Punjab University Journal of Zoology

OPEN ACCESS

41(1): 65-74 (2026)
https://dx.doi.org/10.17582/journal.pujz/2026/41.1.65.74

Research Article

Integrated *In Vitro* and *In Silico* Assessment of Anti-Quorum Sensing and Anti-Biofilm Activities of *Chlorella vulgaris* and *Spirulina platensis* against *Acinetobacter baumannii*

Ali Ayad Jabbar[1], Azhar Noory Hussein[2*]

[1]*Al-Qadisiyah Education Directorate, Iraq*
[2]*Department of Biology, College of Education, University of Al-Qadisiyah, Al-Diwaniyah, Iraq*

Article History
Received: December 27, 2025
Revised: February 18, 2026
Accepted: February 24, 2026
Published: April 24, 2026

Authors' Contributions
AAJ and ANH worked together on designing the study and planning the experiments. AAJ carried out the laboratory work, including the biofilm and quorum-sensing inhibition tests, and also performed the molecular docking analysis. ANH supervised the project, provided guidance throughout the research, and helped steer the scientific direction. Both authors contributed to analyzing the results and discussing the findings. AAJ wrote the first draft of the manuscript. ANH reviewed it and improved the final version. All authors approved the final manuscript.

Keywords
Chlorella vulgaris, *Spirulina platensis*, *Acinetobacter baumannii*, QS receptor proteins, Biofilm inhibition, AbaI/AbaR system

Abstract | Quorum sensing (QS) regulates virulence and biofilm formation in pathogenic bacteria and represents a promising target for alternative antimicrobial strategies. This study aimed to evaluate the anti-biofilm and anti-quorum sensing potential of methanolic extracts of *Chlorella vulgaris* and *Spirulina platensis* against *Acinetobacter baumannii*. Twelve clinical isolates were examined under in vitro conditions. Both algal extracts demonstrated significant, concentration-dependent inhibition of biofilm formation. GC–MS profiling identified several bioactive compounds, four of which were selected for molecular docking analysis. Docking simulations were performed against quorum sensing-related proteins (AbaI and AbaR) and biofilm-associated proteins (CsuE, OmpA, and Bap). The selected ligands exhibited favorable predicted binding affinities ranging from −5.56 to −9.94 kcal/mol, with the 1,2,4-oxadiazole derivative showing the strongest predicted interaction across multiple targets. Although docking analysis provides theoretical insight into ligand–protein interactions, further experimental validation is required to confirm quorum sensing inhibition mechanisms. These findings highlight the potential of *C. vulgaris* and *S. platensis* as natural sources of bioactive compounds for controlling biofilm-related infections caused by multidrug-resistant pathogens.

Novelty Statement | This study provides an integrated in vitro and in silico evaluation of the anti-quorum sensing and anti-biofilm potential of *Chlorella vulgaris* and Spirulina platensis against *Acinetobacter baumannii*. The research identifies bioactive compounds from algal extracts and demonstrates their predicted interactions with quorum sensing and biofilm-associated proteins, suggesting microalgae as promising natural sources of quorum sensing inhibitors.

To cite this article: Jabbar, A.A. and Hussein, A.N., 2026. Integrated *in vitro* and *in silico* assessment of anti-quorum sensing and anti-biofilm activities of *Chlorella vulgaris* and *Spirulina platensis* against *Acinetobacter baumannii*. *Punjab Univ. J. Zool.*, **41**(1): 65-74. https://dx.doi.org/10.17582/journal.pujz/2026/41.1.65.74

Introduction

The emergence of multidrug-resistant pathogenic bacteria and their biofilms at high rates is commonly

Corresponding author: Azhar Noory Hussein
azhar.almousawi@qu.edu.iq

linked to QS the production of antipathogenic and anti-QS agents has become urgent in order to reduce bacterial virulence (Haddadin *et al*., 2019). Bacterial quorum sensing (QS) is a cell density–dependent molecular communication system that regulates gene expression and coordinated bacterial behavior (Rashiya *et al*., 2021). Recently, quorum sensing inhibition strategies, which aim to disrupt quorum signaling, have gained significant attention. These strategies can inhibit the synthesis and accumulation of virulence factors by disrupting signaling molecules (Haque *et al*., 2018; Wang *et al*., 2024; Zhang *et al*., 2025). Interrupting molecular communication pathways is essential to control critical physiological and pathogenic processes in bacteria, including Biofilm formation, Sporulation, Motility, and release of virulence from pathogenic bacteria, Pigmentation (Banerji *et al*., 2020). Quorum sensing regulation can be effectively disrupted through three main mechanisms (Zhao *et al*., 2015). They are the inhibition of AIs synthase, Inactivation or enzymatic degradation of signaling molecules -Quorum quenching, and Blocking the signal receptor (Bhargava, 2010). The QS system of *A. baumannii* consists of the AbaI/AbaR regulatory pair, where AbaI synthesizes AHL signals and AbaR acts as the corresponding transcriptional regulator. The abaI gene is a gene that codes an autoinducer synthases enzyme that is in charge of catalyzing the synthesis of the acyl-homoserine lactone (AHL signals) (Sun *et al*., 2021). The greatest AHLs produced by *A. baumannii* is 3-hydroxy-C12-homoserine lactones. The gene abaR codes the receptor protein that interacts with AHLs, and acts as transcriptional regulatory factors (Sun *et al*., 2021; Saipriya *et al*., 2020). The biofilm system consists mainly of extracellular polymer (EPS) materials, which protect bacterial cells from harsh conditions and antibiotics, thus disrupting therapeutic efficacy (Mishra *et al*., 2023; Salama *et al*., 2016; Azeez and Al-Daraghi, 2019).

The anti-quorum sensing effect of red seaweed extracts has been investigated by examining their unique compounds (Piruthiviraj *et al*., 2024; Phiri, 2017). These compounds can be used to inhibit QS and to control biofilms in medical devices such as catheters, ventilators, and membrane bioactive agents (Shahid *et al*., 2019; Saverina *et al*., 2023).

However, the anti-QS and antibiofilm potential of algal-derived compounds against *A. baumannii* remains poorly explored. Therefore, this study hypothesizes that bioactive metabolites from *C. vulgaris* and *S. platensis* can inhibit QS receptors and biofilm-associated proteins.

Materials and Methods

Clinical isolates and Ethical approval

The research was carried out with a grant of department of Biology, College of Education, University of Al-Qadisiyah (Ref. No. 294, dated 8 October 2024). Institutional review board gave prior consent to the research and the study was conducted according to the stipulations of the institution and the Declaration of Helsinki. Clinical isolates of *Acinetobacter baumannii* were obtained from hospitalized patients in Al-Diwaniyah City, Iraq. Burn, sputum, urine, and blood samples were isolated. All personal information about patients was anonymized prior to analysis, and no identifiable data were recorded.

Identification of Acinetobacter baumannii

The initial identification of bacterial isolates was carried out on the basis of colony morphology on MacConkey agar and Gram staining. Further confirmation was performed by selecting non-lactose fermenting Gram-negative coccobacilli colonies.

The VITEK 2 Compact system (bioMerieux, France) was used to identify the species at a species level based on the instructions of the manufacturer. The study was restricted to the isolates that were considered *Acinetobacter baumannii* with high levels of confidence. Samples were collected between January 2025 and June 2025 from adult hospitalized patients at Al-Diwaniyah Teaching Hospital, Iraq.

Algae extraction

Dry powder was used for algae *C. vulgaris* and *S. platensis*. 20 g of dried algae were ground and then put in 300 mL of methanol and put on a rotating shaker turning 75 times per minute at room temperature, the mixtures prepared were filtered using Whitman filter paper. The solvents were removed using a rotating evaporator at 80°C. The extracts were dried and further process was going on Michalak and Chojnacka (2014).

Anti-biofilm assay and determination of sub-MIC

To determine the anti-biofilm of the *Chlorella vulgaris* and the *Spirulina platensis* against *Acinetobacter baumannii*, the crystal violet microtiter plate assay described by Al-Kafaween *et al*. (2019) with slight alterations was used. In a word, nutrient broth was inoculated with bacterial suspensions that were adjusted to 0.5 McFarland standard, inoculated with various concentrations of algal extracts and in microtiter plate with 96 wells. Bacterial suspension that was without extract in wells formed the positive controls whereas sterile broth in wells was the negative controls. Solvents control (methanol at equivalent concentrations) was also added, to eliminate solvent-related effects. Planktonic cells were washed with phosphate-buffered saline (PBS) after incubating at 37 °C over 24 h. Biofilms were incubated using crystal violet (0.1) in 10 min, rinsed and dried with air. A microplate reader was used to measure absorbance at 570 nm at which bound dye was solubilized in 95% ethanol.

All experiments were performed in triplicate and independently repeated three times. Sub-inhibitory concentrations (sub-MICs) were considered as the concentrations which did not influence the planktonic growth visually, but decreased the biofilm biomass.

GC-MS analysis

GC–MS analysis was performed using an Agilent 5973N GC–MS system equipped with an HP-5MS capillary column (30 m × 0.25 mm internal diameter × 0.25 µm film thickness). The oven temperature was initially set at 50 °C (held for 2 min), then increased at a rate of 10 °C/min to 280 °C and maintained for 10 min. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The injector temperature was maintained at 250 °C.

Mass spectra were recorded under electron ionization (EI) at 70 eV, with an ion source temperature of 230 °C and a mass scan range of m/z 40–550. Compound identification was performed by comparing the obtained spectra with those in the W10N14 mass spectral library database using ChemStation software. Only compounds with similarity index values ≥ 90% were considered for further analysis.

Molecular docking analysis

GC–MS analysis identified 20 compounds in Chlorella vulgaris and 15 compounds in Spirulina platensis extracts. Based on peak area and relative abundance, two major compounds from each extract were selected for molecular docking analysis (Table 1).

Table 1: Two compounds from Chlorella vulgaris and two compounds from Spirulina platensis.

Symbol	Name
A (chlorella)	1H-Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethyl
B (chlorella)	3-Chloro-1,4-dimethyl-2-quinolone
C (spirulina)	Glycine, N-[N-(2-hydroxybenzoyl)-β-alanyl]-, methyl ester
D (spirulina)	1,2,4-Oxadiazole-5-carboxamide, N-[ethyl]-3-[(4-nitro-1H-pyrazol-1-yl)]

Figure 2 illustrates the two-dimensional structures of the selected ligands. Three-dimensional conformations were retrieved from the PubChem database and subjected to geometry optimization. Initial optimization was performed using the semi-empirical AM1 method, followed by full optimization at the DFT level (B3LYP/6-31G) to obtain stable conformations suitable for docking simulations.

The three-dimensional structures of quorum sensing and biofilm-associated proteins, including AbaI (AF-B0FLN1-F1-v4), AbaR (A0A013UPU7), CsuE (A0A5N5YGE1), OmpA (PDB ID: 3TD3), and Bap (A0A140CTC9), were obtained from the Protein Data Bank and AlphaFold Protein Structure Database. Figure 1 presents the structural representation of the selected target proteins.

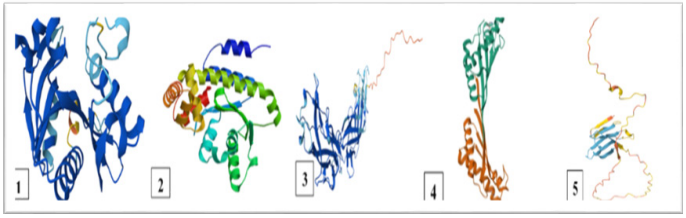

Figure 1: (1) AF-B0FLN1-F1-v4 protein (Abal), (2) A0A013UPU7 protein (AbaR), (3) A0A5N5YGE1 protein (Csu), (4) 3TD3 protein (ompA), (5) A0A140CTC9 protein (bap).

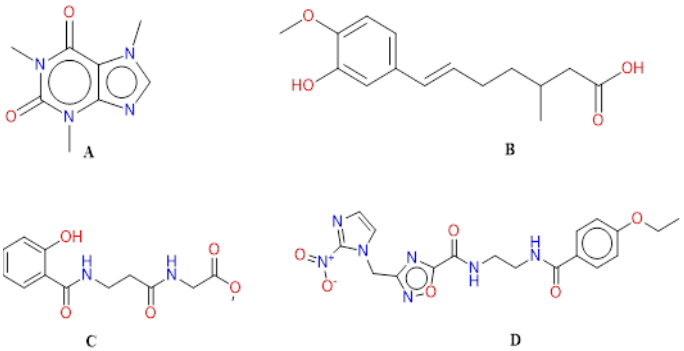

Figure 2: 2D structure of selected compounds (ligand A, B, C, D).

Docking simulations were performed using the Molecular Operating Environment (MOE, 2019). Prior to docking, protein structures were prepared by removing water molecules, adding hydrogen atoms, and correcting partial charges. Active binding sites were identified using the MOE Site Finder tool based on pocket geometry and key residue distribution. Energy-minimized ligands were docked using the London dG scoring function for initial ranking, followed by refinement with the GBVI/WSA dG scoring method. Multiple poses were generated for each ligand, and the best pose was selected based on the lowest binding energy score and acceptable RMSD values. Protein–ligand interactions were analyzed in terms of hydrogen bonding, π–π stacking, π–cation interactions, and hydrophobic contacts.

Statistical analysis

Statistical analysis was performed using SPSS software (Version 23.0; IBM Corp., USA). Data were initially assessed for normality. Biofilm inhibition data that followed a normal distribution (Table 2) were expressed as mean ± standard deviation (SD) and analyzed using one-way analysis of variance (ANOVA) followed by the Least Significant Difference (LSD) post-hoc test for multiple comparisons.

Table 2: effect of *Spirulina* extract on *Acinetobacter baumannii* biofilm formation.

Concentration	N	Mean of O.D.	Std. deviation	P- value
Positive control	12	1.25344	0.098674	0.0001
Negative control	6	0.15994	0.021320	
7.80	12	1.11436	0.107880	
15.60	12	1.11799	0.068967	
31.25	12	0.84014	0.112607	
61.50	12	0.78842	0.082380	
125.00	12	0.23042	0.032977	
250.00	12	0.37669	0.082766	
500.00	12	0.39587	0.080168	
1000.00	12	0.24321	0.051361	

Table 3: Effect of *chlorella* extract to *A. baumanii* biofilm formation.

Concentration	Median	IQR	P- value
1000	0.24	0.115	0.0001
500	0.336	0.148	
250	0.321	0.103	
125	0.403	0.177	
61.5	0.368	0.071	
31.25	0.186	0.023	
15.6	0.921	0.09	
7.8	0.997	0.05	
Positive control	1.326	0.093	
Negative control	0.171	0.107	

Data that did not meet the assumption of normality (Table 3) were expressed as median and interquartile range (IQR) and analyzed using the non-parametric Kruskal–Wallis test.

A p-value of < 0.05 was considered statistically significant. All assays were performed in triplicate and independently repeated three times.

Results

Twelve isolates of *A. baumannii* were collected from various clinical sources and studied.

Anti-biofilm assay (sub mic) determining the minimum inhibitory concentration (sub mic) of spirulina extract on biofilm formation

The efficacy of the compounds extracted from spirulina algae was dose-dependent and measured by optical density (OD). The influence of spirulina on *A. baumannii* was compared at various concentrations, that is, (p= 0.0001) based on the optical signal (OD) as the indicator of biofilm biomass. Statistical analysis showed that there were highly significant differences among the treatment groups (p = 0.0001), which shows that spirulina has a concentration-dependent inhibitory effect on biofilm formation. The mean OD of the positive group was (1.253 ± 0.099) and the negative control group recorded low growth of biofilms (0.160 ± 0.021), Interestingly, Biofilm inhibition increased with extract concentration, with significant reduction observed at concentrations ≥125 µg/mL. Although the mean OD values were 0.230-0.396, results with several LSD comparisons demonstrated that the concentrations of 0.230 and 0.396 were significantly different to the positive control group (p < 0.05), and no significant difference was observed between the 125 and 1000 µg/ml groups (p > 0.05). Moreover, biofilm formation with the concentration of 125 µg/ml was not significantly different as compared to the negative control group (p = 0.087) and this was an indication that the biofilm growth was almost fully inhibited. These findings demonstrate that Spirulina extract exhibited concentration-dependent inhibition of *A. baumannii* biofilm formation.

Determining the minimum inhibitory concentration (sub mic) of chlorella extract on biofilm formation

The effect of Chlorella algae extracts on the ability to inhibit the biofilm of *A. baumannii* bacteria was evaluated. Statistical analysis revealed that there was a significant difference in the mass of biofilm in central treatment concentrations (p= 0.0001), which means that a concentration effect was seen as an inhibitory effect. The mean OD values decreased with increasing extract concentration. The lowest biofilm biomass was observed at 1000 µg/mL (OD= 0.24), representing a marked reduction compared with the untreated positive control (OD= 1.326), while the negative control (medium only) showed an OD value of 0.171 (Table 3). Pairwise comparisons with Bonferroni-adjusted significance levels demonstrated that concentrations of 1000, 500, 250, and 125 µg/mL showed no statistically significant differences from the negative control or from each other ($P > 0.05$), indicating strong and consistent biofilm suppression. These findings support that *chlorella* extract significantly inhibits *A. baumannii* biofilm formation, with maximal inhibitory effects observed at concentrations ≥125 µg/mL.

GC–MS analysis

All methanolic extracts of the study algae underwent chemical analysis. Based on peak area ratios and potential bioactivity, four major compounds were selected for subsequent molecular docking studies (Table 4).

Molecular docking

All selected ligands demonstrated predicted binding interactions with quorum sensing-related proteins (AbaI and AbaR) as well as biofilm-associated proteins (CsuE, OmpA, and Bap). The detailed binding energies, RMSD values and key interacting residues are summarized in Tables 5–9, while representative two-dimensional and

three-dimensional interaction models are illustrated in Figure 4.

Table 4: Major bioactive compounds identified in methanolic extracts of *C. vulgaris* and *S. platensis* for molecular docking.

M/Z	Retention time (R.t, min)	Peak area	Area (%)	Reported activity	Compound name
73.10	62.160	8	0.32	Antibacterial and bioactive	1H-Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethyl
73.10	64.367	10	7.75	Antibacterial and bioactive	3-Chloro-1,4-dimethyl-2-quinolone
55.10	65.361	26	8.96	Antibacterial and bioactive	Glycine, N-[N-(2-hydroxybenzoyl)-β-alanyl]-, methyl ester
55.10	67.756	9	6.69	Antibacterial and bioactive	1,2,4-Oxadiazole-5-carboxamide, N-[ethyl]-3-[(4-nitro-1H-pyrazol-1-yl)]

Table 5: Best docking poses of ligands (A–D) against AbaI protein (AF-B0FLN1-F1-v4).

Compound	Best pose	Binding energy (kcal/mol)	RMSD (Å)	Key interacting residues	Main interaction types
A	Pose 1	-6.17	2.00	ARG103, PHE104	H-bond, π–H
B	Pose 1	-7.90	1.96	ARG103, THR147, SER105	H-bond, π–H
C	Pose 1	-7.82	1.93	ARG103, PHE104, SER105	H-bond, π–H
D	Pose 3	-9.27	1.97	ARG103, LEU78, TRP33, SER105	H-bond, π–H, π–π

Table 6: Best docking poses of ligands (A–D) against AbaR protein (A0A013UPU7).

Compound	Best pose	Binding energy (kcal/mol)	RMSD (Å)	Key interacting residues	Main interaction types
A	Pose 3	-6.55	1.97	TRP62, TYR58	H-bond, π–H
B	Pose 1	-7.83	1.91	MET82, MET129, TYR58	H-donor, π–H
C	Pose 1	-8.15	1.96	ASP75, TYR58, TRP90	H-bond, π–H
D	Pose 1	-9.94	2.05	ASP75, THR77, MET129, MET54, TYR66	H-bond, π–H, π–π

Table 7: Best docking poses of ligands (A–D) against CsuE protein (A0A5N5YGE1).

Compound	Best pose	Binding energy (kcal/mol)	RMSD (Å)	Key interacting residues	Main interaction types
A	Pose 1	-5.84	1.93	MET261, VAL215	H-bond, π–H
B	Pose 1	-7.19	1.96	TYR329, PHE217, ALA326	H-bond, π–H
C	Pose 1	-7.31	2.02	GLY327, GLY218, MET261, TYR329, PHE217	H-bond, π–H
D	Pose 2	-9.23	2.02	VAL215, TYR329, ASP331, ASN216, PHE217, THR219, ALA220, LEU226	H-bond, π–H, π–cation

Table 8: Best docking poses of ligands (A–D) against OmpA protein (3TD3).

Compound	Best pose	Binding energy (kcal/mol)	RMSD (Å)	Key interacting residues	Main interaction types
A	Pose 1	-5.56	2.01	ALA302, ARG281	H-acceptor
B	Pose 1	-6.56	2.07	GLU254, LYS238, TYR298	H-donor, π–H
C	Pose 1	-6.90	1.96	TYR298, THR307, ASN237, LYS246, SER306, LYS291	H-bond, π–cation
D	Pose 1	-8.44	2.05	SER303, LYS238, ASN237, LYS251, GLU254	H-bond, π–H, π–cation

Table 9: Best docking poses of ligands (A–D) against Bap protein (A0A140CTC9).

Compound	Best pose	Binding energy (kcal/mol)	RMSD (Å)	Key interacting residues	Main interaction types
A	Pose 1	-5.69	2.03	SER54	H-acceptor
B	Pose 3	-6.71	1.99	SER54, ASP80	H-donor
C	Pose 1	-7.29	1.90	ASP80, TYR58	H-bond
D	Pose 1	-8.52	2.06	ASP80, ARG32, PHE53, LYS77	H-bond, π–H

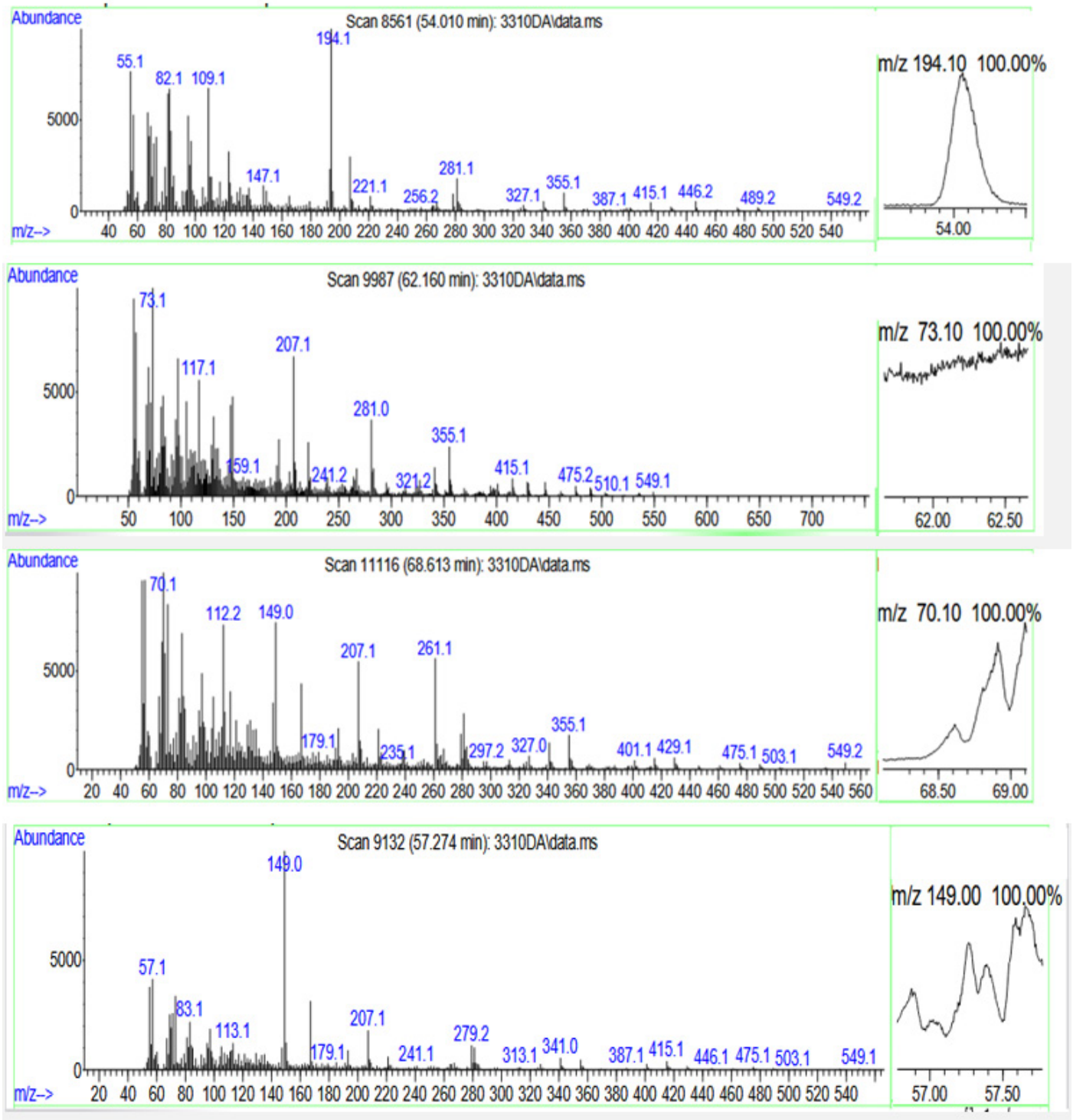

Figure 3: GC–MS chromatogram showing the major detected compounds, with peak intensities corresponding to their relative abundance.

Among the investigated compounds, the 1, 2, 4-oxadiazole derivative (Compound D) consistently exhibited the strongest predicted binding affinity across all five target proteins. The highest binding affinity was observed with AbaR (−9.94 kcal/mol), followed by AbaI (−9.27 kcal/mol), CsuE (−9.23 kcal/mol), OmpA (−8.44 kcal/mol), and Bap (−8.52 kcal/mol). These interactions were primarily stabilized through hydrogen bonding, π–π stacking, and π–cation interactions within the active sites of the respective proteins.

Compound C also showed favorable binding energies, particularly against AbaR and CsuE, whereas compounds A and B demonstrated comparatively moderate binding affinities. The RMSD values for all selected poses were within acceptable ranges, indicating stable and reliable docking conformations.

Overall, the docking results suggest that selected algal-derived compounds, especially compound D, may interfere with quorum sensing signaling pathways and biofilm-associated protein functions in *A. baumannii*.

The strong predicted interactions of compound D with both quorum sensing and biofilm-associated proteins may partially explain the concentration-dependent biofilm inhibition observed in vitro.

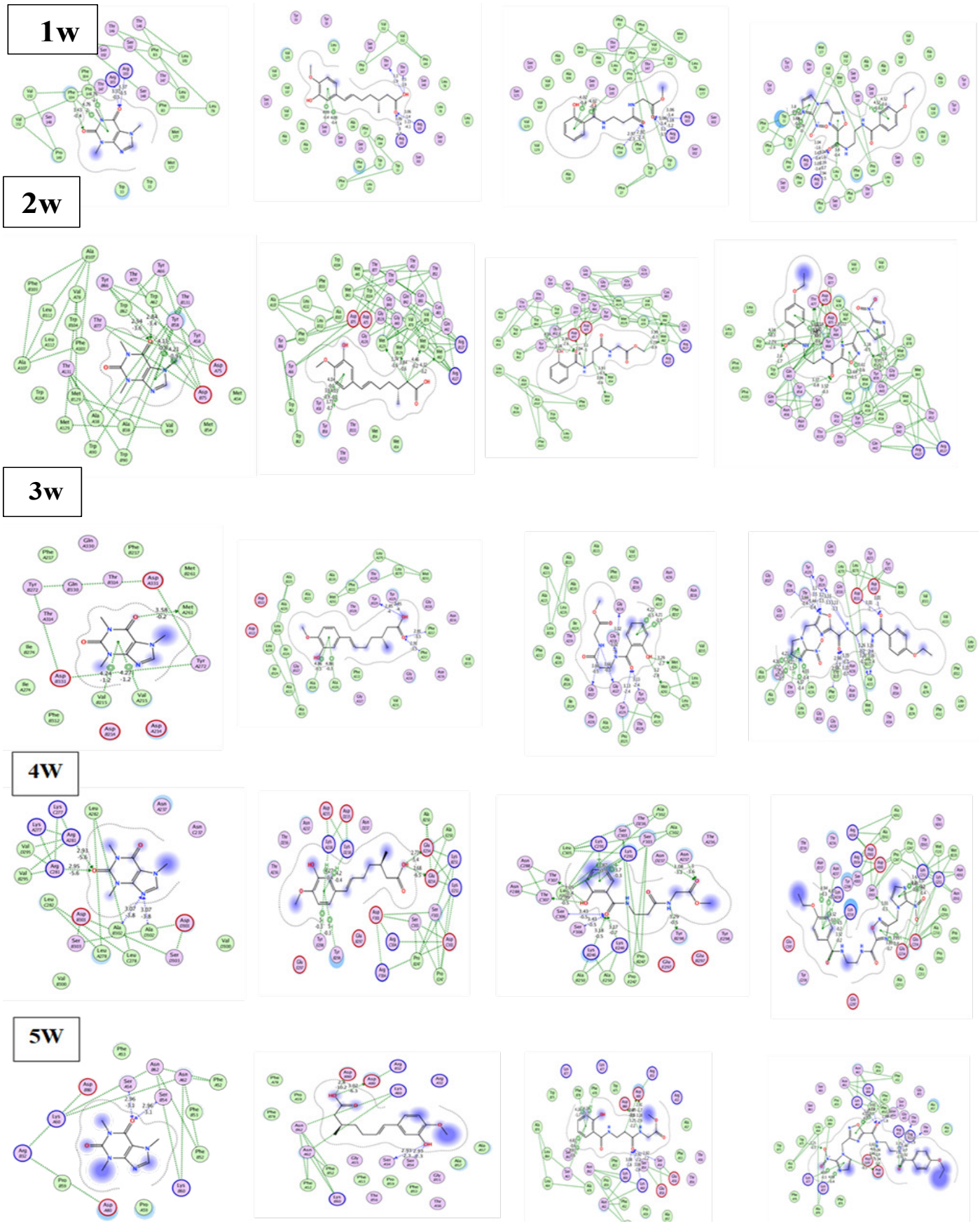

Figure 4: (1w) Abal, (2w) AbaR, (3w) Csu, (4w) OmpA and (5w) bap protein docked with selected compounds.

Discussion

The results of the study showed that the percentage of *A. baumannii* isolated from various clinical sources in Al-Diwaniyah Governorate was 5.6%. The greatest rate was observed in the burn samples, which indicates the presence of prevalence of hospital-acquired infections. This was proceeded by sputum samples because it is one of the most hazardous escapist pathogens in respiratory tract infections. The urine and blood rates were lower. These findings concur with local and international studies that show the high prevalence of *A. baumannii* in burn and surgical hospitals. We also have similar finding with (Al-Sahlawi *et al*., 2014; Abid, 2019). Our present study findings are slightly different than the world trend, which is inclined to the respiratory system as the primary site of *A. baumannii* isolates. Isolation rates have been found to be variable in local studies (Muhaisen and Al-Zeyadi, 2025; Abid, 2019) as there has been epidemiological diversity in terms of patient characteristics and clinical settings. This highlights the importance of the proper epidemiological maps that can facilitate the programs aimed at controlling infections and the choice of experimental treatment. results indicate that all the isolates formed biofilms at a rate of 100 percent with a difference in production of strong to moderate to weak. These findings fit with the research conducted by Babapour *et al.* (2016), and even with local research that registered biofilm production by a rate of 85% (Al-Sehlawi *et al*., 2014).

The effects of the algal extracts on the biofilm forming capacity of *A. baumannii* was tested in this study by prepare the extracts at different concentrations. We found these results to be in agreement with the study by Lykov *et al.* (2023), who tested the antibacterial activity of microalgae extracts, such as *Spirulina platensis* and *Chlorella vulgaris*, against *A. baumannii*. This research showed that microalgae extracts were highly active in *A. baumannii* growth, this is in accordance with our results where we have shown that algal extracts have a similar effect on this bacterium, with regard to inhibiting its growth. This justifies the possible use of algae as a natural source of antibacterial activity compounds. We also find our findings are in full agreement with the findings of (Ismaeil *et al*., 2024) who also established a strong similarity in the effect of algal extracts on biofilm formation genes. It revealed that such extracts have an inhibitory effect on the genes related to the quorum sensing system including IasR/IsaL in Pseudomonas aeruginosa. In the case of molecular docking, Abdel-Aziz *et al.* (2022) carried out a molecular docking experiment of *A. baumannii* with cinnamic acid and Gallic acid. The findings indicated that the binding energies (ΔG) between the two compounds and the biofilm forming proteins were similar whereby the energies obtained were -8.1 kcal/mol with cinnamic acid, and -9.7 kcal/mol with Gallic acid. The predicted binding energies obtained in the present study fall within a comparable range to previously reported quorum sensing inhibitors targeting *A. baumannii*, further supporting the potential relevance of these algal-derived compounds. The pattern of interaction between both compounds was also similar with the amino acid residues Lys238 and Ser239, the both studies also indicated that there were strong π-cation interactions as well as hydrogen bonds with the other amino acids and therefore the importance of the remaining amino acids in stabilizing the binding of compounds with the active protein site. The computational analysis suggested favorable binding interactions between selected algal compounds and QS-related proteins.

The observed reduction in biofilm biomass, combined with predicted docking interactions, suggests a possible role of these extracts in modulating quorum sensing-associated pathways. The extracted compounds may potentially influence quorum sensing-associated pathways in pathogenic bacteria. Diseases caused by harmful microbes such as bacteria and viruses pose a threat to the public health. The majority of these microbes have the ability to contaminate medical equipment and often touched surfaces, and in this process, they form resistant biofilms which are hard to fully eliminate using disinfectants. Thus, there is a necessity to come up with antimicrobial agents containing anti-QS and anti-biofilm.

Although molecular docking provides valuable theoretical insight into ligand–protein interactions, it does not fully represent dynamic biological conditions. Therefore, further molecular validation, gene-expression analysis, and in vivo studies are required to confirm quorum sensing inhibition mechanisms.

Conclusion

This study demonstrated that methanolic extracts of *Chlorella vulgaris* and *Spirulina platensis* significantly reduced biofilm formation in *Acinetobacter baumannii* under *in vitro* conditions. GC–MS analysis identified several bioactive compounds, and molecular docking predicted favorable interactions between selected ligands and quorum sensing- and biofilm-related proteins. Although docking provides theoretical insight into possible ligand–protein interactions, further molecular and gene-expression studies are required to confirm quorum sensing inhibition mechanisms.

Declarations

Acknowledgements

The authors appreciate the University of Al-Qadisiyah/Iraq for their support facilitations.

Funding

No funding was obtained for this study.

IRB approval

The study protocol was reviewed and approved by the Institutional Review Board of the Department of Biology, College of Education, University of Al-Qadisiyah, Iraq (Approval No. 294; October 8, 2024). The research was conducted in accordance with institutional guidelines and ethical standards.

Ethical statement

Clinical samples were collected from hospitalized patients at Al-Diwaniyah Teaching Hospital with prior institutional approval. All patient information was anonymized, and no identifiable personal data were recorded. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Data availability

All data used and generated in this study are included directly within the manuscript. The laboratory results, GC–MS findings, molecular docking outputs, and all tables and figures are presented in the paper. No external data repository was used, and no additional datasets are available outside the manuscript.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declare no conflict of interest.

References

Abdel-Aziz, N.A., Elkhatib, W.F., Sherif, M.M., Abourehab, M.A.S., Al-Rashood, S.T., Eldehna, W.M., Mostafa, N.M. and Elleboudy, N.S., 2022. In silico docking, resistance modulation and biofilm gene expression in multidrug-resistant Acinetobacter baumannii via cinnamic and gallic acids. *Antibiotics,* **11**: 870.

Abid, I.N., 2019. The bacteriological study of *Acinetobacter baumannii* isolated from burn patients in Al-Nasiriyah city-Iraq. *J. Univ. Thi-Qar.*, **14**.

Al-Kafaween, M.A., Mohd-Hilmi, A.B., Jaffar, N., Al-Jamal, H.A.N. and Zahri, M.K., 2019. Determination of optimum incubation time for formation of *Pseudomonas aeruginosa* and *Streptococcus* pyogenes biofilms in microtiter plate. *Bull. Natl. Res. Centre*, **43**: 100. https://doi.org/10.1186/s42269-019-0131-9

Al-Sehlawi, Z.S., Almohana, A.M. and Al-Thahab, A.A., 2014. Isolation and identification of *Acinetobacter baumannii* clinical isolates using novel methods. *J. Babylon Univ.*, **22**: 1041-1050.

Azeez, Z.F. and Al-Daraghi, W.H., 2019. Isolation of lytic *Acinetobacter baumannii* Phage VB_Acib_C_A10 from Iraq pond waters and comparing its antibacterial effect with cefotaxime antibiotic. *Int. J. Pharm. Qual. Assur.*, **10**: 84-89. https://doi.org/10.25258/ijpqa.10.1.14

Babapour, E., Haddadi, A., Mirnejad, R., Angaji, S.A. and Amirmozafari, N., 2016. Biofilm formation in clinical isolates of nosocomial *Acinetobacter baumannii* and its relationship with multidrug resistance. *Asian Pac. J. Trop. Biomed.*, **6**: 528-533. https://doi.org/10.1016/j.apjtb.2016.04.006

Banerji, R., Kanojiya, P. and Saroj, S.D., 2020. Role of interspecies bacterial communication in the virulence of pathogenic bacteria. *Crit. Rev. Microbiol.*, **46**: 136-146. https://doi.org/10.1080/1040841X.2020.1735991

Bhargava, N., Sharma, P. and Capalash, N., 2010. Quorum sensing in Acinetobacter: An emerging pathogen. *Crit. Rev. Microbiol.*, **36**: 349–360. https://doi.org/10.3109/1040841X.2010.512269

Haddadin, R.N., Alsous, M., Wazaify, M. and Tahaineh, L., 2019. Evaluation of antibiotic dispensing practice in community pharmacies in Jordan: A cross sectional study. *PLoS One*, **14**: e0216115. https://doi.org/10.1371/journal.pone.0216115

Haque, S., Ahmad, F., Dar, S.A., Jawed, A., Mandal, R.K., Wahid, M., Lohani, M., Khan, S., Singh, V. and Akhter, N., 2018. Developments in strategies for Quorum Sensing virulence factor inhibition to combat bacterial drug resistance. *Microbial Pathogen.*, **121**: 293-302. https://doi.org/10.1016/j.micpath.2018.05.046

Ismaeil, A.S., Toma, J.J., Hasan, N.S., Ahmed, A.A. and Abdulwahid, M.J., 2024. Impact of algal extract on quorum sensing and biofilm formation genes of multidrug-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Jordan J. Biol. Sci.*, **17**. https://doi.org/10.54319/jjbs/170413

Lykov, A., Salmin, A., Gevorgiz, R., Zheleznova, S., Rachkovskaya, L., Surovtseva, M. and Poveshchenko, O., 2023. *Antimicrobial potential of the microalgae extracts.* https://doi.org/10.20944/preprints202307.1601.v1

Michalak, I. and Chojnacka, K., 2014. Algal extracts: Technology and advances. *Eng. Life Sci.*, **14**: 581-591. https://doi.org/10.1002/elsc.201400139

Mishra, S., Gupta, A., Upadhye, V., Singh, S.C., Sinha, R.P. and Häder, D.P., 2023. Therapeutic strategies against biofilm infections. *Life*, **13**: 172. https://doi.org/10.3390/life13010172

MOE (Molecular Operating Environment), 2019. MOE: Molecular Operating Environment 2019.

Chemical Computing Group Inc.

Muhaisen, M.H.M. and Al-Zeyadi, A.A.K., 2025. Phenotypic and molecular characterization of *Acinetobacter baumannii* and its ability to produce beta-lactamase isolated from different clinical samples in diwaniyah governorate. *Central Asian J. Med. Natl. Sci.*, **6**: 704-710.

Phiri, P.X., 2017. *Investigating the antimicrobial, anti-biofilm and anti-quorum sensing potential of South African seaweed-associated bacteria*. University of KwaZulu-Natal, Westville.

Piruthiviraj, P., Swetha, B.M., Balasubramanian, C., Krishnamoorthy, R., Gatasheh, M.K., Ahmad, A., Parthasarathi, R., Pandurangan, P., Bhuvaneshwari, V. and Vijayakumar, N., 2024. Exploring the potential: inhibiting quorum sensing through marine red seaweed extracts–a study on Amphiroa fragilissima. *J. King Saud Univ. Sci.*, **36**: 103118. https://doi.org/10.1016/j.jksus.2024.103118

Rashiya, N., Padmini, N., Ajilda, A.A.K., Prabakaran, P., Durgadevi, R., Ravi, A.V., Ghosh, S., Sivakumar, N. and Selvakumar, G., 2021. Inhibition of biofilm formation and quorum sensing mediated virulence in *Pseudomonas aeruginosa* by marine sponge symbiont *Brevibacterium casei* strain Alu 1. *Microb. Pathog.*, **150**: 104693. https://doi.org/10.1016/j.micpath.2020.104693

Saipriya, K., Swathi, C., Ratnakar, K. and Sritharan, V. 2020. Quorum-sensing system in *Acinetobacter baumannii*: A potential target for new drug development. *J. Appl. Microbiol.*, **128**: 15-27. https://doi.org/10.1111/jam.14330

Salama, Y., Chennaoui, M., Sylla, A., Mountadar, M., Rihani, M. and Assobhei, O., 2016. Characterization, structure, and function of extracellular polymeric substances (EPS) of microbial biofilm in biological wastewater treatment systems: A review. *Desalinat. Water Treat.*, **57**: 16220-16237. https://doi.org/10.1080/19443994.2015.1077739

Saverina, E.A., Frolov, N.A., Kamanina, O.A., Arlyapov, V.A., Vereshchagin, A.N. and Ananikov, V.P., 2023. From antibacterial to antibiofilm targeting: An emerging paradigm shift in the development of quaternary ammonium compounds (QACs). *ACS Infect. Dis.*, **9**: 394-422. https://doi.org/10.1021/acsinfecdis.2c00469

Shahid, A., Rasool, M., Akhter, N., Aslam, B., Hassan, A., Sana, S., Rasool, M.H. and Khurshid, M., 2019. *Innovative strategies for the control of biofilm formation in clinical settings*. Bacterial Biofilms. IntechOpen. https://doi.org/10.5772/intechopen.89310

Sun, X., Ni, Z., Tang, J., Ding, Y., Wang, X. and Li, F. 2021. The abaI/abaR quorum sensing system effects on pathogenicity in *Acinetobacter baumannii*. *Front. Microbiol.*, **12**: 679241. https://doi.org/10.3389/fmicb.2021.679241

Wang, D., Wang, L., Liu, Q. and Zhao, Y. 2024. Virulence factors in biofilm formation and therapeutic strategies for Staphylococcus aureus: A review. *Anim. Zoon.*, **1**: 188-202..

Zhang, X., Li, S., Zhang, D., Zheng, S., Zhou, D., Hou, Q., Li, G. and Han, H., 2025. A comprehensive review of the pathogenic mechanisms of Pseudomonas aeruginosa: Synergistic effects of virulence factors, quorum sensing, and biofilm formation. *Front. Microbiol.*, **16**: 1619626. https://doi.org/10.3389/fmicb.2025.1619626

Zhao, J., Chen, M., Quan, C. and Fan, S., 2015. Mechanisms of quorum sensing and strategies for quorum sensing disruption in aquaculture pathogens. *J. Fish Dis.*, **38**: 771-786. https://doi.org/10.1111/jfd.12299