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Elucidating the Quorum Sensing Inhibitory Mechanism of Flavonoid Quercetin by Molecular Docking, Molecular Dynamics Simulation, and MM-GBSA Study

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Abstract: Quercetin, a naturally occurring flavonoid found in plants, has gained attention as a powerful quorum sensing (QS) inhibitor. However, the exact mechanism of its inhibitory effects is not yet fully understood. The *Pseudomonas* quinolone signal PQS has been demonstrated to trigger the formation of outer-membrane vesicles (OMVs) in Gram-negative bacteria, including *Pseudomonas aeruginosa*. Various other bacterial species have also responded to PQS, suggesting a shared biophysical mechanism. This study investigated the quorum-sensing inhibitory properties of Quercetin by assessing its binding affinity to the active site of PqsE (PDB: 2Q0J) using molecular docking, molecular dynamics simulations, and MM-GBSA assay. The results of molecular docking envisaged that the compound interacts with the target protein PqsE, exhibiting a binding energy value of (-7.46 kcal/mol; IC=3.39 µM). MD simulation over a time scale of 100 ns corroborated the interaction results obtained from molecular docking between Quercetin and the PqsE protein. In addition, MM-GBSA calculations confirmed that the compound Quercetin has high binding energies of -19.532 ± 16.048 Kcal/mol against PqsE protein. The strong binding affinity of the flavonoid quercetin, as demonstrated by *in silico* inhibition experiments, indicates its potential as a quorum-sensing inhibitor and its promise as a synergistic component in non-antibiotic therapeutic options for treating infections. Our findings reveal the molecular interactions involved in inhibiting the PqsE protein, resulting in blocking bacterial outer membrane vesicles. This sheds light on the essential aspects of the biophysical process underlying the antibacterial effects induced by Quercetin by inhibiting quorum sensing.

Keywords: Quercetin; quorum sensing; antibacterial activity; molecular docking; molecular dynamics simulation.

1. Introduction

The increasing prevalence of multi-drug-resistant bacteria has prompted the search for effective and innovative antibacterial treatments or supplementary agents to combat resistant pathogens. These novel treatments should focus on distinct mechanisms separate from current antibacterial therapies. One such intriguing mechanism is quorum sensing (QS), commonly found in Gram-negative bacteria [1–4].

Hence, by targeting quorum sensing mechanisms that pertain to signal production, dissemination, or reception, we can interrupt QS pathways, reducing bacterial virulence and resistance. Furthermore, this approach could tackle numerous long-lasting and harmful chronic infections without depending on growth-inhibiting agents like antibiotics, preservatives, and disinfectants, which inadvertently foster the emergence of resistant microorganisms [5]. ESKAPE bacteria, including *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp.*, use quorum sensing, which poses a major global health threat. This communication system contributes to antibiotic resistance, virulence, and biofilm formation, reducing treatment options for serious infections. Further insight into QS mechanisms could uncover new aspects of antimicrobial resistance and bacterial disease[6].

Pseudomonas aeruginosa is a widely distributed environmental bacterium that causes opportunistic healthcare-associated infections. It particularly affects immunocompromised and hospitalized individuals, posing a major challenge due to antibiotic resistance [7]. *Pseudomonas aeruginosa* primarily regulates the production of its virulence factors in response to population density using a cellular communication system known as QS. The investigation of *Pseudomonas aeruginosa* QS mechanism has been extensive due to the potentially severe consequences of *Pseudomonas aeruginosa* infections. This research contributes to developing quorum-sensing inhibitors (QSIs) aimed at reducing bacterial pathogenicity, virulence, and resistance, in addition to directly inhibiting growth. Among the various proteins involved in the *Pseudomonas aeruginosa* QS mechanism, LasR and PqsE play pivotal roles in its signal cascade [8].

Throughout history, bioactive compounds derived from plants have displayed significant promise in combatting infectious diseases. Nonetheless, the rising prevalence of multi-drug-resistant bacteria has rekindled the quest for powerful, innovative plant-based substances with unique modes of action distinct from existing antibacterial treatments [9–11]. In the current year, there is a rising interest in developing novel, safe, and broad-spectrum quorum quenching (QQ) drugs obtained from microorganisms and medicinal plants. Compounds originating from plants, specifically derived from secondary metabolites such as flavonoids, alkaloids, terpenoids, and phenolics, play a pivotal role in therapeutic and biological activities to combat quorum-sensing pathogens [12–14]. Quercetin, a naturally occurring flavonoid renowned for its therapeutic properties, antimicrobial and anticancer effects, is commonly found in fruits and vegetables. Recent studies have unveiled its efficacy as a quorum sensing (QS) inhibitor in *Pseudomonas aeruginosa*. The presence of Quercetin resulted in the inhibition of QS, leading to reduced biofilm formation and decreased production of virulence factors like pyocyanin, protease, and elastase [15]. While the flavonoid compound Quercetin, originating from secondary metabolites, has the capacity to block quorum sensing, the exact mechanism of its antimicrobial effectiveness remains to be fully elucidated.

Quercetin has undergone extensive research owing to its potential benefits in addressing diabetes, bacterial infections, inflammation, Alzheimer's disease, cardiovascular issues, wound healing, and its anticancer properties against various cancer cell lines. Quercetin and its derivatives are commonly present in diets through fruits, seeds, vegetables, ferns, coffee, tea, and other plants. Incorporating them into meals or as supplements may offer preventive advantages [16, 17]. The increasing need for groundbreaking and innovative antimicrobials to combat severe infections caused by the widespread dissemination of multidrug-resistant bacterial pathogens is not aligned with the current progress in therapeutic development for

these treatments. This is especially evident in the realms of natural-product-derived and synthetic small compounds. There is a global demand for new antimicrobial drugs with novel modes of action to effectively combat the public health threat posed by antimicrobial resistance [18].

Hence, drawing from prior research, we hypothesized that the flavonoid compound Quercetin exhibits quorum sensing (QS) inhibitory properties in *Pseudomonas aeruginosa* through its interaction with the *Pseudomonas* Quinolone Signal Response protein PqsE (Figure 1). Hence, this study sought to elucidate the quorum sensing mechanism in *Pseudomonas aeruginosa* and investigate the intricate binding interactions through *in silico* molecular docking, molecular dynamics simulation, and MM-GBSA binding energy calculations involving Quercetin and the PqsE protein.

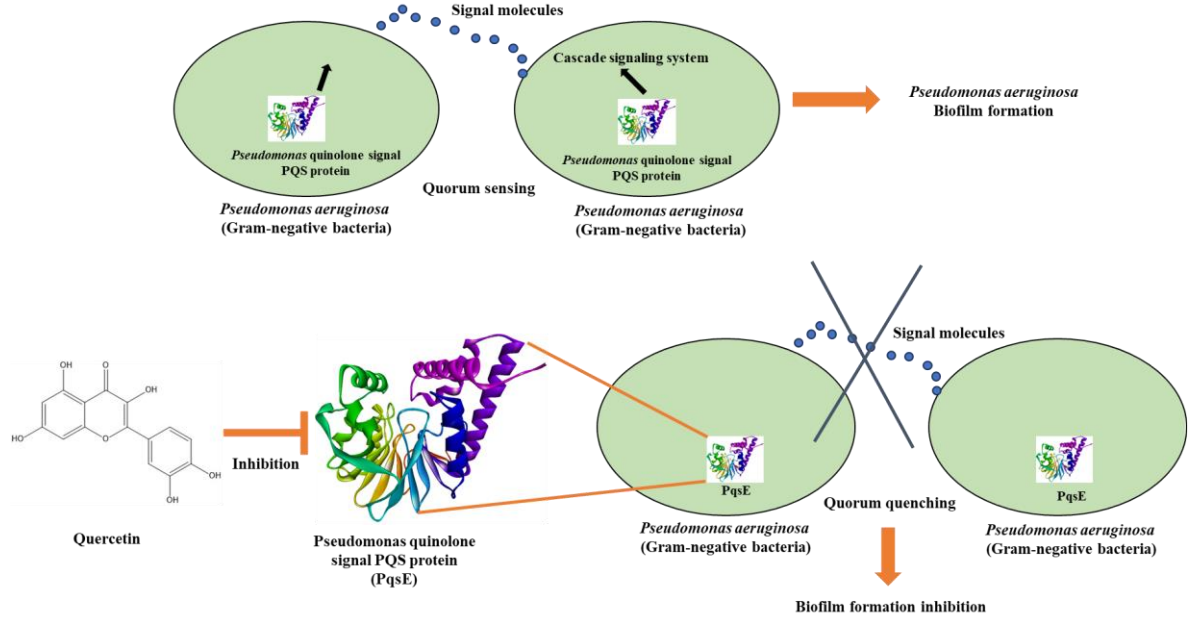

Figure 1. Overall hypothetical mechanism of Quorum sensing inhibitory potential of flavonoid Quercetin.

2. Materials and Methods

2.1. Preparation of ligands and receptors.

The 3D structure of the compound Quercetin (CID: 5280343) was obtained from the PubChem database [19] in SDF format and subsequently converted into pdb format using Open Babel 2.2.3 [20]. The crystal structure of the target protein *Pseudomonas* Quinolone Signal Response Protein PqsE (PqsE) (PDB ID-2Q0J) protein was obtained from the protein data bank (PDB) repository (https://www.rcsb.org) [21] with a resolution of 2.10 Å. The protein's water molecules, inhibitors, and other heteroatoms were removed and used for molecular docking study.

2.2. Molecular docking of PqsE protein with Quercetin using AutoDock 4.2.6.

Autodock version 4.2.6. was used for molecular docking studies. Protein and ligand preparation was done using AutoDock Tools (v.1.5.6) [22]. The protein underwent the addition of polar hydrogen and Gasteiger charges prior to conversion into PDBQT format. Similarly, the ligand molecules had Gasteiger charges added before their conversion into PDBQT format. A grid point spacing of 0.500Å was set, centered on x = 10.558, y = 8.947, and z = 11.748 Å. Docking was done, keeping the protein molecule rigid and ligands flexible. Molecular docking

was done via Autodock 4.2 [23] using the Lamarckian Genetic Algorithm (LGA) scoring function. The docking procedure was carried out by employing the AutoDock executable and utilizing DPF files as inputs, resulting in the generation of a DLG file. To analyze interactions, we utilized BIOVIA Discovery Studio Visualizer (DSV) Client 2021 (https://discover.3ds.com/discovery-studio-visualizer-download)) for creating two-dimensional (2D) and three-dimensional (3D) interactions.

2.3. MD simulations.

The MD simulations were performed for Quercetin in a complex with PqsE protein to understand better the binding nature of the targeted protein and the compound. The whole MD simulation was run using the Schrodinger Desmond MD simulation program. The Quercetin - PqsE complex was solvated in an orthorhombic box shape of the SPC water model [24]. The OPLS-2005 force field was used in this system [25–27]. Furthermore, to achieve a neutralized complex system, we introduced suitable quantities of counter ions, comprising 15 sodium ions (Na^+) and 28 chloride ions (Cl^-), while maintaining a physiologically relevant salt concentration of 0.15 M NaCl throughout the simulation. After the initial step, an equilibration and minimization process were carried out using an NPT ensemble. The NPT ensemble setup involved the Nose-Hoover chain coupling method. Pressure control was managed through the Martyna-Tuckerman–Klein chain coupling scheme barostat with a relaxation time of 2 ps. Long-range electrostatic interactions were calculated using the Particle Mesh Ewald method, maintaining a Coulomb interaction radius consistently set at 9 Å. For each trajectory, the RESPA integrator was applied [28–30]. Using the MD trajectory acquired, the stability and binding orientation of the Quercetin - PqsE complex were studied using the simulation interaction diagram (SID) pane. The root means square deviation (RMSD), radius of gyration (Rg), root mean square fluctuation (RMSF), and the number of hydrogen (H-bonds) were calculated to monitor the stability of the MD simulations.

2.4. Molecular mechanics generalized born surface area (MM-GBSA) calculations.

The MM-GBSA method is used to compute the binding affinity of Quercetin (in kcal/mol) with the target PqsE protein. As a result, the Prime Module's Python script *thermal mmgbsa.py* was utilized to execute post-simulation MM-GBSA analysis. For binding free energy calculations of Quercetin in complex with PqsE protein, 100 ns MD simulation trajectory was studied. Individual energy modules such as van der Waals, columbic, covalent, self-contact, solvation, hydrogen bond, lipophilic, and π- π stackings of ligand and protein were used collectively to compute the Prime MM-GBSA binding free energy (kcal/mol) [31, 32]. The following formula was used to calculate the total free energy binding:

$$\Delta G_{bind} = G_{complex} - (G_{protein} + G_{ligand}) \quad (1)$$

Where ΔG_{bind} = binding free energy, $G_{complex}$ = free energy of the complex, $G_{protein}$ = free energy of the target protein, and G_{ligand} = free energy of the ligand.

3. Results and Discussion

3.1. Molecular docking analysis.

The interactions of compound Quercetin with the target protein Pseudomonas Quinolone Signal Response Protein PqsE (PqsE) were analyzed using the *in silico* molecular docking method. Previous studies have demonstrated that the Quercetin compound serves as an effective QS inhibitor and exhibits antibiofilm activity in *Pseudomonas aeruginosa* [15]. Consequently, PqsE was identified as the target protein for investigating the underlying mechanism. The molecular docking interaction energies of the compound Quercetin showed significant binding energy (-7.46 kcal/mol; IC=3.39 µM) with the target protein PqsE (Figure 2). As depicted in Figure 2, the bioactive compound quercetin interacts with the protein PqsE by establishing two hydrogen bonds with the amino acid residues at the active site of the protein, namely His 71 (3.74 Å) and Leu 281 (3.67 Å). In addition, Quercetin forms hydrophobic van der Waals interactions with His 69, His 74, Val 108, Leu 112, Leu 193, His 282, Gly 284, Ser 285, and His 221. Tyr 72, Asp 73, Leu 277, and Arg 288 interact by forming π interaction. Prior research indicates that oxicam derivatives like Meloxicam and Piroxicam exhibit the most pronounced inhibitory effects on the PqsE protein. Specifically, these compounds possess the lowest inhibition constants (Ki), with Meloxicam having a Ki of 4.0 µM and Piroxicam at 4.88 µM [8]. In comparison, our studied Quercetin demonstrated an even lower inhibition constant value, with an IC of 3.39 µM. Thus, the computed binding energy data suggested that the flavonoid Quercetin had a stronger tendency to inhibit PqsE. Previous findings demonstrated that Meloxicam binds to the active site of PqsE, forming a hydrogen bond with Ser 285, and engages in an electrostatic interaction with one of the ferrous ions (Fe^{2+}) within the PqsE binding site [8].

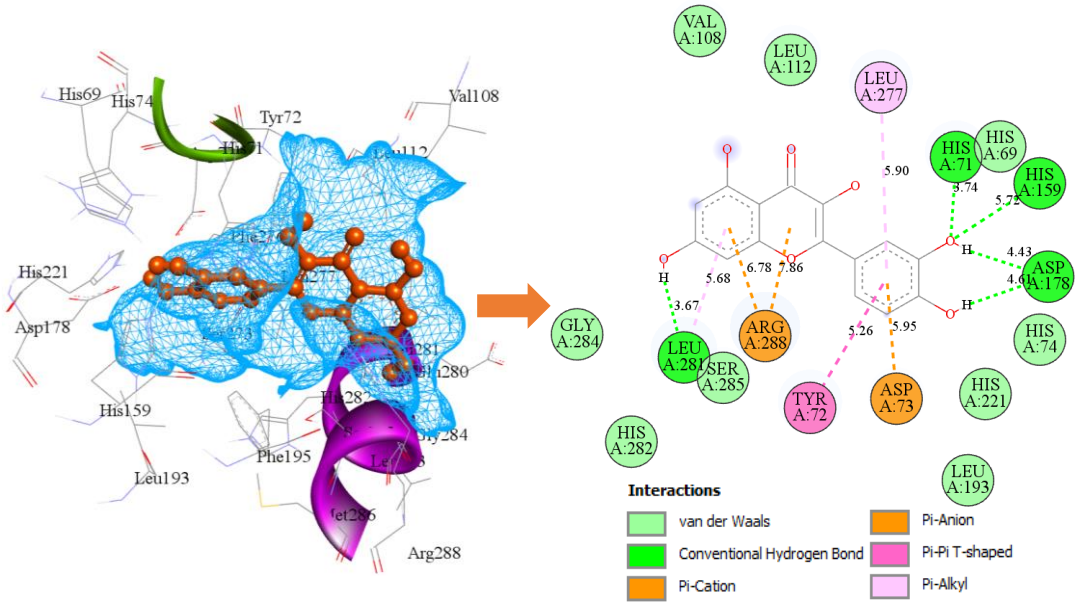

Figure 2. 2D and 3D Molecular docking interactions in the active site of PqsE protein (PDB ID: 2Q0J) with ligand Quercetin.

3.2. Molecular dynamics (MD) simulation study.

To assess the binding stability of the complex formed by the ligand and protein, as well as the essential intermolecular interactions throughout the simulated path, we conducted an MD simulation study on the Quercetin-PqsE protein docking complex. We scrutinized the

simulated trajectory of the complex according to standard simulation parameters, including RMSD, RMSF, Rg, and the count of H-bonds formed between the protein and ligand over the course of a 100 ns MD simulation. The RMSD of Cα atoms stands as a significant parameter in MD simulation trajectories, assessing the structural changes of Cα atoms within a dynamic context. Prior studies have demonstrated that the stability of the protein-ligand complex is associated with a lower RMSD value throughout the duration of the MD simulation. The Cα atoms RMSD is one of the important parameters of the MD simulation trajectory that is used to investigate the Cα structure deviation of a single frame generated in a dynamic environment. Previous research showed that the stability of the protein-ligand complex is determined by the lower RMSD value during the course of the MD simulation [33]. The RMSD analysis utilized Cα atoms of the PqsE protein, and the resulting data was plotted against the simulation time, as depicted in Figure 3A. When Quercetin was bound to PqsE, the RMSD values for the Cα atoms of PqsE ranged from 1.04 Å to 3.92 Å, with an average value of 3.04 Å. The findings indicate that the complex remained consistently stable throughout the entire MD simulation, implying that the protein-ligand complex achieved and maintained a stable conformation. RMSF provides insight into the level of dynamism exhibited by the interaction between the protein and ligand. Individual amino acid residues play a crucial role in maintaining the stability of the protein-ligand complex throughout the MD simulation. The degree of residue fluctuation during the simulation is assessed using the RMSF parameter. The RMSF values were derived from the MD simulation trajectory, as displayed in Figure 3B. The results of RMSF analysis indicated that the Cα atoms of the PqsE protein, when bound to Quercetin, exhibited an average RMSF value of 0.994 Å, suggesting minimal fluctuations in the complex's structure. Nevertheless, certain residues, such as Gln 272 (2.98 Å), Ser 273 (3.119 Å), Asp 275 (2.769 Å), Phe 276 (2.606 Å), His 282 (3.662 Å), Leu 283 (3.256 Å), Leu 300 (3.004 Å), and Asp 301 (4.009 Å), displayed slight fluctuations within the PqsE-Quercetin complex during the 100 ns simulation. Although some amino acid residues exhibited more significant fluctuations compared to others, the overall fluctuation of amino acid residues during the interaction remained below 2.4 Å, which is considered satisfactory and acceptable [33]. The elevated RMSF values observed at the C-terminal residues represent the tails or ends of the protein structure. These residues correspond to the terminations or tails of the protein, have greater freedom of movement, and exhibit higher reactivity [34]. The Rg property was also examined to demonstrate the stability of Quercetin within the binding pockets of PqsE over the course of the 100 ns simulation (Figure 3C). The Rg is a parameter that calculates how much a protein's structure changes during MD simulations. The Rg assesses how the protein's compactness and flexibility change within a biological context [35]. When Quercetin was in complex with the PqsE protein, it displayed an average Rg value of 19.67 Å, ranging from 19.32 Å to 19.95 Å. Notable fluctuations in the Rg value were absent, indicating consistent and stable behavior. Consequently, our comprehensive MD simulation results unambiguously affirm the favorable interactions of Quercetin, highlighting its stability within the PqsE active site and reinforcing its potential as a viable PqsE inhibitor. The binding interactions between PqsE and the ligand Quercetin were examined by assessing hydrogen bond formations. The figure displayed in Figure 3D illustrates the number of intermolecular hydrogen bonds established during the simulation of the compound with the PqsE protein. An examination of the outcomes revealed that, on average, Quercetin formed 1 hydrogen bond over the course of a 100 ns MD simulation with PqsE. This finding substantiates the potent inhibitory effect of Quercetin on PqsE throughout the MD simulation. These findings are inconsistent with the

molecular docking results, which demonstrated the creation of 2 hydrogen bonds within the active site residues of the PqsE protein. This discrepancy can be attributed to the formation of relatively weak hydrogen bonds during the molecular docking process.

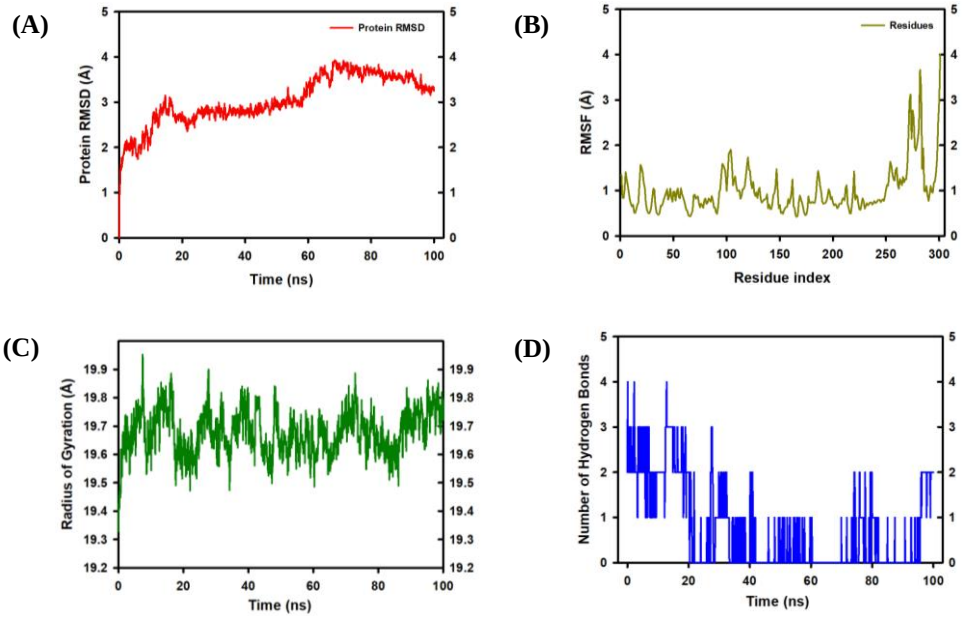

Figure 3. MD simulation analysis of Quercetin in complex with PqsE **(a)** Plot of RMSD (C-α atom of PqsE protein); **(b)** Plot of RMSF values; **(c)** Plot of the number of H-bonds interactions; **(d)** Plot of Rg during 100 ns MD simulation time.

3.3. Molecular mechanics generalized born surface area (MM-GBSA) calculations.

While molecular docking methods are effective at predicting the optimal ligand position within a protein's binding site, they often lack reliability when it comes to ranking compounds based on their binding affinities and, consequently, their biological activity. This discrepancy can be attributed to the inherent limitations in docking scoring algorithms, which may significantly magnify errors in these computations [36–38]. Recent advancements have revealed that the integration of more physically meaningful energy parameters, such as solvation energy and surface accessibility area, along with a molecular mechanical force field, can enhance the precision of predictions concerning ligand binding energy [39, 40]. As a result, the MM-GBSA approach was employed to make relatively accurate forecasts of compound binding affinity. The computed post-simulation MMGBSA-based binding free energy for the protein-ligand complex, along with its standard deviation, is presented in Table 1. The mean ΔG Bind value for the Quercetin-PqsE complex was calculated to be -19.532 ± 16.048 kcal/mol. A more negative value indicates a stronger binding of the compound to the target protein. The results in Table 1 indicated that the primary contributions to ΔGbind, affecting the stability of the simulated complexes, stemmed from ΔGbindvdW, ΔGbindLipo, and ΔGbindCoulomb, while ΔGbindCovalent and ΔGbindSolvGB had destabilizing effects on the Quercetin-bound PqsE complex.

Table 1. Binding free energy components for the docking complexes of PqsE protein with ligand Quercetin calculated by MM-GBSA analysis

MM-GBSA (kcal/mol)	**Quercetin - PqsE**
ΔG_{bind}	-19.532 ± 16.048
ΔG_{bind}Lipo	-5.401 ± 4.669
ΔG_{bind}vdW	-16.750 ± 12.707
ΔG_{bind}Coulomb	-4.645 ± 6.501

MM-GBSA (kcal/mol)	Quercetin - PqsE
$\Delta G_{bind}H_{bond}$	-0.531 ± 0.741
ΔG_{bind}SolvGB	9.133 ± 6.103
ΔG_{bind}Covalent	0.121 ± 1.515
ΔG_{bind} Packing	-1.457 ± 1.513

4. Conclusions

Since Quercetin, an active compound, and flavonoid, is commonly found in many plants consumed by humans, the active compounds derived from such plants, with the capability to inhibit QS, offer the potential advantage of being safe for human consumption and presenting minimal to no risk to human cells. This attribute holds promise for the development of new anti-quorum sensing compounds as possible alternatives to current therapeutic agents. The current study indicates that the bioactive compound Quercetin could potentially inhibit the *Pseudomonas* Quinolone Signal Response Protein PqsE (PqsE) protein in *Pseudomonas aeruginosa*. *In-silico* molecular interaction studies of the complex express higher binding interaction. Therefore, the molecule Quercetin can inhibit the PQS signaling, which was regulated to form a shown to initiate outer-membrane vesicles (OMVs) formation in *Pseudomonas aeruginosa* by interacting with the outer membrane (OM). Hence, the flavonoid Quercetin successfully suppressed the biofilm development in *Pseudomonas aeruginosa* by inhibiting the QS mechanism. These results imply that Quercetin has the potential to serve as an inhibitor for regulating the *Pseudomonas aeruginosa* quorum sensing signaling system and inhibiting the formation of biofilms.

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Conflicts of Interest

The authors declare no conflict of interest.

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