The Cuvierian tubules were then dissected, preserved in 70% ethanol, and stored at – 20 °C until analysis.

Saponin extraction and purification

Following the procedures described in earlier research, saponin extraction and purification were carried out (Campagnuolo et al. 2001; Van Dyck et al. 2009, 2010b). Following two extractions using a 70:30 ethanol mixture, the homogenized tissue was filtered. After that, a rotary evaporator (Laborota 4001 efficient, Heidolph) was used to concentrate the filtrate at 30 °C under reduced pressure. The resulting dry extract was redissolved in 90% methanol and partitioned against n-hexane (v/v). After that, the methanol–water phase was partitioned against CCl_4 and $CHCl_3$, respectively, after being adjusted to 20% and 40% water content (v/v). For chromatographic purification, the last methanol–water mixture was evaporated and then reconstituted in water. The crude aqueous extract was applied to an Amberlite XAD-4 column (Sigma-Aldrich, St. Louis, MO). Elution with methanol was used to extract saponins after the column had been cleaned with water to get rid of inorganic salts. Following the evaporation of the methanolic eluate, the dry extract was reconstituted in water for additional partitioning against iso-butanol (v/v). This final partition yielded a butanolic fraction enriched with purified saponins extracted from sea cucumbers (SPS).

Bacterial growth condition

Aeromonas hydrophila MTCC 1739 was procured from the Iranian Biological Resource Centre IBRCIBRC in Tehran, Iran. The strain was regularly cultivated at 30 °C with rotary shaking at 120 rpm in Luria Bertani LBLB broth. A UV–visible spectrophotometer was used to standardize the bacterial culture's optical density (OD) to 0.4 at 600 nm. This adjustment was based on an overnight culture with a density of 1×10^5CFU/ml. The standardized cell suspension was used in all in vitro assays.

MIC and MBC measurements

One of the most important metrics for assessing an agent's antibacterial efficacy is its minimum inhibitory concentration (MIC) (Elshikh et al. 2016). Antibiotics are classified into bactericidal, which kill bacteria, and bacteriostatic, which inhibit bacterial growth. Bactericidal antibiotics are generally regarded as more potent than bacteriostatic ones (Gomes et al. 2020). The MIC is essential for determining the susceptibility of organisms to antimicrobials and is widely used in vitro to identify promising new treatments (Benkova et al. 2020). The MIC of saponins extracted from sea cucumbers (SPS) was determined using a serial dilution method in a 96-well microplate. The extracts were initially dissolved in DMSO to create a 200 µg/mL stock solution. Serial dilutions were prepared in Mueller–Hinton Broth with 0.5% Tween 80, ranging from 100 to 5 µg/mL, and tested against *A. hydrophila* at a concentration of 10^5 CFU/mL. Control wells included sterile Mueller–Hinton broth (sterile control), antibiotics (positive controls), and DMSO (negative control). The plates were incubated for 18–20 h at 30 °C in an aerobic environment. The lowest concentration that totally stopped bacterial growth after incubation was known as the minimum inhibitory concentration, or MIC. Furthermore, bacteriostatic and bactericidal activities were evaluated using the minimum bactericidal concentration (MBC) test (Elshikh et al. 2016). Bacteria from the MIC wells were cultivated on sterile Mueller–Hinton agar and incubated for 24 h at room temperature in order to assess bactericidal activity. Bacteriostatic activity was indicated by the presence of bacterial colonies, whereas bactericidal activity was confirmed by their absence.

Inhibition of QS

Aeromonas hydrophila was cultured at 30 °C for 24 h with and without sub-MIC levels (1/2, 1/3, and 1/4 MIC) of SPS. Cell-free culture supernatants (CFCS) were obtained by harvesting the cultures following incubation. After that, these supernatants were filtered through a 0.22 µm membrane filter (Millipore Corp. USA) for additional research.

β-Hemolysin quantification assay

Aeromonas hydrophila production of extracellular hemolysin was assessed using the Scheffer et al. method (1988). Briefly, 100 mL of *A. hydrophila* cell-free culture supernatant (CFCS), treated with sub-MICs of SPS (1/2, 1/3, and 1/4 MIC) and an untreated control, was mixed with 900 mL of phosphate-buffered saline (PBS; pH 7.4) containing 2% sheep erythrocytes. After 20 min of ice-based incubation, the mixtures were centrifuged. At 530 nm, the hemoglobin absorbance that was released into the supernatant was measured (Scheffer et al. 1988).

Protease quantification assay

An azocasein assay was used to measure the total proteolytic activity of *A. hydrophila*, in accordance with the procedure outlined by Ding et al. (2018). In brief, 75 µL of cell-free culture supernatant (CFCS) from *A. hydrophila* treated with sub-MICs of SPS (1/2, 1/3, and 1/4 MIC) and an untreated control were each mixed with 125 µL of a 0.3% azocasein solution in 50 mM Tris–HCl and 0.5 mM $CaCl_2$. After the mixtures were incubated for 15 min at 30 °C, 600 µL of 10% trichloroacetic acid was added to stop the reaction. The absorbance at 440 nm was measured after 700 µL of 1 M NaOH was added to the supernatant after centrifugation at 10,000 rpm for 10 min (Ding et al. 2018).

Lipase quantification assay

With p-nitrophenyl palmitate as the substrate, lipase production was measured quantitatively. In this experiment, 100 mL of cell-free culture supernatant (CFCS) from *A. hydrophila*, treated with sub-MICs of SPS (1/2, 1/3, and 1/4 MIC), and an untreated control, were mixed with 900 mL of substrate solution. The substrate solution contained 50 mM Na_2PO_4 buffer with 0.2 percent (w/v) sodium deoxycholate and 0.1 percent (w/v) gum arabic (9 parts, pH 8.0) and 0.3 percent (w/v) p-nitrophenyl palmitate in isopropanol (1 part). For one hour, the mixture was incubated at room temperature. To halt the reaction after incubation, 1 mL of 1 M sodium carbonate buffer was added. After centrifuging the mixture at 800 g, a spectrophotometer was used to measure the supernatant's absorbance at 410 nm (Srinivasan et al. 2020).

Swarming motility

Swarming agar plates were prepared with a medium consisting of 0.8% nutrient broth, 0.5% glucose, and 0.3% agar, adjusted to a pH of 7.2. Two microliters of an overnight culture of *A. hydrophila* were inoculated at the center of each plate, which contained sub-MICs of SPS at 1/2, 1/3, and 1/4 MIC concentrations. DMSO was used as a control. Each concentration was tested in triplicate. The plates were incubated at 30 °C for 24 h, and

Table 1 The primer sequences of genes in this study

Gene	Primers sequences and directions (5′-3′)	Tm (℃)	Product size (bp)	References
ahyI	TCT GGA GCA GGA CAG TTT CG ATG ATG CAG GTC AGT TCG CT	54	249	Patel et al. (2017), Tanhay Mangoudehi et al. (2020)
ahyR	TTT ACG GGT GAC CTG ATT GAG CCTGG ATG TCC AAC TAC ATCTT	54	206	Patel et al. (2017), Tanhay Mangoudehi et al. (2020)
16s rRNA	GCA CAA GCG GTG GAG CAT GTGG CGT GTG TAG CCC TGG TCG TA	55	299	Patel et al. (2017), Tanhay Mangoudehi et al. (2020)

the diameters of the swarming zones were subsequently measured (Sun et al. 2021).

Biofilm formation

A biofilm formation assay was performed using 96-well plates, based on the method described by Dong et al. (2021). An overnight culture of *A. hydrophila* was subcultured in fresh BHI medium to an OD600 of 1.0, then diluted 1:20 with fresh medium. This diluted culture was added to wells containing various sub-MIC concentrations of SPS (1/2, 1/3, and 1/4 MIC). The plates were incubated at 30 °C for 24 h without agitation. Fresh medium served as the negative control, and wells with a defined concentration of DMSO were used as the positive control. After confirming that all wells had reached the stationary phase, the medium and non-adherent bacteria were removed, and the wells were washed with PBS to remove residual planktonic cells. Biofilms were then stained with 0.5% crystal violet for 30 min, air-dried, and subsequently washed. The stain was solubilized with 30% glacial acetic acid, and biofilm formation was quantified by measuring the absorbance at 570 nm using a microplate reader (Dong et al. 2021).

Level of ahyI and ahyR transcript

An overnight culture of *A. hydrophila* was grown in LB broth supplemented with sub-MIC levels of SPS (1/2, 1/3, and 1/4 MIC). The cultures were incubated at 30 °C with shaking at 80 rpm for 18 h, reaching the late exponential phase (OD600 of 1.5). Centrifugation at 4500 × g for 10 min was used to extract the bacterial cells after incubation. Following the manufacturer's instructions, RNA was extracted using TriZol™ reagent (Thermo Fisher Scientific, USA) and treated with DNase I to eliminate any contaminants from the DNA. After that, cDNA synthesis was carried out according to the given protocol using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA). The master mix composition included 4 µL of 5 × reaction buffer, 1 µL RiboLock RT RNase Inhibitor, 2 µL of 1 mM dNTP mix, 0.2 µg/µL Random Hexamer Primers, and 1 µL reverse transcriptase, topped up to 20 µL with 12 µL nuclease-free water. The reaction conditions were as follows: initial denaturation at 64 °C for 4 min, annealing at 25 °C for 12 min, extension at 41 °C for 59 min, and a final elongation at 70 °C for 5 min. Quantitative polymerase chain reaction (qPCR) was conducted using a LightCycler real-time PCR system (Bio-Rad, California, USA), with the 16S rRNA gene serving as the internal control for data normalization. The reaction mixture consisted of 12 µL of 2X SYBR Green qPCR Master Mix (Fermentas Co., Germany), 1 µL each of forward and reverse primers (Table 1), 5 µL of nuclease-free water, and 1 µL of cDNA. Thermal cycling included an initial step at 50 °C for 3 min, followed by 95 °C for 12 min, and 45 cycles of denaturation at 94 °C for 20 s, annealing at 59 °C for 30 s, and extension at 72 °C for 40 s. Relative gene expression was quantified using the $2^{-\Delta\Delta CT}$ method (Pfaffl 2001).

Table 2 Effect of sub-MICs of saponins extracted from sea cucumbers (SPS) on inhibition of virulence factors in *A. hydrophila MTCC* 1739

SPS	Hemolysin activity (530 nm)	Protease activity (440 nm)	Lipase activity (410 nm)
Control (DMSO)	0.18 ± 0.02^{a}	0.93 ± 0.04^{a}	540.3 ± 11.30^{a}
15 µg/mL	0.10 ± 0.01^{b}	0.33 ± 0.01^{b}	311.1 ± 18.12^{b}
10 µg/mL	0.09 ± 0.03^{b}	0.61 ± 0.03^{c}	314.1 ± 15.10^{b}
7.5 µg/mL	0.10 ± 0.02^{b}	0.62 ± 0.02^{c}	316.1 ± 13.45^{b}

Different letters in each column indicate significant differences ($P < 0.05$) (Mean ± SD)

Statistical analyses

Three duplicates of each experiment were carried out, and the results were reported as mean ± SD. SPSS (IBM, Chicago, IL, USA) was used to conduct statistical analyses. SPSS (IBM, Chicago, IL, USA) was used to conduct statistical analyses.

Results

MIC and MBC

In this study, the MIC was employed to assess the antibacterial strength of saponins extracted from sea cucumbers. The MIC and MBC of SPS against *A. hydrophila* was determined to be 30 µg/mL and 80 µg/mL.

QS inhibition assay

Concentrations of 1/2 MIC (15 µg/mL), 1/3 MIC (10 µg/mL), and 1/4 MIC (7.5 µg/mL) of SPS significantly decreased the activity of hemolysin, protease, and lipase in the treatment groups compared to the control group ($P < 0.05$) (Table 2). Treatment with sub-MIC levels of SPS reduced hemolysin activity in *A. hydrophila* by 44%.

The greatest decrease in protease activity, 64.5%, was observed with 15 µg/mL SPS (Table 2). Lipase activity in *A. hydrophila* decreased by 41% after treatment with sub-MIC concentrations of SPS (Table 2).

Swarming motility

In the DMSO control, the swarming motility of *A. hydrophila* was evident (Fig. 1). However, with SPS supplementation at concentrations ranging from 7.5 to 15 µg/mL, the swarming motility of *A. hydrophila* was significantly repressed (Fig. 1). The greatest reduction in the swarming zone diameter, observed at 15 µg/mL, resulted in a 77% inhibition of motility ($P < 0.05$) (Fig. 1).

Inhibition of biofilm formation

As shown in Fig. 2, sub-MIC concentrations of SPS significantly inhibited biofilm formation ($P < 0.05$). The biofilm inhibition rates were approximately 87.1%, 33.8%, and 33.5% for bacteria treated with SPS at 15, 10, and 7.5 µg/mL, respectively.

ahyI and ahyR expression

The relative expression of ahyI and ahyR in A. hydrophila cultures treated with sub-MICs of SPS was compared to untreated cells (Fig. 3). The results indicated a significant reduction ($p < 0.05$) in the expression of both genes, with a more pronounced decline observed for ahyR. Specifically, exposure to 15 µg/mL of SPS significantly reduced ahyR expression more than any other treatment group (Fig. 3).

Discussion

QS is a mechanism by which bacteria modulate gene expression in response to population density. This regulatory process impacts several virulence factors, including extracellular enzyme production, biofilm formation, motility, and the activity of secretion systems. As a result, QS is a crucial determinant of bacterial pathogenicity. (Zhao et al. 2015). Given this, the inhibition of QS has emerged as a promising strategy for controlling bacterial pathogenicity. Research has explored numerous natural and synthetic compounds for their potential to inhibit QS (Asfour 2018). In this study, the quorum sensing (QS) inhibitory effects of saponins extracted from sea

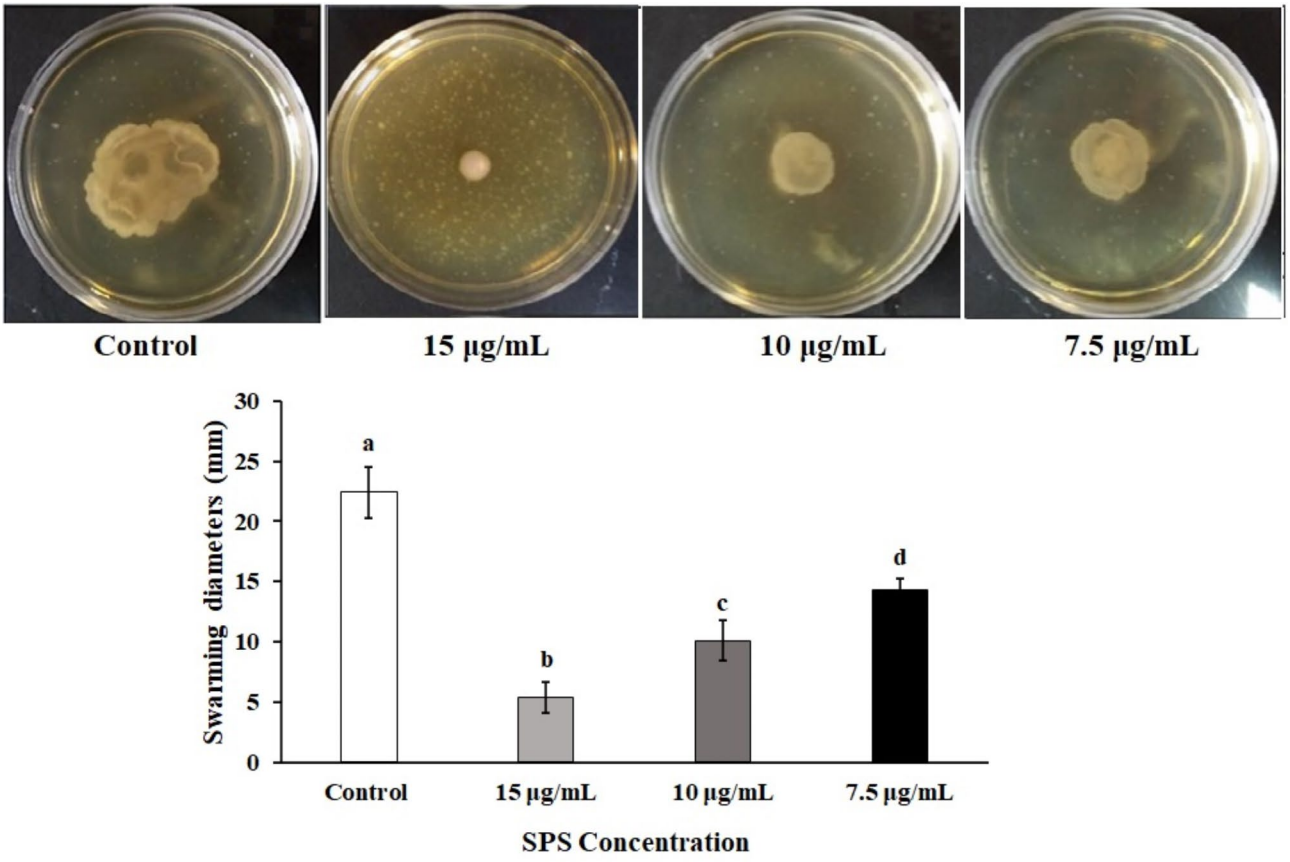

Fig. 1 Effect of sub-MICs of saponins extracted from sea cucumbers (SPS) on swarming motility of *A. hydrophila* MTCC 1739 (up). Swarming zone diameter(down). Data are presented as the swarming diameter of mean ± SD of three independent experiments. Different letters in each group indicate significant differences ($P < 0.05$)

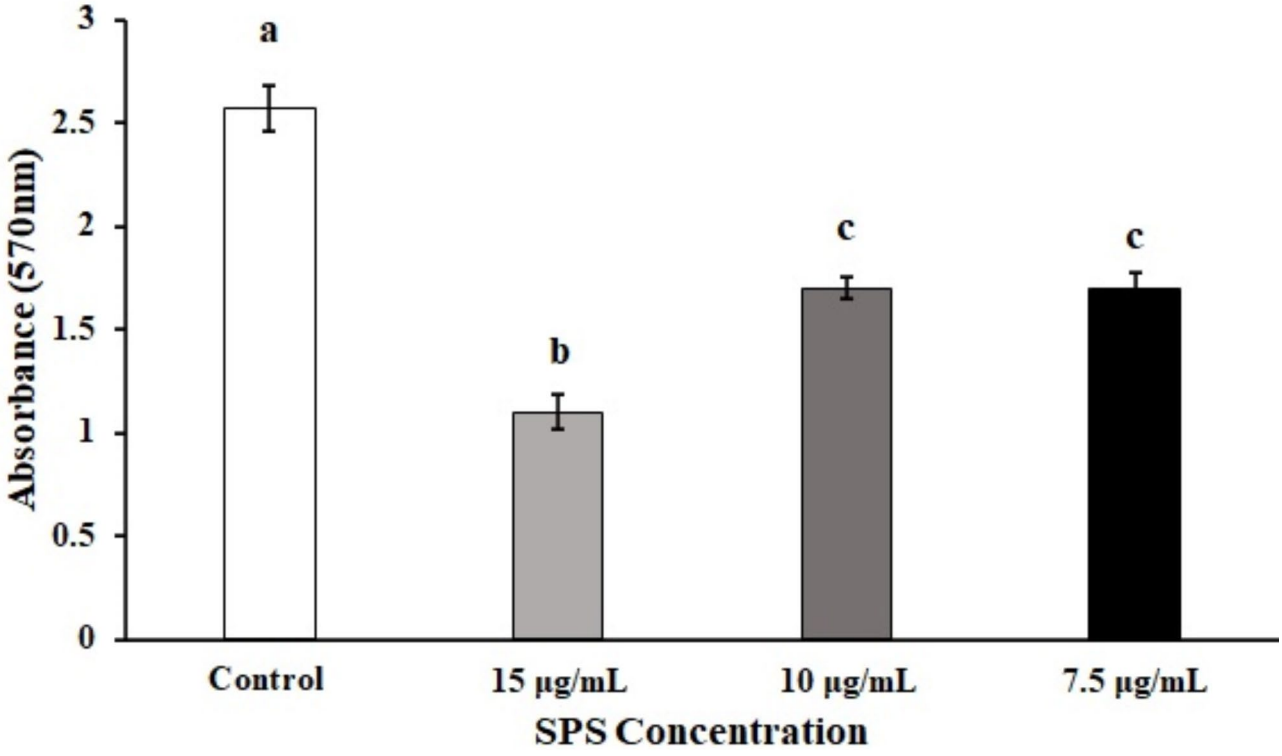

Fig. 2 Effect of sub-MICs of saponins extracted from sea cucumbers (SPS) on biofilm formation of *A. hydrophila* MTCC 1739. Data are presented as the swarming diameter of mean ± SD of three independent experiments. Different letters in each group indicate significant differences ($P < 0.05$)

cucumbers (SPS) on *A. hydrophila* were investigated. The effects of SPS on various pathogenic traits of the bacteria were evaluated, including hemolysin, protease, and lipase activities, as well as bacterial motility and biofilm formation. Additionally, the expression of the quorum sensing genes *ahyI* and *ahyR* in response to SPS treatment was assessed.

The antibacterial and antibiofilm properties of bioactive compounds extracted from sea cucumbers, including saponins, against various bacterial pathogens have been documented (Eissa et al. 2021; Kamyab et al. 2020a; Kartikaningsih et al. 2018; Salari et al. 2018). However, the anti-quorum sensing (QS) potential of saponins against *A. hydrophila* has not been previously investigated. This study demonstrates that saponin extracts (SPS) exhibit significant antibacterial activity against *A. hydrophila*, with a MIC of 80 µg/mL. Previous reports have confirmed that sea cucumbers contain saponins with antibacterial activity (Sukmiwati et al. 2018). Saponins, a subclass of terpenoids, inhibit or kill microbes by interacting with membrane sterols, leading to the release of proteins and enzymes from bacterial cells. Consequently, saponins are effective in inhibiting cell growth (Sukmiwati et al. 2020).

This study demonstrated that sub-MIC levels of SPS significantly inhibit hemolysin production by *A. hydrophila*. Hemolysin, a key virulence factor and exotoxin (also known as aerolysin) produced by the bacterium, is a single polypeptide molecule. This hemolytic activity, detectable both in vivo and in vitro, contributes to the pathogen's ability to induce bleeding in infected animals (Dooley and Trust 1988). Previous research has underscored the significance of aerolysin in *A. hydrophila* pathogenicity. Allan and Stevenson (1981) found that injection of this exotoxin caused disease in rainbow and speckled trout (Allan and Stevenson 1981). Additionally, Thune et al. (1986) identified β-hemolysin from a protease-deficient *A. hydrophila* strain, which was lethal to catfish (Thune et al. 1986). The aerolysin genes are highly conserved among Aeromonas species, underscoring their essential role in the bacterium's pathogenicity (Nam and Joh 2007).

Sub–MIC levels of SPS, particularly at 1/2 MIC, significantly inhibit protease activity in *A. hydrophila*. Extracellular proteases, which include metalloproteases and serine proteases, are key virulence factors in *A. hydrophila* and are prevalent within the bacterium (Weihua and Chengping 2000). In addition to their direct pathogenic effects, these proteases also trigger other virulence factors. For instance, the exotoxins secreted by *A. hydrophila* are initially in an inactive precursor form and require activation by extracellular proteases (Majeed et al. 2023).

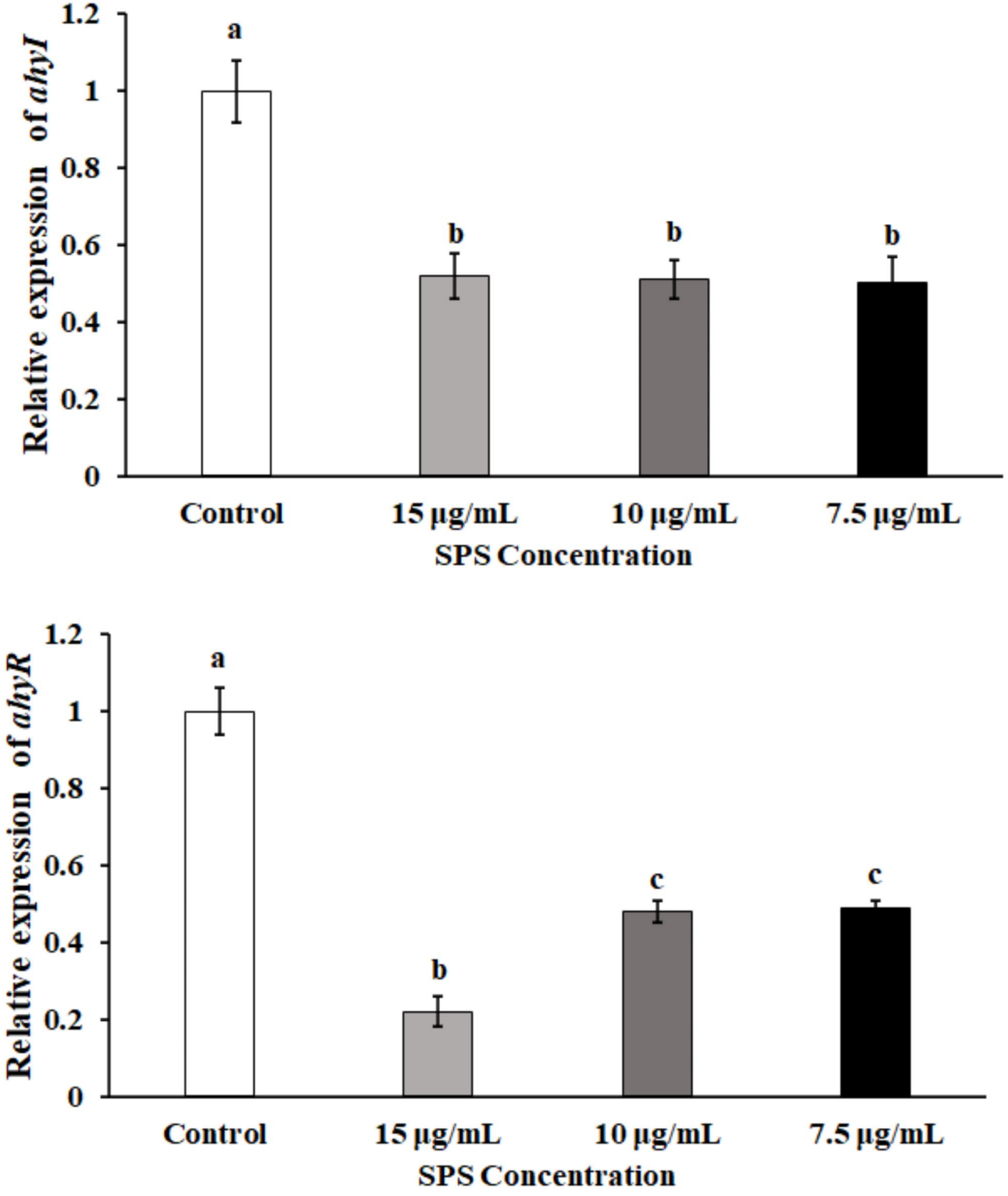

Fig. 3 Effect of sub-MICs of saponins extracted from sea cucumbers (SPS) on the expression of QS associated genes of *A. hydrophila* MTCC 1739. Data are presented as the swarming diameter of mean ± SD of three independent experiments. Different letters in each group indicate significant differences ($P < 0.05$)

The findings of this study indicate that reducing protease activity diminishes the pathogenicity of *A. hydrophila*. Specifically, the impaired activation of exotoxins, such as hemolysin, due to reduced protease activity further decreases the bacterium's pathogenic potential.

Sub-MIC levels of SPS significantly inhibited lipase activity, a key virulence factor in *A. hydrophila*. Lipase compromises the plasma membranes of host cells, while elastase contributes to diseases in both fish and humans (Ahangarzadeh et al. 2022). This reduction in virulence factor production highlights the effectiveness of SPS in inhibiting quorum sensing (QS) and mitigating bacterial pathogenicity.

Bacterial swarming motility, mediated by flagella and regulated by the QS system (Muduli et al. 2021; Sun et al. 2021), is crucial for pathogenicity and is believed to enhance biofilm formation (Li et al. 2023). In this study, the swarming motility of *A. hydrophila* was significantly inhibited by sub-MIC levels of SPS, indicating that SPS interferes with the QS system in the pathogen, thereby inhibiting its motility.

Biofilm formation in *A. hydrophila* was significantly inhibited by SPS at sub-MIC levels. A self-produced matrix of lipids, proteins, nucleic acids, and exopolysaccharides (EPS) surrounds bacterial cells in biofilms, which are microbial communities. This matrix can obstruct antimicrobial agents from penetrating the cells (Chu et al. 2011; Tanhay Mangoudehi et al. 2020), and is closely associated with multi-drug resistance (Kjelleberg and Molin 2002). Consequently, disrupting or inhibiting biofilm formation can effectively reduce bacterial pathogenicity and drug resistance (Kjelleberg and Molin 2002). Our study found that SPS significantly impaired biofilm formation in *A. hydrophila*, resulting in a looser and sparser biofilm. This effect likely stems from SPS interfering with the synthesis of biofilm matrix components, such as EPS.

The expression of the quorum-sensing associated genes, *ahyR* and *ahyI,* in *A. hydrophila* was notably reduced following exposure to sub-MIC of SPS. These genes are critical for synthesizing the AhyI protein during the exponential growth phase, a process regulated by the AHL/AhyR complex that upregulates *ahyI* gene expression (Kirke et al. 2004). During this phase, AhyR and AhyI engage in a feedback loop within the AI-1 system, resulting in auto-amplification known as "autoinduction." AHLs diffuse freely across the cellular membranes of *Aeromonas*, accumulating in the extracellular environment (Talagrand-Reboul et al. 2017). However, once the stationary phase is reached and external AHL concentrations exceed a certain threshold, this autoinduction is inhibited, and intercellular communication predominates, as demonstrated in *A. hydrophila* (Garde et al. 2010). The expression of *AhyI* is contingent upon the growth phase. The precise mechanisms by which saponins suppress *ahyI* and *ahyR* gene expression remain unclear, though they are presumed to involve either direct interactions with bacterial cell membranes or disturbances in growth process via signaling pathways (Bhattacharya et al. 2023). This impact of saponins on quorum sensing genes underscores their potential as natural antimicrobial agents or quorum sensing inhibitors, offering promising strategies for managing bacterial infections and mitigating antibiotic resistance.

The finding of this study demonstrates that saponins, at sub-MICs, significantly downregulate the expression of the QS genes *ahyI* and *ahyR*, which are crucial for the regulation of bacterial communication and pathogenicity in *A. hydrophila*. Consequently, the production of key virulence factors such as hemolysin, proteases, and lipases are diminished, and both swarming motility and biofilm formation are notably suppressed. These findings underscore the potential of natural compounds like saponins as effective quorum sensing inhibitors (QSIs) that can modulate bacterial behavior and pathogenicity. The use of such natural QSIs could represent a novel approach to combat bacterial infections, particularly in the context of increasing antibiotic resistance. The study contributes to the growing body of knowledge on the antimicrobial and anti-QS properties of marine-derived compounds and highlights the potential of sea cucumber saponins as a promising natural resource for the development of new therapeutics against bacterial pathogens.

Abbreviations

AQ	Quorum sensing
QSIs	Quorum sensing inhibitors
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
AHL	N-acyl-homoserine lactone
SPS	Saponins extracted from sea cucumbers

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Author contributions

B. P. and M.S. carried out the experiments and analyzed data. M.S.M designed experiments and reviewed the manuscript. H.R.I and M.N wrote the manuscript.

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Availability of data and materials

The data will be available if it is necessary.

Declarations

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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