Journal of Applied Biology & Biotechnology Vol. 13(3), pp. 106-113, May-June, 2025
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2025.197114

Quorum quenching of virulence traits expression in human and plant pathogens by Isoxazolone and its molecular docking studies

Komal S. Salkar[1], Lakshangy S. Charya[1]*, Milind M. Naik[1], Hari K. Kadam[2], Vishnu Chari[2]

[1]Microbiology Programme, School of Biological Sciences and Biotechnology, Goa University, Taleigao Plateau, Goa, India.
[2]School of Chemical Sciences, Goa University, Taleigao Plateau, Goa, India.

ARTICLE INFO

Article history:
Received on: December 02, 2024
Accepted on: February 18, 2025
Available online: March 25, 2025

Key words:
Biofilm formation,
EPS production,
Quorum sensing,
Isoxazolones,
Quorum quenching,
Human and plant pathogen.

ABSTRACT

Inhibiting quorum sensing (QS) to hinder extracellular polymeric substances (EPS) production and biofilm formation in pathogenic bacteria was studied as an efficient alternative for controlling the infections caused by multiple drug resistant (MDR) bacteria. In the present study, the isoxazolone derivative (4-(2-hydroxy-5-methoxybenzylidene)-3-methylisoxazol-5(4*H*)-one) was tested for its ability to inhibit EPS production and biofilm formation in human as well as plant pathogenic bacteria. The binding affinity of the derivative to the quorum sensing regulatory proteins (AgrA and LasR) was investigated by carrying out molecular docking studies. The derivative was capable of substantially inhibiting EPS production and biofilm formation in *Pseudomonas aeruginosa, Staphylococcus aureus, Erwinia carotovora,* and *Ralstonia solanacearum* at subinhibitory concentrations. Furthermore, molecular docking studies confirmed our results with notable binding affinity –7.5 kcal/mol to transcriptional activator protein LasR and binding affinity –6.8 kcal/mol to AgrA (transcription factor), both controlling expression of virulence factors in *P. aeruginosa* and *S. aureus,* respectively. This is a first report that proves that isoxazolone derivatives have quorum quenching potential (QQ) against both human and plant pathogens, which can be applied in medical and agricultural fields.

1. INTRODUCTION

Isoxazolones, an important class of heterocyclic compounds, are widely known for their ability to interact with biomolecules of various biological pathways. Isoxazolone derivatives have applications in numerous fields because of their antimicrobial, antioxidant, and anticancer activities [1-3]. Quorum sensing (QS) is a communication between bacterial cells that helps bacteria regulate gene expression based on the cell density. Quorum sensing mediates expression of those phenotypes that are metabolically and energetically steep to be performed by a single bacterial cell. Communication between the bacterial cells occurs with the help of autoinducers, which are AHLs (Acyl homoserine lactones) in Gram negative bacteria and cyclic peptides in Gram positive bacteria [1]. Regulation of a variety of genes involved in functions such as pigment production, biofilm formation, toxin production, EPS production, virulence factor production, enzyme production, conjugation, and motility is controlled by quorum sensing mechanisms. Quorum sensing mechanisms play a major role in the pathogenicity and virulence of bacterial pathogens [4-5].

One of the most important phenotypes contributing to the virulence of pathogenic bacteria is their ability to form biofilms. Biofilms are spatial structures made up of a self-synthesized matrix of EPS, which helps to increase the protection of microbial cells by several folds. Bacteria in biofilm are more resistant to host immune responses and to the action of antimicrobials as compared to planktonic cells [6-7]. Within the biofilm, bacteria communicate through quorum sensing mechanisms thus modulating pathogenesis, motility, nutrient acquisition, production of secondary metabolites, as well as further spread and maturation of the biofilm [8].

Use of antibiotics is a common practice for the treatment of bacterial infections; however, bacteria when present in the biofilm are several fold more resistant to antibiotics [9]. Also, indiscriminate use of antibiotics has led to the development of antibiotic resistance and the emergence of multiple drug resistant bacteria. Therefore, inhibiting quorum sensing (quorum quenching) by interfering in communication between the bacterial cells will have a profound negative effect on biofilm forming potential in pathogenic bacteria. This serves as an efficient alternative to impede bacterial colonization and tackle infections caused by MDR bacteria [1]. Since quorum quenching does not involve inhibition of growth and proliferation of bacteria, there will not be any selection pressure on bacteria, and development of resistance towards quorum quenchers is less likely to occur [10].

In the present study, we are reporting for the first time an isoxazolone derivative having QQ potential (EPS production and biofilm formation)

**Corresponding Author:*
Lakshangy S. Charya, Microbiology Programme, School of Biological Sciences and Biotechnology, Goa University, Taleigao Plateau, Goa, India.
E-mail: lakshangy@unigoa.ac.in

against both gram-positive and gram-negative human (*Pseudomonas aeruginosa* and *Staphylococcus aureus*) and plant pathogens (*Erwinia carotovora* and *Ralstonia solanacearum*), which can be applied in medical and agricultural fields. Additionally, *In-silico* studies (molecular docking) were carried out to validate our QQ results.

2. MATERIALS AND METHODS

Bacterial cultures used: Clinical isolates of *Pseudomonas aeruginosa* and *Staphylococcus aureus* were obtained from Goa Medical College, Bambolim, Goa. Plant pathogenic bacteria used were *Ralstonia solanacearum* strain MK1 (Accession number: OQ788252), isolated at Goa University from diseased (wilted) eggplant, and *Erwinia carotovora* BL0005 was procured from ICAR-IARI, New Delhi.

Chemical compound: Isoxazolone derivative (4-(2-hydroxy-5-methoxybenzylidene)-3-methylisoxazol-5(4*H*)-one) was prepared by our previously reported procedure [1]. The spectral data of this compound is in accordance with the reported spectrum. *4-(2*-hydroxy-*5*-methoxybenzylidene)-*3*-methylisoxazol-*5(4H)*-one; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 2.246 (s, 3H), 3.727 (s, 3H), 6.935 (d, J = 8.8 Hz, 1H), 7.144 (dd, J = 9.2 Hz, J = 3.2 Hz, 1H), 8.047 (s, 1H), 8.468 (d, J = 3.2 Hz, 1H) ppm.

Effect of isoxazolone derivative on growth of human and plant pathogenic bacteria: Bacteria were grown in the presence of different concentrations (10–500 µg/mL*)* of isoxazolone derivative prepared in DMSO at 110 rpm, 37ºC (for *Pseudomonas aeruginosa* and *Staphylococcus aureus*) or RT (for *Erwinia carotovora* and *Ralstonia solanacearum*). Appropriate solvent control and media control were kept. After incubation, the bacterial growth was monitored by checking the OD of culture broth at 600 nm. Overnight grown culture broth from each flask was serially diluted using 0.85% saline and spread plated on nutrient agar plates (for *Pseudomonas aeruginosa, Staphylococcus aureus*, and *Erwinia carotovora*) and Bacto-glucose (BG) plates (for *Ralstonia solnacearum*) and incubated for 24 hrs. After incubation, colonies were counted, and viable count was determined, and percentage survival was calculated using the formula:

Percentage survival of bacteria = (viable count in the presence of derivative / viable count in the absence of derivative) × 100.

Effect of isoxazolone derivative on EPS production by human and plant pathogenic bacteria: The test culture was grown in appropriate media (i.e. nutrient broth media for *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Erwinia carotovora;* BG media for *Ralstonia* solanacearum) containing different concentrations (10–500 µg/mL) of isoxazolone derivative. The culture broth was centrifuged at 10000 rpm for 10 min. Appropriate solvent control and media control were kept. To the supernatant, 3 volumes of chilled absolute ethanol were added. The mixture was then incubated at 4ºC for 18 h to precipitate EPS. After incubation, the mixture was centrifuged, and the pellet obtained was dissolved in 0.5 mL of distilled water. Total sugar content and total protein content in each sample were quantified by phenol sulfuric acid method and the Folin-Ciocalteau method, respectively [11-12]. In addition, the dry weight of precipitated EPS was also examined. All experiments were conducted in triplicates.

2.1. Effect of Isoxazolone Derivative on Biofilm Formation in Human and Plant Pathogenic Bacteria

Crystal violet assay: Overnight grown bacterial culture was diluted (1:100) with sterile nutrient broth (for *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Erwinia carotovora*) or BG medium (for *Ralstonia solanacearum*). The diluted culture broth along with different concentrations (10-500 µg/mL) of isoxazolone derivative as well as DMSO (solvent control) was added to the wells of a 96-well microtiter plate. The plate was incubated at 37ºC (for *Pseudomonas aeruginosa* and *Staphylococcus aureus*) or RT (for *Erwinia carotovora* and *Ralstonia solanacearum*) with constant shaking at 80 rpm for 24 hrs. Following incubation, the culture broth was discarded from each well, and the wells were washed gently with distilled water twice to remove the loosely attached cells. The wells were allowed to air dry completely and were later stained with 200 µL of 0.1% crystal violet. The plate was incubated for 45 min at room temperature. After incubation, the crystal violet was discarded, and wells were washed with distilled water to remove the excess dye. The microtiter plate was allowed to dry, and following that, 30% acetic acid (200 µL) was added to each well. The bound crystal violet was thus extracted, and its absorbance was read at 590 nm using 30% acetic acid as blank. The percent decline in biofilm formation in the presence of isoxazolone derivative was calculated [13-14]. All experiments were conducted in triplicate.

SEM analysis: Overnight-grown bacterial cultures were diluted (1:100) with sterile nutrient broth (for *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Erwinia carotovora*) and BG broth (for *Ralstonia solanacearum*). The diluted culture broth was treated with different concentrations (10 µg/mL, 50 µg/mL, and 100 µg/mL) of isoxazolone derivative prepared in DMSO. Appropriate solvent control and media control were kept. The biofilm was allowed to grow on glass pieces (1 cm × 1 cm) by placing the glass pieces in diluted culture broth and incubating for 24 hrs at 37ºC (for *Pseudomonas aeruginosa* and *Staphylococcus aureus*) and RT (for *Erwinia carotovora* and *Ralstonia solanacearum*). After incubation, the culture broth was discarded, and the slides were gently rinsed with sterile 0.85% saline to remove unadhered cells and were then allowed to air dry. Biofilm formed on glass pieces was fixed overnight with 2.5% glutaraldehyde solution and subsequently dehydrated by a series (20%, 40%, 60%, 80%, 90%, and 100%) of acetone treatments for 10 minutes each. The treated glass pieces were air dried, sputter coated with gold, and visualized under a scanning electron microscope (Zeiss EVO 18) [15].

2.2. Computational Methodology: Molecular Docking Studies

AutoDock Vina v.1.1.2 was employed for molecular docking studies as described by Trott and Olson [16]. The crystal structure with PDB code 2uv0 for the (https://doi.org/10.1074/jbc.M700556200) protein LasR receptor of *Pseudomonas aeruginosa* and 4G4K for the protein AgrA LytTR domain of *Staphylococcus aureus* was employed as the receptors for Gram negative and Gram positive bacterium, respectively, and ligands, namely isoxazolone derivative (4-(2-hydroxy-5-methoxybenzylidene)-3-methylisoxazol-5(4*H*)-one), were docked into the binding site. LasR, a transcriptional activator of virulence genes in *Pseudomonas aeruginosa,* and AgrA control expression of over 200 virulence-related genes in *S. aureus*. Water molecules and existing ligands present within the protein structures were removed prior to docking. Polar hydrogen atoms were then added to the receptor structure using AutoDock tools v.1.5.6. Successively, a grid box of size 25 × 25 × 25 Å^3 was applied to encompass the binding

pocket. The grid box was defined with its center coordinates set at x = 23.793, y = 14.969, z = 77.95 for 2uv0, and x = 32.0255, y = 24.947, z = 40.691 for 4G4K.

The docking parameters were set with an exhaustiveness value of 32, and the search was randomized using seed=1 to ensure reproducibility. The entire process aimed to predict the binding modes and affinities of ligands isoxazolone derivatives (4-(2-hydroxy-5-methoxybenzylidene)-3-methylisoxazol-5(4*H*)-one). The ligand-protein interactions were analyzed using LigPlot^{+} v.2.2 software [17].

3. RESULTS AND DISCUSSION

Quorum quenchers disrupt the colonization and spread of bacterial cells by interfering in the expression of various quorum sensing regulated traits. The potential of the isoxazolone derivative to act as a quorum quencher by inhibiting EPS production and biofilm formation in human as well as plant pathogenic bacteria was checked. The isoxazolone derivative [Figure 1] synthesized by the reported procedure was confirmed by H^1-NMR analysis [1]. Prior to studying the quorum sensing inhibitory potential of isoxazolone derivative I, its effect on the growth of all four bacteria was checked. This was done to make sure that inhibition of EPS production and biofilm formation is due to quorum quenching and not growth inhibition. It was observed that there was no significant growth inhibition up to the concentration of 100 µg/mL. A considerable growth inhibition was seen only at the concentrations 250 µg/mL and above [Figure 2]. Therefore, for further studies, the decrease in EPS production and biofilm formation up to the isoxazolone derivative concentration of 100 µg/mL was considered.

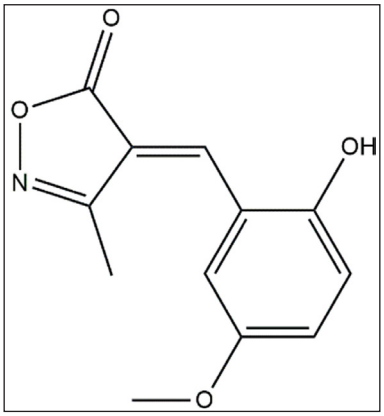

Figure 1: Structure of Isoxazolone derivative (4-(2-hydroxy-5-methoxybenzylidene)-3-methylisoxazol-5(4*H*)-one).

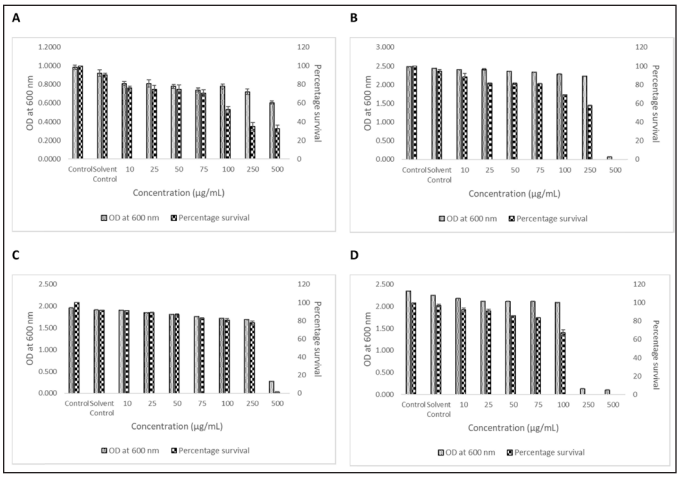

Figure 2: Graphs showing effect of increasing concentration (10 – 500 µg/mL; Solvent control: 0 µg/mL) of Isoxazolone derivative on growth of **(A)** *Pseudomonas aeruginosa,* **(B)** *Staphylococcus aureus,* **(C)** *Erwinia carotovora,* **(D)** *Ralstonia solanacearum.*

EPS is an integral part of the biofilm matrix and is a prerequisite for the formation of biofilm, making the adhesion process more favorable [18-20]. Extracellular polymeric substance (EPS) is predominantly made up of exopolysaccharides; however, it also comprises proteins, lipids, deoxyribonucleic acids, and other macromolecules; however, the basic composition differs in different pathogenic bacteria [21]. EPS helps in maintaining the structural integrity of the biofilm, making pathogens more resistant and impermeable to antibiotics [18]. EPS within the biofilm acts as a barrier against penetration of antimicrobial agents. Various cationic and anionic molecules present within the EPS bind to the charged antimicrobials and prevent its further spreading [22]. The isoxazolone derivative showed a substantial decrease in EPS production by *Pseudomonas aeruginosa, Staphylococcus aureus, Erwinia carotovora,* and *Ralstonia solanacearum* in a concentration dependent manner, as summarized in Table 1 and Figure 3. A significant decrease was observed in EPS production w.r.t. total sugar content, total protein content, and dry weight of EPS at subinhibitory concentrations. The highest decrease in total sugar content and EPS dry weight was shown in *Pseudomonas aeruginosa*, whereas that of total protein content was shown in *Ralstonia solanacearum.* Accordingly, on average, the inhibitory activity of EPS production in the pathogens was in the

Table 1: Percentage reduction observed in EPS production (w.r.t total sugar content, total protein content and EPS dry weight) by human pathogenic bacteria *Pseudomonas aeruginosa* (PA) and *Staphylococcus aureus* (SA) and plant pathogenic bacteria *Erwinia carotovora* (EC) nd *Ralstonia solanacearum* (RS) in the presence of increasing concentration of (10-100 µg/mL) of Isoxazolone derivative.

Concentration (µg/mL)	Percentage (%) reduction in Total Sugar content				Percentage (%) reduction in Total Protein content				Percentage (%) reduction in EPS dry Weight			
	PA	SA	EC	RS	PA	SA	EC	RS	PA	SA	EC	RS
10	39.81	0.47	5.38	7.10	25.26	3.87	7.9	8.45	66.67	18.18	20	45.61
25	43.67	10.84	6.46	14.51	27.45	14.83	9.74	40.73	76.81	39.39	34.28	59.64
50	44.51	22.46	7.08	42.93	28.28	37.35	25.86	56.05	85.07	51.51	57.14	66.67
75	70.50	42.10	8.16	50.49	46.32	61.81	39.69	60.35	92.75	66.67	62.85	73.68
100	76.43	55.77	15.39	75.43	50.33	67.03	63.23	89.11	97.10	78.78	85.17	89.47

Table 2: Percentage reduction observed in biofilm formation in human pathogenic bacteria *Pseudomonas aeruginosa* and *Staphylococcus* and plant pathogenic bacteria *Erwinia carotovora* and *Ralstonia solanacearum* in the presence of increasing concentration (10–100 µg/mL) of Isoxazolone derivative.

Concentration (µg/mL)	Percentage reduction (%) in biofilm formation			
	Pseudomonas aeruginosa	*Staphy -lococcus aureus*	*Erwinia carotovora*	*Ralstonia solanacearum*
10	47.61	21.27	20.77	7.75
25	61.22	20.92	33.66	9.08
50	67.62	25.17	53	64.15
75	67.22	38.65	68.55	81.79
100	74.83	47.87	80	87.67

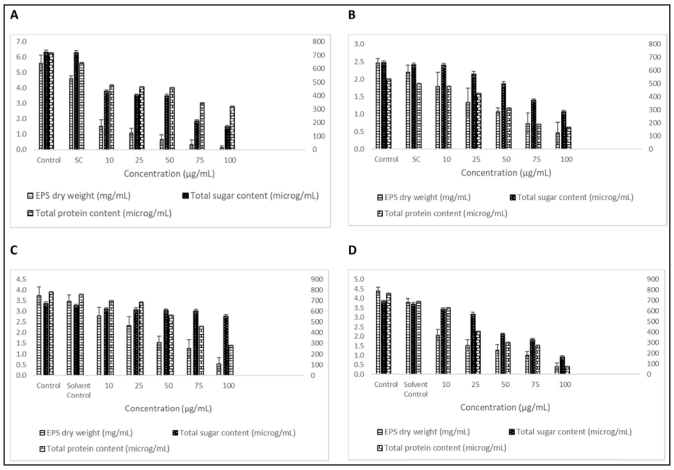

Figure 3: Graphs showing effect of increasing concentration (10 – 100 µg/mL; Solvent control: 0 µg/mL) of Isoxazolone derivative on EPS production (w.r.t. Total sugar content, Total protein content and EPS dry weight) by **(A)** *Pseudomonas aeruginosa,* **(B)** *Staphylococcus aureus,* **(C)** *Erwinia carotovora,* **(D)** *Ralstonia solanacearum.*

following order: *Pseudomonas aeruginosa* > *Ralstonia solanacearum* > *Staphylococcus aureus* > *Erwinia carotovora*.

Isoxazolone derivative I also showed considerable antibiofilm activity. Biofilm formation is a quorum sensing mediated process that contributes to the spread of virulence encoding genes in pathogenic bacteria through horizontal gene transfer, and it also helps the bacteria to become more resistant to the action of antibiotics [23] Bacteria within the biofilm are resilient and are very difficult to eradicate, enabling them to survive in harsh environmental conditions [8]. On carrying out the crystal violet assay, it was observed that the isoxazolone derivative considerably inhibited biofilm formation in *Pseudomonas aeruginosa, Staphylococcus aureus, Erwinia carotovora,* and *Ralstonia solanacearum* in a dose dependent fashion [Figure 4]. Percentage decline in biofilm formation is as depicted in [Table 2]. Percent reduction observed at subinhibitory concentration, i.e. 100 µg/mL, was 74.83%, 47.87%, 80%, and 87.67% in *Pseudomonas aeruginosa, Staphylococcus aureus, Erwinia carotovora,* and *Ralstonia solanacearum,* respectively, with the best antibiofilm

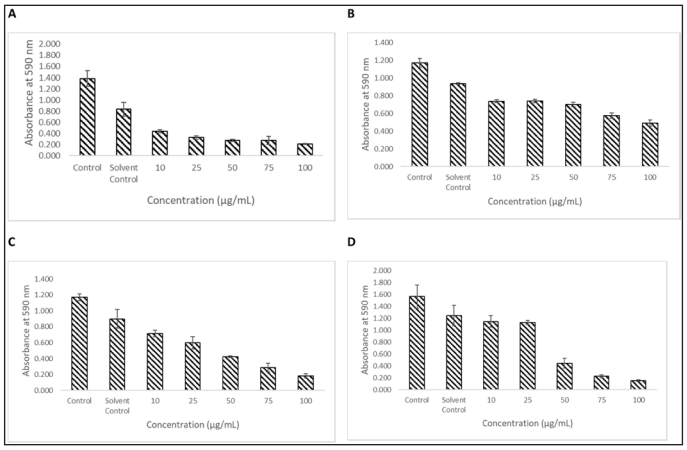

Figure 4: Graphs showing effect of increasing concentration (10 – 100 µg/mL; Solvent control: 0 µg/mL) of Isoxazolone derivative on biofilm formation in **(A)** *Pseudomonas aeruginosa,* **(B)** *Staphylococcus aureus,* **(C)** *Erwinia carotovora,* **(D)** *Ralstonia solanacearum.*

activity observed in *Ralstonia solanacearum*. The results obtained by the crystal violet assay were also supported by scanning electronic microscopic analysis of bacterial biofilm grown on glass surfaces in the presence of increasing concentrations (10 µg/mL, 50 µg/mL, and 100 µg/mL) of derivative. In control samples as well as in the presence of solvent control, clumped and aggregated bacterial cells in a well-developed mature biofilm were observed. Bacterial cell density in biofilm decreased, and solitary bacterial cells were observed as the concentration of isoxazolone derivative was increased [Figure 5]. Since EPS production and biofilm formation are related, the antibiofilm activity shown by the isoxazolone derivative is in accordance with the decrease observed in EPS production.

Staphylococcus aureus and *Pseudomonas aeruginosa* are common opportunistic pathogens known to cause severe nosocomial infections. *Pseudomonas aeruginosa* is known to even cause deadly infections in patients with compromised immune systems infected with HIV, patients with severe burns, cancer, or post-surgery trauma [24]. *Staphylococcus aureus* is known to cause a range of infections, starting from skin or soft tissue infections to life threatening blood infections, septicemia, and toxic shock syndrome [25]. The ability of these bacteria to form biofilm greatly contributes to their pathogenicity. Biofilm formation in these bacteria is regulated by the Agr QS system in *Staphylococcus aureus,* whereas in *Pseudomonas aeruginosa* it is regulated by the LasI/ LasR, RhlI/RhlR, and PQS QS systems [24-25]. The biofilm forming ability of these bacteria has several implications, most importantly in the health sector. *Pseudomonas aeruginosa* is known to cause highly structured biofilm in patients with chronic infections such as chronic wound infection, chronic lung infection, and chronic rhinosinusitis. *Pseudomonas aeruginosa* because of their biofilm forming ability, dominates and multiplies in the polymicrobial environment of cystic fibrosis lungs [24]. Patients with severe skin infections such as atopic dermatitis are highly susceptible to *Staphylococcus aureus* infection because of its biofilm forming potential. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are also known to colonize medical materials such as medical catheters, contact lenses, and implants, thus causing the spread of chronic nosocomial infections [8, 24-25]. Infections caused by these biofilm-forming nosocomial pathogens are difficult to eradicate since antibiotics are unable to penetrate through the layers of biofilm. Also, bacteria within the biofilm have the ability to evade

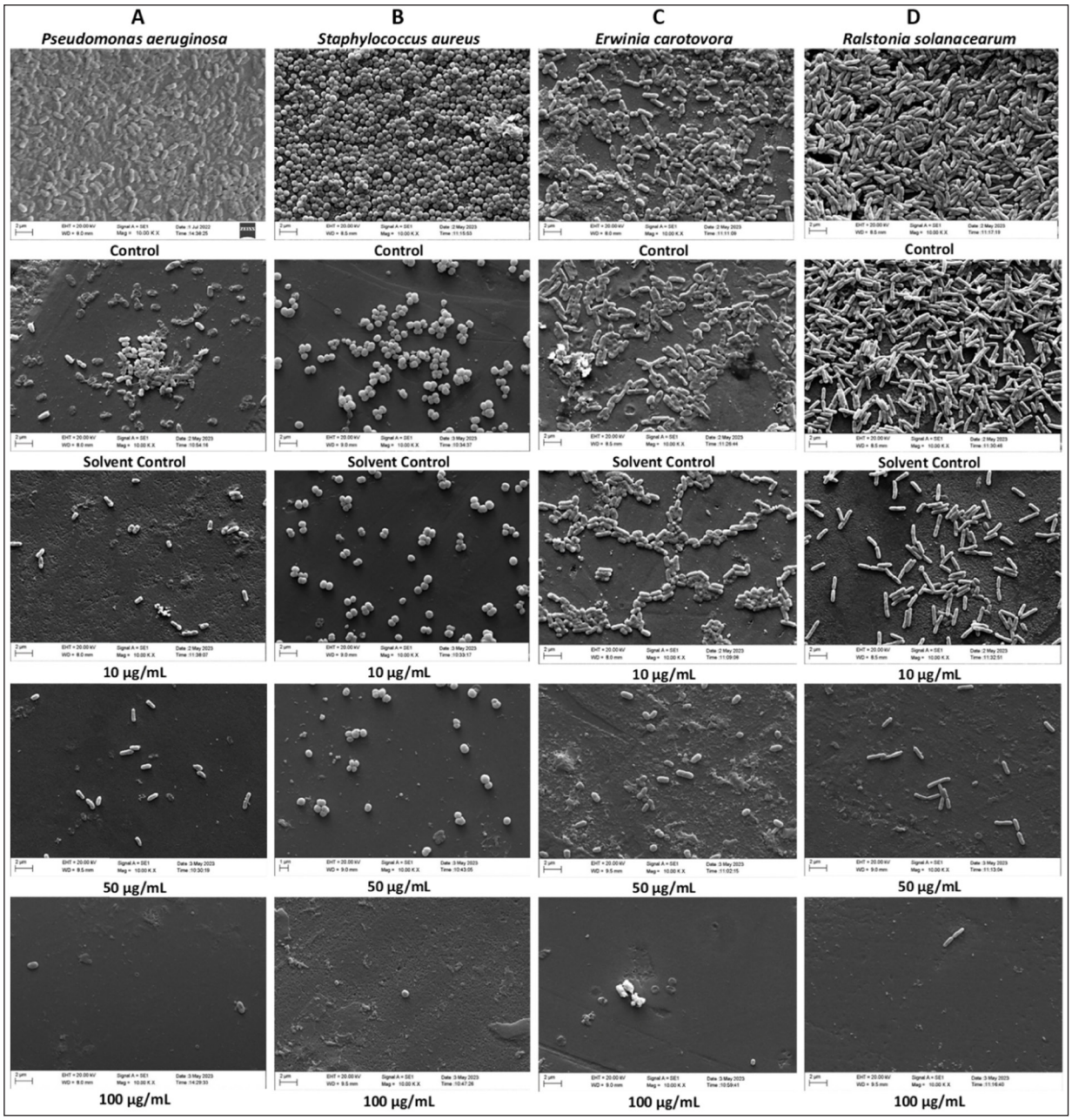

Figure 5: SEM images of **(A)** *Pseudomonas aeruginosa,* **(B)** *Staphylococcus aureus,* **(C)** *Erwinia carotovora* and **(D)** *Ralstonia solanacearum* biofilm grown in the presence of increasing concentration (10 µg/mL, 50 µg/mL and 100 µg/mL) of Isoxazolone derivative.

host immune response, impede phagocytosis, have genome plasticity, and also the exchange of genetic material between the bacterial cells through horizontal gene transfer improves their adaptability [24-25]. As a result, the application of a limited amount of antibiotics has no effect on the pathogens, and use of indiscriminate amounts of antibiotics leads to the development of antibiotic resistance and the emergence of MDR bacteria such as Methicillin Resistant *Staphylococcus aureus* (MRSA) [25]. Consequently, to tackle infections caused by deadly pathogens, an alternative strategy is a need of an hour.

Plant pathogenic bacteria *Erwinia carotovora* and *Ralstonia solanacearum* also have biofilm forming potential regulated by ExpI/ExpR and SolI/SolR, respectively [4]. *Erwinia carotovora* causes soft rot diseases in monocot and dicot plant including various food plants, as well as ornamental crops. The development of soft rot symptoms by the bacterial pathogen is because of its ability to produce various plant cell wall degrading enzymes [26-27]. Biofilm formation by *Erwinia carotovora* is very crucial for its pathogenesis, since it helps the bacteria to attach to surfaces and promote cell aggregation [19]. *Ralstonia solanacearum* is a soil bacterium responsible for one of the most damaging plant diseases called bacterial wilt. Bacteria invade the secondary roots through small wounds or openings, colonize plant tissues, enter xylem eventually, and through the xylem spreads into stems and leaves. *Ralstonia solanacearum* forms biofilm like aggregates containing a large number of cells and made up of EPS,

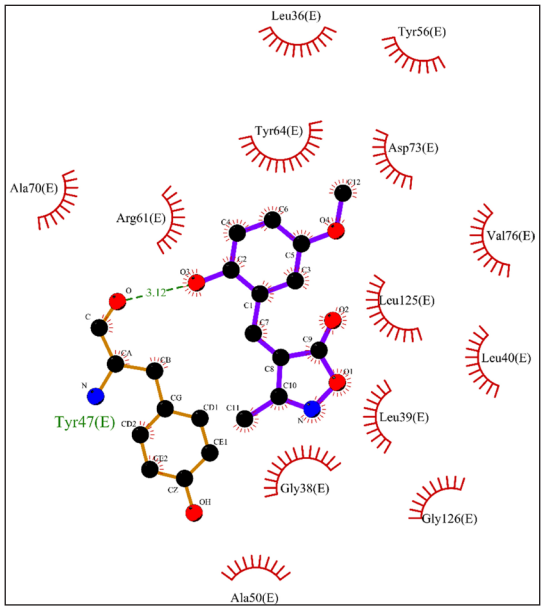

Figure 6: 2D Protein-ligand interactions of selected isoxazolone derivative with 2UV0 of *P. aeruginosa*.

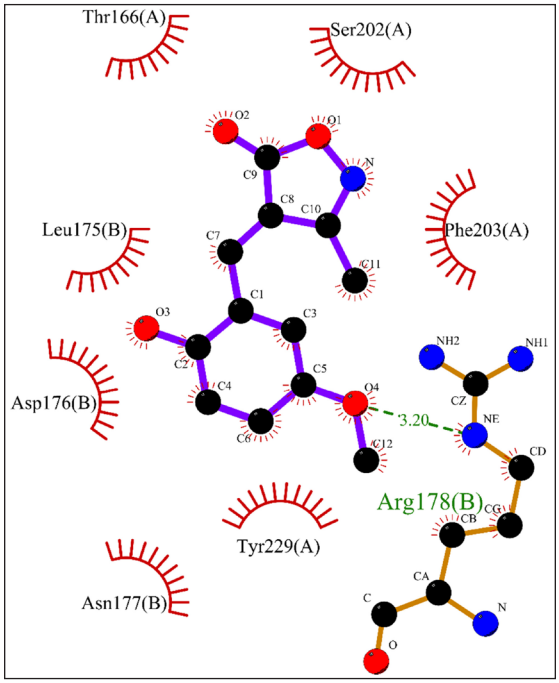

Figure 7: 2D Protein-ligand interactions of selected isoxazolone derivative. with 4G4K of *S.aureus*.

which blocks the xylem vessels, preventing the flow of xylem sap, thus obstructing the water and nutrient transport, eventually leading to the wilting of the plant [19, 28-29]. Biofilm formation also helps the bacteria in surface attachment to the plant tissue and also increases their resistance to antimicrobial compounds [28]. EPS production and biofilm formation are very crucial for pathogenesis in human as well as plant pathogenic bacteria, and in addition, they also make these pathogens resistant to several antibiotics. Since biofilm formation and EPS production are quorum sensing mediated phenomena, the use of quorum quenching can be used as an alternative to antibiotics to prevent the expression of these virulent phenotypes.

In our earlier investigation, we had reported (preliminary studies) the ability of isoxazolone derivative I to inhibit quorum sensing mediated pigment production in bioreporter *Chromobacterium violaceum* [1] and in the present investigation, we are reporting its potential in inhibiting EPS production and biofilm formation in human and plant pathogens *Pseudomonas aeruginosa, Staphylococcus aureus, Erwinia carotovora,* and *Ralstonia solanacearum.* This potential makes it an efficient alternative to antibiotics to control infections caused by human as well as plant pathogenic bacteria and prevent the development of human/plant diseases. Since inhibition of EPS production and biofilm formation was due to QQ without growth inhibition, the development of resistance to isoxazolone derivatives is less likely to occur. Isoxazolone derivatives can be used to target multiple quorum sensing systems because of their ability to show quorum quenching against both gram-positive and gram-negative bacterial human and plant pathogens. This derivative can therefore be used as a single weapon to tackle multiple bacterial infections.

3.1. Molecular Docking Studies

In the present research, in addition to antibiofilm studies against *Pseudomonas aeruginosa, Staphylococcus aureus, Erwinia carotovora,* and *Ralstonia solanacearum*, *in-silico* validation of the isoxazolone derivative (4-(2-hydroxy-5-methoxybenzylidene)-3-methylisoxazol-5(4*H*)-one) was tested for receptor protein LasR of *Pseudomonas aeruginosa* and protein AgrA LytTR domain of *Staphylococcus aureus* via molecular docking analysis, which boosted our findings, making isoxazolone a potential QS inhibitor. Molecular docking studies revealed notable binding affinity -7.4 kcal/mol to transcriptional activator protein LasR and binding affinity -6.8 kcal/mol to AgrA (transcription factor), both controlling expression of virulence factors in *P. aeruginosa* & *S. aureus,* respectively, through QS [Figures 6,7].

In *Pseudomonas aeruginosa,* quorum sensing is mediated by three QS systems, i.e. LasI/LasR, RhlI/RhlR, and pqs. The LasR gene is however at the center of the QS regulon and is required for the activation of other QS genes such as *lasI, rhlI,* and *rhlR* which further activates pqs. Thus, the LasR gene is indispensable for regulating quorum sensing, and its inhibition will lead to diminished expression of QS regulated genes [30]. Ligplot analysis revealed that the isoxazolone derivative (4-(2-hydroxy-5-methoxybenzylidene)-3-methylisoxazol-5(4*H*)-one) formed both hydrophobic as well as hydrophilic interactions within the ligand binding pocket of LasR. Amino acid Tyr47(E) strongly interacted by hydrogen bonding with hydroxyl group of isoxazolone derivative, whereas aromatic ring of isoxazolone derivative displayed hydrophobic interaction with Tyr64(E), Arg61(E), Asp73(E), and Val76(E). Additionally, isoxazolone 5 membered heterocyclic ring (containing oxygen and nitrogen atoms) showed hydrophobic interaction with amino acids Leu39 (E), Leu 125 (E), and Gly38(E).

In *Staphylococcus aureus*, accessory gene regulator (Agr) operon *agrABCD* facilitates the expression of virulence factors responsible for invasive infections. Transcription factor AgrA controls expression of above 200 virulence-related genes. Therefore, disrupting Agr signaling is one proficient strategy to fight invasive *S. aureus* infections. The ligand demonstrated a mixture of hydrophilic and

hydrophobic interactions. Analysis of the protein-ligand interaction for isoxazolone derivative (4-(2-hydroxy-5-methoxybenzylidene)-3-methylisoxazol-5(4*H*)-one) revealed hydrogen bonding with Arg178(B) strongly interacted by hydrogen bonding with the hydroxyl group of the isoxazolone derivative, whereas the aromatic ring of the isoxazolone derivative displayed hydrophobic interaction with Tyr 229 (A), Leu 75(B), and Asp 176 (B). Additionally, isoxazolone 5 membered heterocyclic ring (containing oxygen and nitrogen atoms) showed hydrophobic interaction with amino acids Thr166 (A), Ser 202 (A), and Phe203 (A). In line with the above docking results, the isoxazolone derivative (4-(2-hydroxy-5-methoxybenzylidene)-3-methylisoxazol-5(4*H*)-one) significantly reduced biofilm formation in *P. aeruginosa* and *S. aureus,* confirming its QQ potential.

Isoxazolone derivatives have earlier been reported for antimicrobial, antioxidant, anticancer, antidiabetic, and anti-HIV activities [2-3, 31-32]. However, this is the first study reporting the inhibition of quorum sensing-mediated EPS production and biofilm formation by an the isoxazolone derivative in human as well as plant pathogenic bacteria. This is also the first investigation reporting the docking potential of Isoxazolone derivative to the LasR and Agr genes playing a crucial role in the QS system of *Pseudomonas aeruginosa* and *Staphylococcus aureus,* respectively. Bacterial infections put tremendous pressure on the economy and public health management of the country. Similarly, bacterial plant diseases lead to huge losses in the agricultural sector and also result in famine like situations in developing countries. Therefore, application of QQ molecules to tackle infections caused by these pathogenic bacteria can save millions of lives as well as the country's economy. In this study, the QQ ability of isoxazolone derivatives sheds light on their huge applications in medical as well as agricultural fields. Isoxazolone derivatives can replace or can be used synergistically along with the antibiotic, thus decreasing the usage of antibiotics and moreover, preventing the development of antibiotic resistance.

4. CONCLUSION

This is a first report on isoxazolone derivative showing a substantial inhibition in QS mediated phenotypes, *viz*. biofilm formation and EPS production by both Gram positive and Gram negative infectious humans (*P. aeruginosa* and *S. aureus*) and plant pathogenic bacteria (*E. carotovora* and *R. solanacearum*), attenuating their disease developing potential, thus projecting their tremendous applications in both the medical and agricultural fields in the near future. These results were further validated by molecular docking studies.

5. ABBREVIATIONS

EPS: Extracellular polymeric substances. QS: Quorum sensing. QQ: Quorum quenching. Kcal/mol: Kilocalories per mole. AHL: Acyl homoserine lactone. MDR: Multidrug resistant. DMSO: Dimethyl sulfoxide. OD: Optical density. RT: Room temperature. hrs: hours. mins: minutes. mL: milliliter. µL: microliter.

6. ACKNOWLEDGEMENTS

Komal Salkar is thankful to the Goa University for providing research studentship and Directorate of Higher Education, Government of Goa for Manohar Parrikar Goa Scholars Award 2021-22. Authors also acknowledge UGC-SAP and DST-FIST, Government of India for instrument funding. Hari Kadam is thankful to University Grants Commission, Government of India and Department of Science and Technology, Government of Goa for project funding.

7. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This article does not contain any studies involving human participants or laboratory animals, and no clinical trials have been carried out by any of the authors.

10. DATA AVAILABILITY

The authors confirm that the data supporting the findings of this study are available within the article.

11. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

12. PUBLISHER'S NOTE

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REFERENCES

1. Kadam HK, Salkar K, Naik AP, Naik MM, Salgaonkar LN, Charya, L, et al. Silica supported synthesis and quorum quenching ability of isoxazolones against both gram positive and gram negative bacterial pathogens. Chemistry Select. 2021;6(42):11718-28.
2. Hushare VJ, Rajput PR. Synthesis, characterization and antimicrobial activity of some novel isoxazoles. Rasayan J Chem. 2012;5(1):121-6.
3. Arya GC, Kaur K, Jaitak V. Isoxazole derivatives as anticancer agent: a review on synthetic strategies, mechanism of action and SAR studies. Eur J Med Chem. 2021;221:113511.
4. Baltenneck J, Reverchon S, Hommais F. Quorum sensing regulation in phytopathogenic bacteria. Microorganisms. 2021;9(2):239.

5. Yan C, Li X, Zhang G, Zhu Y, Bi J, Hao H, Hou H. Quorum sensing-mediated and growth phase-dependent regulation of metabolic pathways in Hafnia alvei H4. Front Microbiol. 2021;12:567942.
6. Paluch E, Rewak-Soroczyńska J, Jędrusik I, Mazurkiewicz E, Jermakow KJAM. Prevention of biofilm formation by quorum quenching. Appl Micrbiol Biotechnol. 2020;104:1871-1881.
7. Wright PP, Ramachandra SS. Quorum sensing and quorum quenching with a focus on cariogenic and periodontopathic oral biofilms. Microorganisms. 2022;10(9):1783.
8. Preda VG, Săndulescu O. Communication is the key: biofilms, quorum sensing, formation and prevention. Discov. 2019;7(3):e100.
9. Zhou L, Zhang Y, Ge Y, Zhu X, Pan J. Regulatory mechanisms and promising applications of quorum sensing-inhibiting agents in control of bacterial biofilm formation. Front Microbiol. 2020;11:589640.
10. Li T, Mei Y, He B, Sun X, Li J. Reducing quorum sensing-mediated virulence factor expression and biofilm formation in hafnia alvei by using the potential quorum sensing inhibitor L-carvone. Front Microbiol. 2019;9:3324.
11. Al-Shabib NA, Husain FM, Hassan I, Khan MS, Ahmed F, Qais FA, et al. Biofabrication of zinc oxide nanoparticle from Ochradenus baccatus leaves: broad-spectrum antibiofilm activity, protein binding studies, and in vivo toxicity and stress studies. J Nanomater. 2018;2018:8612158.
12. Richards C, O'Connor N, Jose D, Barrett A, and Regan F. Selection and optimization of protein and carbohydrate assays for the characterization of marine biofouling. Anal Methods. 2020;12(17):2228-36.
13. Naik MM, Naik SP, Dubey SK, Bhat C, Charya LSJ. Enhanced exopolysaccharide production and biofilm forming ability in methicillin resistant Staphylococcus sciuri isolated from dairy in response to acyl homoserine lactone (AHL). Food Sci Technol. 2018;55:2087-94.
14. Singh VK, Mishra A, and Jha B. 3-Benzyl-hexahydro-pyrrolo [1, 2-a] pyrazine-1, 4-dione extracted from Exiguobacterium indicum showed anti-biofilm activity against Pseudomonas aeruginosa by attenuating quorum sensing. Front Microbiol. 2019;10:1269.
15. Nithya C, Begum MF, Pandian SK. Marine bacterial isolates inhibit biofilm formation and disrupt mature biofilms of Pseudomonas aeruginosa PAO1. Appl Microbiol Biotechnol. 2010;88(1):341-58.
16. Trott O, Olson AJ. AutoDock vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J Comput Chem. 2010;31(2):455-61.
17. Laskowski RA, Swindells MB. LigPlot+: multiple ligand-protein interaction diagrams for drug discovery. J Chem Inf Model. 2011;51(10):2778-86.
18. Myszka K, and Czaczyk K. Characterization of adhesive exopolysaccharide (EPS) produced by Pseudomonas aeruginosa under starvation conditions. Curr Microbiol. 2009;58:541-6.
19. Mina IR, Jara NP, Criollo JE, Castillo JA. The critical role of biofilms in bacterial vascular plant pathogenesis. Plant Pathol J. 2019;68(8):1439-47.
20. Husain FM, Ahmad I, Al-Thubiani AS, Abulreesh HH, AlHazza IM, Aqil F. Leaf extracts of Mangifera indica L. Inhibit quorum sensing–regulated production of virulence factors and biofilm in test bacteria. Front Microbiol. 2017;8:727.
21. Fong J, Mortensen KT, Nørskov A, Qvortrup K, Yang L, Tan CH, et al. Itaconimides as novel quorum sensing inhibitors of Pseudomonas aeruginosa. Front Cell Infect. 2019;8:443.
22. Dincer S, Uslu FM, Delik A. Antibiotic resistance in biofilm. In: Dincer S, Ozdenefe MS, Arkut A, eds. Bacterial Biofilms. London: IntechOpen; 2020. pp. 135-48.
23. Li Y-H, Tian X. Quorum sensing and bacterial social interactions in biofilms. *Sensors*. 2012;12(3):2519-38.
24. Thi MTT, Wibowo D, Rehm BH. Pseudomonas aeruginosa biofilms. Int J Mol Sci. 2020;21(22):8671.
25. Idrees M, Sawant S, Karodia N, Rahman A. Staphylococcus aureus biofilm: morphology, genetics, pathogenesis and treatment strategies. Int J Environ Res Public Health. 2021;18(14):7602.
26. Joshi JR, Khazanov N, Senderowitz H, Burdman S, Lipsky A, Yedidia I. Plant phenolic volatiles inhibit quorum sensing in pectobacteria and reduce their virulence by potential binding to ExpI and ExpR proteins. Sci Rep. 2016;6(1):38126.
27. Ha NT, Minh TQ, Hoi PX, Thuy NTT, Furuya N, Long HH. Biological control of potato tuber soft rot using N-acyl-L-homoserine lactone-degrading endophytic bacteria. Curr Sci. 2018;115(10):1921-7.
28. Mori Y, Inoue K, Ikeda K, Nakayashiki H, Higashimoto C, Ohnishi K, et al. The vascular plant-pathogenic bacterium R alstonia solanacearum produces biofilms required for its virulence on the surfaces of tomato cells adjacent to intercellular spaces. Mol Plant Pathol. 2016;17(6):890-902.
29. Lowe-Power TM, Khokhani D, Allen C. How Ralstonia solanacearum exploits and thrives in the flowing plant xylem environment. Trends Microbiol. 2018;26(11):929-42.
30. Kostylev M, Kim DY, Smalley NE, Salukhe I, Greenberg EP, Dandekar AA. Evolution of the Pseudomonas aeruginosa quorum-sensing hierarchy. Proc Nat Acad Sci. 2019;116(14):7027-32.
31. Kushwaha PK, Srivastava KS, Kumari N, Kumar R, Mitra D, Sharon A. Synthesis and anti-HIV activity of a new isoxazole containing disubstituted 1, 2, 4-oxadiazoles analogs. Bioorg Med Chem. 2022;56:116612.
32. Nie JP, Qu ZN, Chen Y, Chen JH, Jiang Y, Jin MN, et al. Discovery and anti-diabetic effects of novel isoxazole based flavonoid derivatives. Fitoterapia. 2020;142:104499.

How to cite this article:
Salkar KS, Charya LS, Naik MM, Kadam HK. Quorum quenching of virulence traits expression in human and plant pathogens by Isoxazolone and its molecular docking studies. J App Biol Biotech. 2025;13(3):106-113. DOI: 10.7324/JABB.2025.197114