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Impact of Algal Extract on Quorum Sensing and Biofilm Formation genes of Multidrug-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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Abstract

Nowadays, bacteria become resistant to different types of drugs posing a significant global health challenge; therefore, scientists are constantly searching for new alternatives to combat them. Algae have been recognized as a significant supply of biological components and have the capacity to function as valuable resources in the fight against bacterial infections. This investigation assessed the activities of two types of blue-green algae against multidrug resistant pathogenic *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The efficacy of three solvents (water, diethyl ether and acetone) utilized in the extraction of two algae, *Chlorella vulgaris* and *Spirulina platensis*, was tested to show the effectiveness on both bacterial virulence and quorum sensing genes on the level of expression. Results emphasized that acetone and diethyl ether fractions of *Chlorella vulgaris* were effective at 25mg/ml. However, both tested pathogenic bacteria showed more resistance against *Spirulina platensis* fractions. Biofilm formation by *Staphylococcus aureus* was reduced significantly by diethyl ether fraction of *Chlorella vulgaris, w*hile the reduction of formation of biofilm in *Pseudomonas aeruginosa* was more efficient by *Spirulina platensis* acetone fraction. *MvfR* and *ndvF* genes of *Pseudomonas aeruginosa* downregulated when treated with diethyl ether fraction of both *Chlorella vulgaris* and *Spirulina platensis*. Interestingly, all algal extracts showed a remarkable effect on *icaC, hla* and *RNAIII* genes of *Staphylococcus aureus.* According to the findings, algal extracts can be utilized to interrupt growth and encourage the creation of biofilms and pathogenicity, downregulation of QS-related and biofilm-associated genes in MDR strains.

Key words: algal extract, antibiotic resistance, quorum sensing, virulence.

1. Introduction

Antibiotics were introduced in the last century, and they are considered as one of the most significant medical innovations ever (Ghosh *et al*., 2020). Antibiotics, which were discovered more than seventy years ago, saved countless lives by treating bacterial diseases that were previously fatal (Uddin *et al.*, 2021, Laws et al., 2019, Ventola, 2015). Antibiotic resistance has developed due to the overuse of antimicrobials for an extended period of time as well as the excessive use of these medicines, inadequate infection control measures, and a lack of innovation in pharmaceutical development research (Aslam *et al*., 2021, Ventola, 2015). In 2019, the agency World Health Organization (WHO) released a report stating that antimicrobial resistance was responsible for a minimum of at least 700,000 deaths worldwide annually. If this current situation is not addressed, it is predicted that by 2050, the number would increase to 10 million (Nji et al., 2021). The proliferation and dissemination of drug-resistant pathogens in countries with low to middle- income levels may have detrimental consequences for public health (Van Boeckel *et al*., 2019). Given that over 70% of pathogenic bacteria exhibit resistance to at least one kind of antibiotic, it is imperative to develop innovative techniques to tackle this worldwide problem (Laws *et al*., 2019 , Uddin *et al*., 2021). Reducing the harmful effects of bacteria by focusing on the quorum sensing (QS) offers a possible alternative for inhibiting or suppressing the proliferation of disease-causing bacteria (Tang *et al*., 2020). QS is a mechanism by which microorganisms communicate with each other through the activation of multiple genes, primarily those that are involved in biofilm formation and virulence factors synthesis. The control of this process relies on the formation and detection auto-inducers, which are signals that depend on population density. Further, as a possible new treatment approach for bacterial infections, "quorum quenching" is being studied. This term describes the interruption of signaling in several biological processes (Fleitas Martínez *et al*., 2019). Several plants exhibited a quorum quencher behaviors against pathogenic bacteria, for instance thyme and cinnamon (Ahmed *et al.* , 2023). Furthermore, recently nanoparticles have also been

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reported to work as anti -bacterial QS agents as conveyed by Hasan and Ahmed (2023).

Microbial biofilm development, swarming, and maturity are all dependent on the QS system. During the formation of a biofilm, cellular communication initiates as chemical signaling reaches a particular concentration threshold. The chemical signals stimulate the production of substances outside the polymer, which triggers the activation of genes responsible for virulence and infectiousness. Biofilms consist of a wide range of bacteria that adhere to surfaces, generate an extracellular matrix using polymeric substances, and become embedded within it. The QS behavior of biofilm-linked bacteria contributes to multidrug resistance (Boominathan *et al*., 2022). Algae are nonvascular, photosynthetic plants that inhabit a variety of aquatic environments, their bioactive compounds possess antimicrobial activity (Toma and Aziz, 2023). Both *Chlorella vulgaris* and *Spirulina platensi*s are unicellular, freshwater organisms that contain bioactive substances like proteins, vitamins, sterols, pigments, long-chain polyunsaturated fatty acids, and other substances (Hussein *et al*., 2018). Methicillin-resistant *Staphylococcus aureus* (MRSA) is the most prevalent pathogen in humans. It forms biofilms which significantly increase the risk of morbidity and mortality from infections acquired in hospitals and communities. *S. aureus*, an opportunistic pathogen, might potentially lead to a variety of diseases, such as endocarditis, acute respiratory infection, septicemia, osteomyelitis, and skin and soft tissue lesions. The majority of the pathogenicity-related gene regulation in *S. aureus* occurs via the action of small RNA and RNAIII, which are part of the agr quorum-sensing system (Huntzinger *et al*., 2005). RNAIII is a significant small regulatory RNAs (sRNAs) which regulates the expression of genes by binding to target mRNAs in an antisense manner (Gupta *et al*., 2015). *S. aureus* produces additional virulence factors, including exotoxins like alpha-hemolysin (also referred to as α-toxin) that is directed by *hyl* gene (Chen *et al*., 2015). The establishment of biofilm by pathogens is the primary determinant in the long-lasting nature of chronic infection (Guilhen *et al.*, 2017). The major obstacle to effectively treating *S aureus* infections is the formation of biofilm, which highlights the bacteria's resistance (Lee *et al*., 2020). The production of polysaccharide intercellular adhesin (PIA) and an export protein, known as *IcaC*, regulate biofilm development in *S. aureus*. The production of PIA is facilitated by proteins that are encoded by the ica ADBC operon, which is located within the ica locus *(Cramton et al*., 1999). *Pseudomonas aeruginosa*, an opportunistic pathogen, can grow biofilms on a wide range of substrates, allowing bacteria to adhere to and proliferate on medical equipment. The high rate of morbidity and mortality among hospitalized patients is attributed to this factor (Lalancette *et al.*, 2017, Maurice *et al*., 2018). The generation of biofilms is influenced by multiple genetic mechanisms, but not in the growth of planktonic cells during the exponential phase paraphrase the second part after the comma with linking it with this paraphrased first part, though these same mechanisms do not affect the growth of planktonic cells during the exponential phase. The rise of biofilm resistance in *P. aeruginosa* is facilitated by these mechanisms as well. The gene ndvB is essential for one of these pathways through an unidentified mechanism (Beaudoin *et al*., 2012). The virulence factor transcriptional regulator MvfR, encoded by *mvfR*, is essential for full virulence in *P. aeruginosa*. It facilitates the production of 4-hydroxy-2-alkylquinolines (HAQs), including the quinolone signal, PQS (pseudomonas quinolone signal), and regulates several QS-controlled virulence factors (Déziel *et al*., 2005, Xiao *et al*., 2006). *MvfR* represents a valuable target for treating the majority of infections associated with *P. aeruginosa*. This is because it regulates the virulence functions that are crucial for both acute and long-term infections (Kitao *et al*., 2018, Cao *et al*., 2001). On top of that, it produces phenazines, which are redox-active pigments that have the ability to change antibiotic susceptibility through their effects on metabolic flux, redox balancing, and gene expression (Price-Whelan *et al*., 2007). The research aims to assess the influence of algal extracts on the expression of QS, virulence and biofilm-related genes in pathogenic bacteria that are resistant to multiple drugs.

2. Materials and techniques

2.1. Algal gathering and identification

In the current study, samples taken from springs in the Shaqlawa district (Aquban and Sarkand villages), which are positioned 32 kilometers to the northwest of Erbil city, were found to contain *Spirulina platensis* and *Chlorella vulgaris*. The taxonomic determination of both algal species was established by analyzing their morphological characteristics using several scientific keys for algal identification (Brook *et al*., 2011, Wehr *et al*., 2015).

2.2. Algae species isolation

The necrotic sections of algae samples were excised. The algae samples were separated and purified using the plate technique method. This method involves placing a tiny bit of the diluted microbial mixture into the middle pf a petri dish. While the petri dish is being spun, a sterile L-shaped bent glass rod is used to spread it evenly over the surface. As the procedure progresses, the curved glass rod deposits individual cells onto the agar surface. After that, a glass container was used to incubate the algae sample. Algal growth was achieved using BG-11(HI Media Laboratories, PvtLtd-India) medium in a glass container with the medium volume of 5 liters. Subsequently, the specimens were subjected to a 14-day incubation period at a temperature of 25 ± 2°C. During this period, they were exposed to a light intensity ranging from 3000 to 5000 lux for 16 hours in the light and 8 hours in the dark. The pH level during the incubation was maintained at 8.2. In order to obtain algae species that are free from impurities, this procedure was carried out more than once. After that, the resultant product was placed in an incubator with 25 ml of BG-11 medium and left to incubate for 14 days under identical conditions in order to generate an algal inoculum. The moss was isolated using streak plating method (Hussein *et al*., 2018).

2.3. Preparation of biomass and harvesting

A 100 ml beaker containing BG-11 medium was supplemented with 25 ml of isolated algae, which were then incubated for 14 days under the same conditions as previously. Following that, the culture medium was moved to a 500 ml glass beaker containing 100 ml of BG-11

medium. After that, it was kept for an additional 14 days during incubation. These procedures were repeated until the algae growth in the container, which was covered with cotton swabs, reached a volume of 4 liters. At this stage, rubber was utilized to provide air (Richmond, 2008). The algae mass was collected on day 20 by centrifuging it for ten minutes at 4000 rpm (Elnabris *et al*., 2013). Afterward, the algae samples were cleaned in sterile water and dried in an oven maintained at a temperature range of 38 to 40°C. Weighed samples of algae were then stored in a refrigerator (Hassan *et al*., 2020).

2.4. Bacterial isolates and sources

A total of 20 clinical strains of *P. aeruginosa* and *S. aureus* were gathered from various microbiology laboratories in the hospitals of Erbil city, located in Kurdistan, Iraq. The clinical isolates of *S. aureus* were re-cultivated on Mannitol salt agar and Blood agar (Merck, Germany), while *P. aeruginosa* was cultured on Cetrimide agar (acumedia, Germany). Plates were incubated aerobically overnight at 37ºC. In order to identify the individual colonies, a variety of biochemical and conventional diagnostic assays were conducted using previously established standard methods: catalase, coagulase, DNase and hemolysin tests on blood agar for identifying *S. aureus* and oxidase, catalase, motility and pigment production assays for identifying *P. aeruginosa* (Tille, 2021), The bacterial isolates were verified using the automated Vitek 2 system (BioMérieux, France) and kept at 4°C and 70°C in nutrient broth with 25% glycerol for future research purposes. The method of disc diffusion was utilized to determine the antibiotic sensitivity of strains of bacteria, this method was uesd in accordance with established guidelines set by the Laboratory and Clinical Sciences Institute (CLSI) (CLSI, 2022). Ciprofloxacin (5µg), ceftaroline (30µg), chloramphenicol (30µg), clindamycin (10µg), gentamicin (10µg), linezolid (10µg), oxacillin (30µg), penicillin (10µg), rifampicin (15µg) and trimethoprim/sulfamethoxazole (20µg) from Oxoid/UK company were used for *S. aureus*. Amikacin (30µg), cefepime (10µg), ceftazidime (10µg), ciprofloxacin (5µg), colistin (15µg), imipenem (30µg), meropenem (30µg), piperacillin (10µg) and tobramycin (30µg) from Oxoid/UK company were used for *P. aeruginosa*. The isolates with the highest resistance were chosen to evaluate inhibitory capacity of the algal extracts. Two independent samples were examined at various time points.

2.5. Algal extracts preparation

An 8-hour soxhlet extraction was performed using 300 ml each of water, diethyl ether and acetone to extract approximately 30 grams of finely ground powder. A duration of approximately three days of incubation at a temperature of 37°C is required to facilitate the evaporation of the extracted products. The extracts were dissolved in dimethyl sulfoxide (DMSO). A volume of 5 ml of DMSO was used to dissolve 1 g of each extract, resulting in a stock solution with a concentration of 200 mg/ml. The samples were then stored at -4°C until they were used (Pina-Pérez *et al.* , 2017).

2.6. Minimum inhibitory and sub-inhibitory concentrations

The algal extracts were tested against the clinical specimens of *P. aeruginosa* and *S. aureus* using broth microdilution method to determine the minimum inhibitory concentration (MIC) of the algal extracts (Wiegand *et al*., 2008). A 96-well polystyrene microtiter plate (MTP) was filled with 100 µL of nutrient broth (NB) containing extracts at concentrations ranging from 0 to 50 mg/ml, and 10 µl of stationary phase cells with an OD550 of 0.5. At 37°C, the aerobic cultures were incubated for one day. The MIC was established as the minimum detectable concentration. The MIC was established as the minimum detectable concentration. The evaluation of anti-biofilm and anti-virulence characteristics within bacterial isolates involved considering concentrations below the minimum inhibitory concentrations (MICs), known as sub-inhibitory concentrations (SICs). Three biological samples were analyzed at different occasions.

2.7. Sub-MIC impact of algae extracts on bacterial strains' biofilm

The impact of algal extract on the production of biofilm was evaluated using polyvinyl chloride biofilm formation assay. Briefly, 24-hour cultures of the isolates of *P.aeruginosa* and *S. aureus* were re-suspended in new and fresh NB medium and cultivated at 37 °C for a full day in a static environment, both with and without SICs of the algal extract. After removing the liquid cultures from the plate wells, PBS was used three times to rinse the wells. A solution of 1% crystal violet dye was added to the biofilm, and then the extra dye was washed away with distilled water. The dye was subsequently dissolved with ethanol. The binding affinity to an abiotic surface was measured using an Elisa reader (Epson, Biotek, UK) at a wavelength of 490 nm (Ismaeil & Saleh, 2019). The standard error was calculated after three biological specimens were subjected to separate analysis.

2.8. Extracting the RNA and quantifying of QS and biofilm-associated genes

Algal extracts at SIC values were assessed using real-time polymerase chain reaction (PCR) for their impact on the expression levels of the *icaC, hla,* and *RNAIII* genes of *S. aureus*, and the *ndvr, phzf,* and *mvfr* genes of *P. aeruginosa.* The manufacturer's instructions were followed to extract total RNA from bacteria exposed to the plant extract as well as from untreated bacteria that were utilized as a control (Total RNA Kit, Favorgen Biotech, Taiwan). The AddScript cDNA synthesis kit was used to reverse transcribe the isolated RNA into cDNA in accordance with the manufacturer's instructions. RealQ Plus 2x Master Mix Green (Ampliqon, Denmark) was used to produce RT-PCR reactions in the PCRmax Eco 48 RT-PCR device. Candidate genes were analyzed by qPCR using the primer sequences listed in table (1), and the results were estimated using ΔCt technique (Livak and Schmittgen, 2001).

Table 1. A list containing the genes and the corresponding primer sequences

Gene	Primer Sequence (5′–3′)		
	Frontward	Reversal	Ref.
ndvr	GGCCTGAACATCTTCTTCACC	GATCTTGCCGACCTTGAAGAC	(Ismail and Altaai, 2021)
Phzf	AACTCCTCGCCGTAGAAC	ATAATTCGAATCTTGCTGCT	(Nowroozi *et al*., 2012)
mvfr	GTTTCGACGAATGCTCGGTTG	GACAAGGTGCTCTTCGTGGA	(Faisal *et al*., 2020)
RNAIII	TTTATCTTAATTAAGGAAGGAGTGA	TGAATTTGTTCACTGTGTCG	(Bezar *et al.*, 2019)
hla	GTACAGTTGCAACTACCTGA	CCGCCAATTTTTCCTGTATC	(Bezar *et al*., 2019)
icaC	CATGAAAATATGGAGGGTGG	TCAAACTGATTTCGCCCACCG	(Gowrishankar *et al*., 2016)

2.9. Statistical Analysis

The obtained data were analysed using Graph-pad Prism 8.0 software. One-way analysis of variance (ANOVA) method was employed to conduct multiple comparisons. The data are presented as mean±SE.

3. Results

The effect of algal extracts on MDR pathogens was determined by measuring the MIC using the MTP method. The results demonstrated that the most effective fraction was *Chlorella vulgaris* extracted by acetone against *S. aureus*, *showing* an MIC of 25 mg/ml (Table 1). In contrast, the most potent extract against *P. aeruginosa* was the diethyl ether fraction of *Chlorella vulgaris*, with an MIC of 25 mg/ml (Table 2).

Table 1. Minimum Inhibitory Concentrations (MICs) and Sub-MICs of *Chlorella vulgaris* and *Spirulina platensis* extracts against *Staphylococcus aureus*

Algal extracts	MIC(mg/ml)	SIC(mg/ml)
Ch-1	40	30
Ch-2	30	20
Ch-3	25	15
S-1	40	30
S-2	30	15
S-3	35	20

Ch-1; aqueous, *Ch-2*; diethyl ether, *Ch-3*; acetone extract of *Chlorella vulgaris, S-1*; aqueous, *S-2*; diethyl ether, *S-3*; acetone extract of *Spirulina platensis*

Table 2. Minimum Inhibitory Concentrations (MICs) and Sub-MICs of Chlorella vulgaris and *Spirulina platensis* extracts against *Pseudomonas aeruginosa*

Algal extracts	MIC(mg/ml)	SIC(mg/ml)
Ch-1	45	30
Ch-2	25	15
Ch-3	30	20
S-1	45	30
S-2	35	25
S-3	40	30

Ch-1; aqueous, *Ch-2*; diethyl ether, *Ch-3*; acetone extract of *Chlorella vulgaris, S-1*; aqueous, *S-2*; diethyl ether, *S-3*; acetone extract of *Spirulina platensis*According to our data, all fractions of *Chlorella vulgaris* demonstrated a significant decrease in the amount of biofilm in both tested bacteria (*S. aureus* & *P. aeruginosa*), as shown in figures (1 & 2).

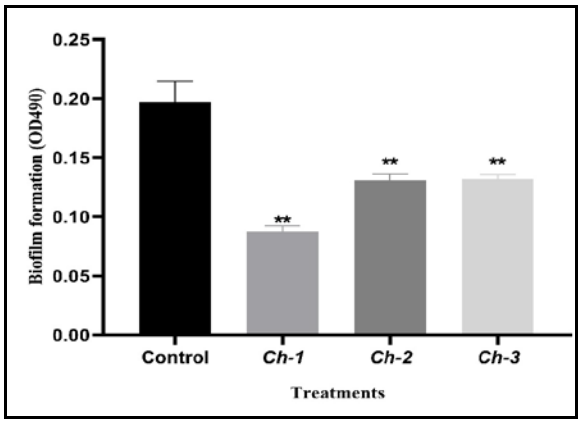

Figure 1. An enumerative analysis of the reduction in *S. aureus* biofilm caused by SICs of algal extracts, measured by absorbance at 490 nm. Data are presented as mean±SE. **Significance at P≤ 0.01. Ch-1; diethylether, Ch-2; acetone, Ch-3; aqueous extract of Chlorella vulgaris.

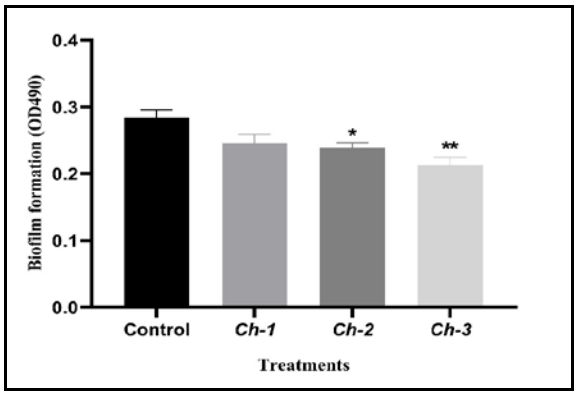

Figure 2. Quantitative assessment of *P. aeruginosa* biofilm suppression using SIC of algal extracts, measured by absorbance at 490 nm. Data are presented as mean±SE. *Significance at P≤ 0.05, **Significance at P≤ 0.01. Ch-1; diethylether, Ch-2; acetone, Ch-3; aqueous extract of Chlorella vulgaris.Furthermore, the fractions of *Spirulina platensis* exhibited a significant decrease in developing a biofilm in both tested bacteria (Figures 3 & 4).

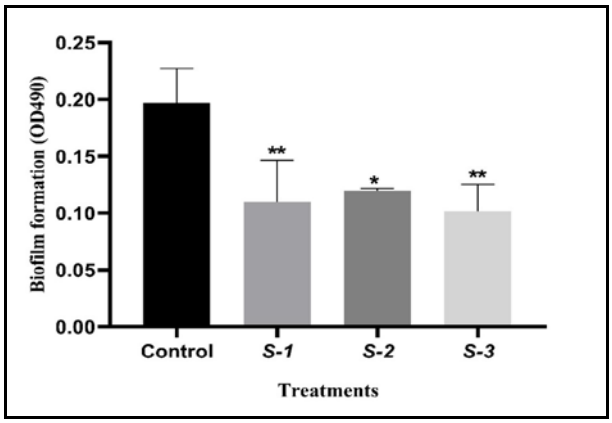

Figure 3. Quantitative assessment of *S. aureus* biofilm suppression using SIC of *algal* extracts, measured by absorbance at 490 nm. Data are presented as mean±SE. *Significance at P≤ 0.05, **Significance at *P*≤ 0.01. *S-1*; diethylether, *S-2*; acetone, *S-3*; aqueous extract of *Spirulina platensis*

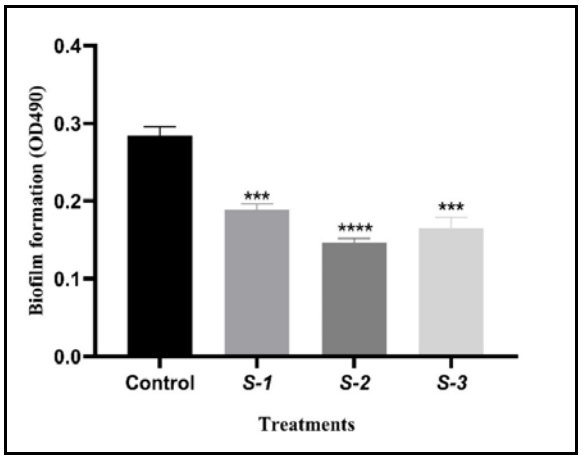

Figure 4. Quantitative assessment of *P. aeruginosa* biofilm suppression using SIC of *algal* extracts, measured by absorbance at 490 nm. Data are presented as mean±SE. ***Significance at P≤ 0.001, ****Significance at *P*≤ 0.0001. *S-1*; diethylether, *S-2*; acetone, *S-3*; aqueous extract of *Spirulina platensis*.The gene expression results showed that fractions of *Chlorella vulgaris* had different impacts on the downregulation of biofilm and QS genes in *P. aeruginosa & S. aureus.* This is illustrated in figures (5 & 6), with a particular impact observed on the *MvfR* gene in *P. aeruginosa* and *icac, hla & RNAIII* genes in *S. aureus.* Moreover, the fractions of *Spirulina platensis* suppressed the expression of genes associated with both biofilm formation and QS, specifically the *ndvF* gene in *P. aeruginosa* and the *icaC and hla* genes in *S. aureus* (Figures 7 &8).

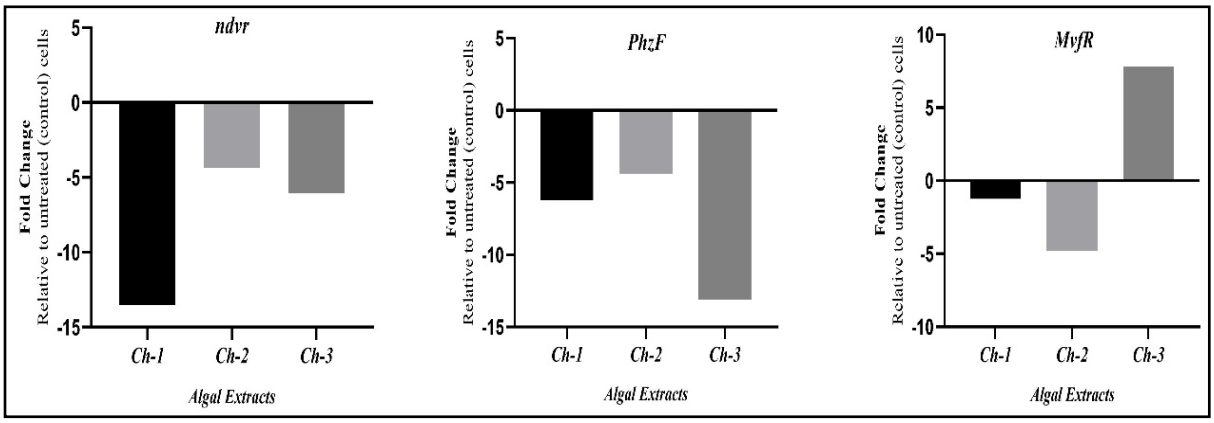

Figure 5. Transcriptional profiles of ndvF, mvfR and phzF genes in *P. aeruginosa* strains after exposure to Chlorella vulgaris SICs extracts. RT-PCR was utilized to calculate the transcriptional profiles. Ch-1; diethylether; Ch-2; acetone; Ch-3; Chlorella vulgaris aqueous extract.

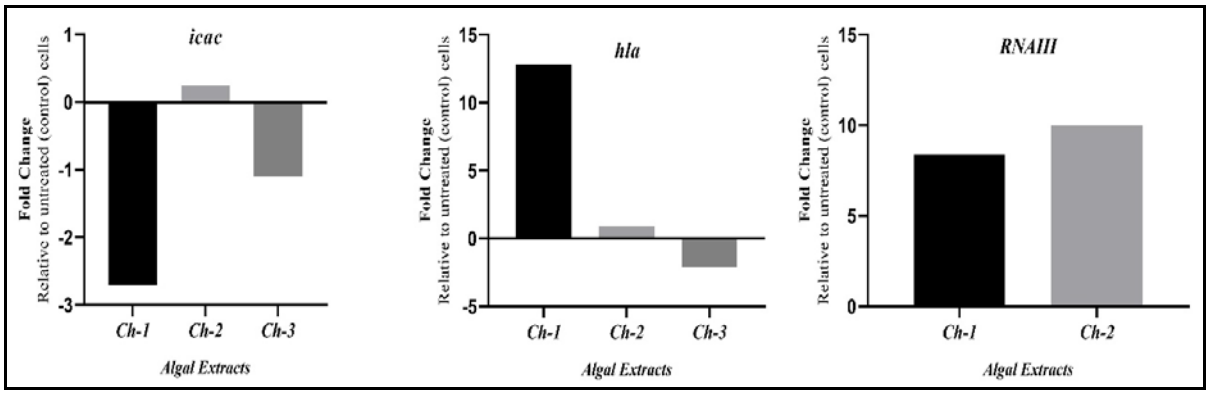

Figure 6. Transcriptional patterns of *S. aureus* isolates treated with SICs of *Chlorella vulgaris* extracts in terms of *icaC, hla*, and *RNAIII* gene expression. By using RT-PCR, transcriptional profiles were quantified. Ch-1; diethylether; Ch-2; acetone; Ch-3; *Chlorella vulgaris* aqueous
extract.

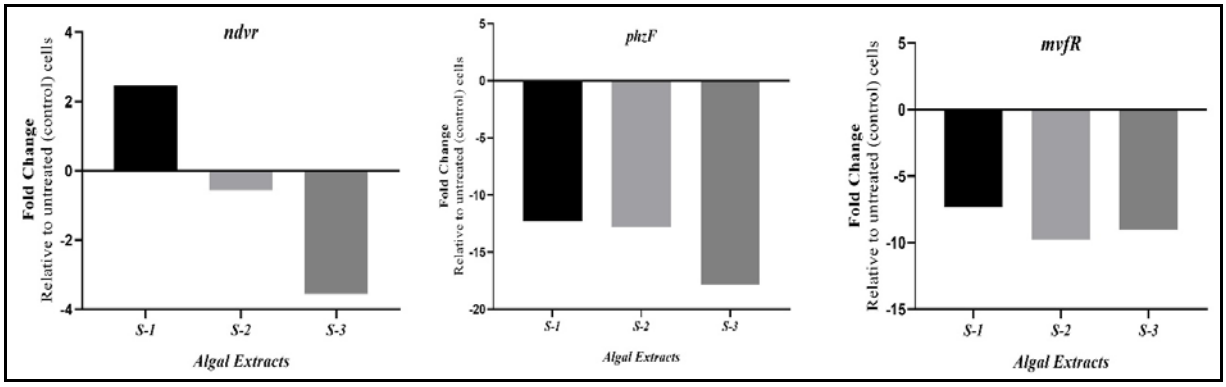

Figure 7. Transcriptional patterns of *P. aeruginosa* isolates treated with *Spirulina platensis* SICs for the *ndvF*, *mvfR*, and *phzF* genes. By using RT-PCR, transcriptional profiles were quantified. S-1; diethylether; S-2; acetone; S-3; *Spirulina platensis* aqueous extract.

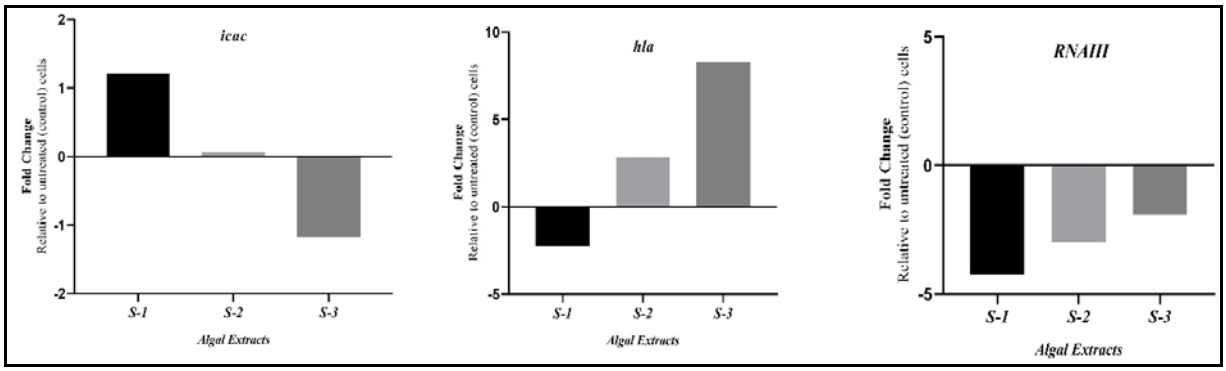

Figure 8. Transcriptional patterns of *S. aureus* isolates treated with *Spirulina platensis* extract-derived SICs for the *icaC, hla*, and *RNAIII* genes. By using RT-PCR, transcriptional profiles were quantified. S-1; diethylether; S-2; acetone; S-3; *Spirulina platensis* aqueous extract.

4. Discussion

Drug resistance is currently recognized as a serious issue that threatens not just worldwide health but food security and development as well (Nji *et al.*, 2021). *S. aureus* and *P. aeruginosa* are both antibiotic-resistant bacteria that generate concerns for human health. (Harding *et al*., 2018).

It has been proven that natural products possess a more potent effect on the pathogenicity of bacterial infections compared to traditional bacteriostatic or bactericidal medications. As a result, new methods for treating bacterial infections were developed. In order to combat diseases that do not respond to standard therapies, it is worth investigating the direct impact on the genes that control virulence activation in a more direct manner (Jiang *et al.*, 2019). Instead of focusing on bactericidal and bacteriostatic methods, researchers have begun to investigate whether QS inhibitory techniques can reduce bacterial pathogenicity in the face of multidrug-resistant bacteria (Yang *et al*., 2015).

One can effectively reduce the factors that contribute to bacterial infections by using a method that involves regulating bacterial QS signaling with QS-targeted substances. In recent times, there has been an increasing curiosity in a unique treatment that does not rely on antibiotics. This treatment has gained attention due to its capacity to prevent infection, inhibit the expression of genes linked to pathogens and reduce the likelihood of bacterial cells developing drug resistance (Jiang *et al*., 2019).

The search for remedies that are natural using innovative methods to prevent and/or treat life-threatening diseases maybe driven by the maritime environment, which is abundant in biodiversity (Guzzo *et al*., 2020). It is believed that marine organisms like algae could provide a variety of bioactive chemicals that could be used to combat the growth of antibiotic-resistant bacteria and inhibit biofilm formation.

Consequently, the objective of this study was to evaluate the antibacterial efficacy of two distinct types of algae, such as *Spirulina platensis* and *Chlorella vulgaris*, and to determine how they affect the virulence and QS-related genes at the level of expression. Results showed that the best MICs of extracts from *Chlorella vulgaris* and *Spirulina platensis* against *S. aureus* were 25 mg/ml and 30 mg/ml, respectively, for acetone and diethyl ether extracts (Table 1). The highest MICs for each of *Chlorella vulgaris* and *Spirulina platensis* extracts against *P. aeruginosa* were observed with diethyl ether (25mg/l and 35mg/l, respectively) (Table 2). The antibacterial properties of medications derived from these algae species offer some unique benefits in preventing bacterial growth, thereby enhancing vector infection control without any adverse effects (Syed *et al*., 2015). Many researchers have deduced the effective antimicrobial action of blue-green algae extracts against different pathogenic bacteria; most compounds derived from these genera are expected to be considered as antimicrobials for medical use, as they are safe for human health and the environment in which they live *(Marrez et al*., 2021). In addition, the data of this research demonstrate that the inhibitory action of *Chlorella vulgaris* extracts was much greater than that of *Spirulina platensis* extracts. This could be due to variations in the concentration and purity of the chemical substances found in each, as was demonstrated by previous research (Alghanmi and Omran, 2020).

Globally, a wide range of pathogenic bacteria present in the environment and possess the ability to form biofilms. Biofilms are physiological states where bacteria attach to a substrate by the formation of a DNA-, polysaccharide-, and protein-based extracellular matrix (Tsuneda *et al*., 2003). Hence, the development of bacterial biofilms is regarded as critical determinant in the pathogenicity of infections, since these biofilms provide comprehensive shielding from both the host immune system and the desired impact of therapeutic drugs (Vestby *et al*., 2020). The findings of reduced biofilm formation with extracts from both *Chlorella vulgaris* and *Spirulina platensis* against both bacterial strains are promising. With an 86% reduction in biofilm formation for *S. aureus* and an 81% reduction for *P. aeruginosa*, the acetone extract of *C. vulgaris* demonstrated the highest efficacy among the extracts. Consistent with previous studies that examined algal extracts against these bacteria, these results indicate that they are effective. An example of this is the 79% and 82% reduction in biofilm formation for *P. aeruginosa* and *S. aureus*, respectively, that was observed in a study using the methanol extract of *C. vulgaris* (Afzal *et al*., 2023). Another study revealed that the ethyl acetate extract derived from *Spirulina* sp. exhibited a significant reduction of 85% in biofilm formation by *S. aureus* and 80% by *P. aeruginosa* (Monteiro *et al*., 2021).

The study evaluated how algal extracts affect the expression levels of genes associated with biofilm development and QS, processes regulated by various signaling molecules in bacteria. The data showed that the extracts decreased the expression of genes associated with these processes in both bacterial species. Specifically, genes such as *MvfR* and ndvr in *P. aeruginosa*, and *icac*, *hla*, and *RNAIII* in *S. aureus*, which encode proteins responsible for regulating the production of virulence factors like exopolysaccharides, pyocyanin, rhamnolipids, intercellular adhesion, hemolysin, and toxins, were found to be downregulated by the algal extracts (Asimakis *et al.*, 2022). The downregulation of these genes suggests that the algal extracts interfere with the signaling pathways that regulates the formation of biofilm and QS in bacteria, hence diminishing their capacity to induce infections and withstand the effects of antibiotics. Previous studies have shown that the methanol extract derived from the marine algae *Asparagopsis taxiformis* had both quorum quenching and antibacterial properties against *Serratia liquefaciens* (Jha *et al*., 2013). The lichen secondary metabolite evernic acid reduces *P. aeruginosa* virulence protein production by blocking the QS system (Gökalsın and Sesal, 2016). Quorum sensing inhibitors (QSIs) from plant-derived natural remedies that affected *P. aeruginosa* included sappanol, butein, and catechin-7-xyloside (C7X). These compounds demonstrated the ability to interact with QS regulators in *P. aeruginosa* (Zhong *et al*., 2020).

According to the results of this study, the biologically active substances derived from *Chlorella vulgaris* and *Spirulina platensis* exhibited antibacterial properties against both gram- negative and gram-positive bacteria (Hussein *et al*., 2018). Syed *et al*. (2015) reported that *Chlorella vulgaris* extracts contain bioactive materials like flavonoids, tannins, terpenoids, phenolic compounds, cardiac glycosides and saponins. Our findings align with those of Hussein *et al*., (2017) and Hussein *et al*., (2018).

The mechanisms underlying the antimicrobial, anti-biofilm and gene regulation effects of algal extracts can be multifaceted. Algal extracts may include bioactive substances directly disrupt bacterial cell membranes, interfere with QS signals, or modulate the expression of virulence genes. The specific compounds responsible for these effects could include secondary metabolites, polyphenols, terpenoids, and fatty acids, which have been previously documented for their antimicrobial and anti-biofilm properties. Moreover, the mode of action by which algal extracts modulate genes expression is not clear, but it may involve binding to or inhibiting receptors or enzymes involved in these pathways.

5. Conclusion

This study demonstrates that algal extracts have inhibitory effectiveness on bacteria growth and biofilms in *S. aureus* and *P. aeruginosa* and modulate their biofilm and QS-related gene expression. These results suggest that algae may serve as a good source of natural compounds for the development of adjuvants or novel antibacterial compounds. Additional researches are required to identify the active components of algal extracts and elucidate their mode of action at the molecular level.

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