REVIEW

Breaking down bacterial communication: a review of quorum quenching agents

Tresa Boban[1], Sahaya Nadar[1*] and Savita Tauro[1]

Abstract

Background Bacterial cells communicate via small extracellular molecules that facilitate gene expression which is dependent on cell density and this mechanism is known as Quorum Sensing (QS). At low cell density, these bacteria show a single cellular type of behavior but once they reach the threshold level they alter to a multicellular type and hence a QS is established by the transfer of signalling molecules called autoinducers. Quorum sensing inhibitors (QSI) are those that hinder the quorum sensing pathway.

Main body of the abstract The emergence of antimicrobial resistance has become a threat to mankind with quorum sensing being one of the mechanisms responsible for this resistance. Hence Quorum Quenching can be considered to interrupt bacterial communication. This review focuses on the effects of different synthetic and natural quorum-sensing inhibitors on different organisms and how it affects their gene regulation.

Conclusion Different natural and synthetic agents can quench quorum sensing by various mechanistic pathways. The various quorum-sensing inhibitors against both Gram-positive and Gram-negative bacteria provide a wider scope to prevent emerging antimicrobial resistance.

Keywords Quorum sensing, Auto-inducers, Quorum sensing inhibitors

Introduction

The discovery of antibiotics in the 1920s made a great revolution in the history of medicine [1]. A century later, the unrestrained, inappropriate, and continuous use of these antimicrobials led to the evolution of Multiple Drug Resistant species [2]. It was identified that one of the main reasons behind this is the biofilm formation of the bacterial colonies [3]. Other bacterial behaviors also contribute to the evolution of resistant species; this is regulated by a phenomenon called Quorum Sensing [4]. Quorum Sensing (QS) is a cell-to-cell communication between bacteria. It regulates gene expression by producing small extracellular signalling chemical molecules known as Auto-Inducers (AIs). QS is a significant mechanism for regulating various physiological activities like virulence [5], motility [6], sporogenesis [7], bioluminescence [8], and biofilm formation [9]. It also regulates other infections related to phenotypes for a signal response, usually small molecules like oligopeptides, furanones, or fatty acid derivatives [10].

Advantage that QS offers the microorganism is to control its response to environmental stimuli. The QS system also strengthens the biotransformation pathways and hence plays an important role in the biodegradation of contaminants [11].

Quorum sensing is a population-dependent mechanism, when the concentration reaches the threshold level, the signalling molecules reach a detectable level which activates the responses of the bacteria [12].

There are three mechanistic concepts through which this QS works.

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I. By density-dependent action—At Low Cell Density (LCD) since, there are fewer signalling molecules, it doesn't reach the threshold level to identify the AIs. At High Cell Density (HCD) there are enough signalling molecules to establish the QS between bacterial cells, as depicted in Fig. 1.

II. There are specific receptors present in the cytoplasm of a bacterial cell that identifies these signalling molecules for the QS activity to initiate.

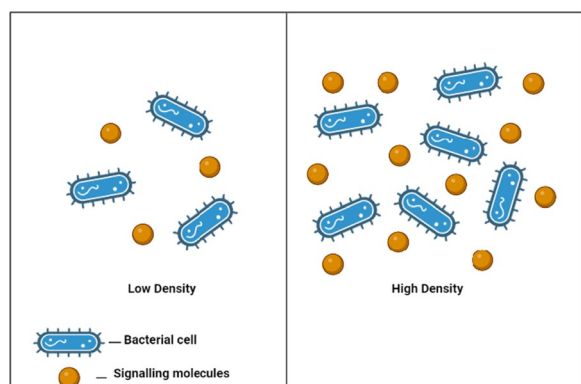III. As the AIs get detected and activation takes place, it would therefore boost more auto-inducers [13].

Fig. 1 The signalling molecules at low and high cell density of the bacterial cells

The AIs are different for Gram-positive and Gram-negative bacteria [14]. In Gram-positive bacteria, the signalling molecules are known as Autoinducing Peptides (AIPs) whereas in Gram-negative bacteria the autoinducer is N-Acyl-homoserine Lactone (AHL). AI-2 are the autoinducers adopted as the communication media between the bacterial cells in both Gram-negative and Gram-positive. The secretion of these signalling molecules is by the diffusion process [15].

Quorum sensing in Gram-negative bacteria
N-Acyl Homoserine Lactone (AHL) is responsible for the signalling in Gram-negative bacteria, and it was first observed in a marine bacteria known as *Vibrio fischeri.* The LuxI/LuxR-type quorum sensing in Gram-negative happens through different operons. LuxI proteins are responsible for the biosynthesis of the signalling molecules specifically for the Homoserine lactone whereas LuxR binds to the autoinducers and activates the target gene transcription once they reach the threshold level (Fig. 2). On high cell density, there will be an increase in the concentration of the signalling molecules [16].

Quorum sensing for Gram-positive bacteria
The autoinducers in Gram-positive are Auto-Inducing Peptides (AIPs). The Gram-positive bacteria produce small oligopeptides in their cells. Once they mature, they are transported across their cell membrane through an ABC transporter. The AIP concentration on reaching
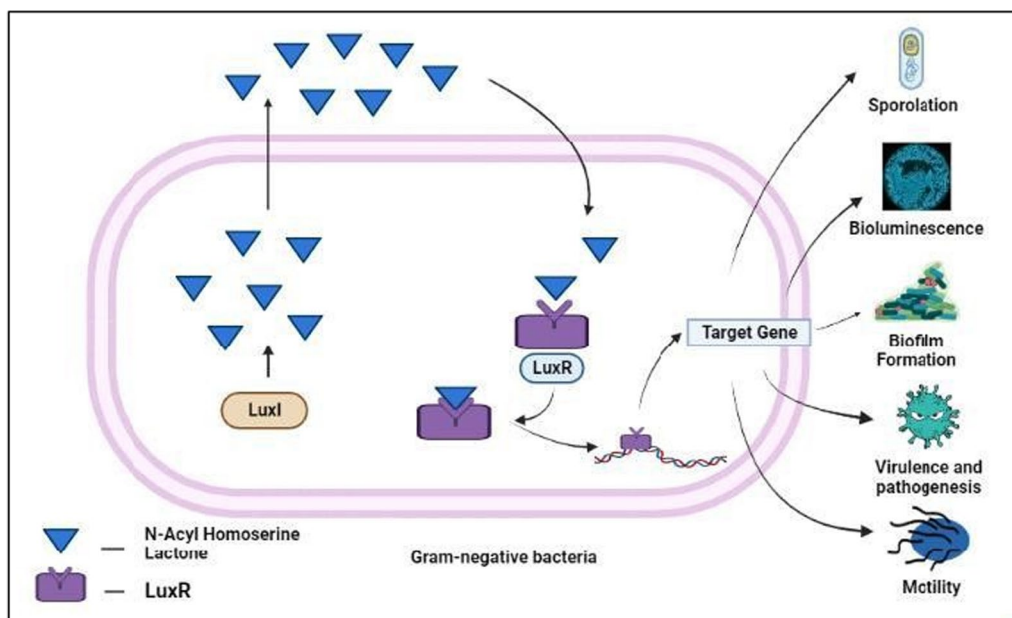

Fig. 2 Quorum sensing mechanism in Gram-negative bacteria involves the biosynthesis of autoinducer, N-Acyl Homoserine Lactone produced from LuxI followed by attachment of the autoinducer on LuxR receptor which leads to target gene transcription

the threshold level binds to a histidine sensor kinase segment, this then activates the kinase and successively auto-phosphorylates on histidine residue, which then passes to a response regulator protein and activates the gene expression (Fig. 3) [16].

Quorum Sensing Inhibitors

QS Inhibitors (QSI) act by inhibiting bacterial cell-to-cell communication without exerting pressure on the bacteria. Thus, QS inhibition disrupts cell-to-cell signalling and inhibits the autoinducer-induced QS system [17]. QS inhibition reduces bacterial virulence [18]. Antimicrobial resistance has been a major concern in various diseases through quorum-sensing inhibition mechanisms we can conquer this obstacle. QS inhibiting agents don't destroy or inhibit bacterial growth, they quench QS-regulated pathogenic activities like toxin production, swimming, swarming, motility, and biofilm formation which leads to loss of the ability of bacteria to cause disease and thus drug-resistant mutations [19].

Various mechanisms of QS inhibition are as follows:

1. Inhibiting the synthesis of signalling molecules [20]
2. Inactivation or enzymatic degradation of signalling molecules [20, 21]
3. Competing with signalling molecules [22]
4. Restricting the signal receptor complex [21]

To select effective quorum-sensing inhibitors, various factors have to be considered as follows [1, 23, 24]:

1. There must be a small molecule for the adequate depletion of the QS-regulated gene expression.
2. There must be no adverse effect with a particular quorum sensing regulator.
3. It must be chemically stable and tolerant to metabolic degradation.
4. The QSI must act longer than the native AHL

There is a wide range of Quorum sensing inhibitors like:

Natural QSIs include Prokaryotic QSIs that consist of various enzymes responsible for quenching the quorum sensing activities. For example, *Bacillus sp.* and *Agrobacterium tumefaciens* consist of Lactonase enzyme that degrade the AHL signalling molecules [1, 25–27], another such example is *Pseudomonas aeruginosa (P. aeruginosa) PAO1* consisting of the enzyme AHL-acylase that degrade the long chains of AHLs [1, 28].

Animal-based QSIs include interaction between the eukaryotic host and bacterial pathogen, which also acts by enzymatic inhibition, as seen in fungi *Penicillium* which consists of patulin and penicillic acid acting as a QSI by inhibiting the biofilm formation in *P. aeruginosa.*
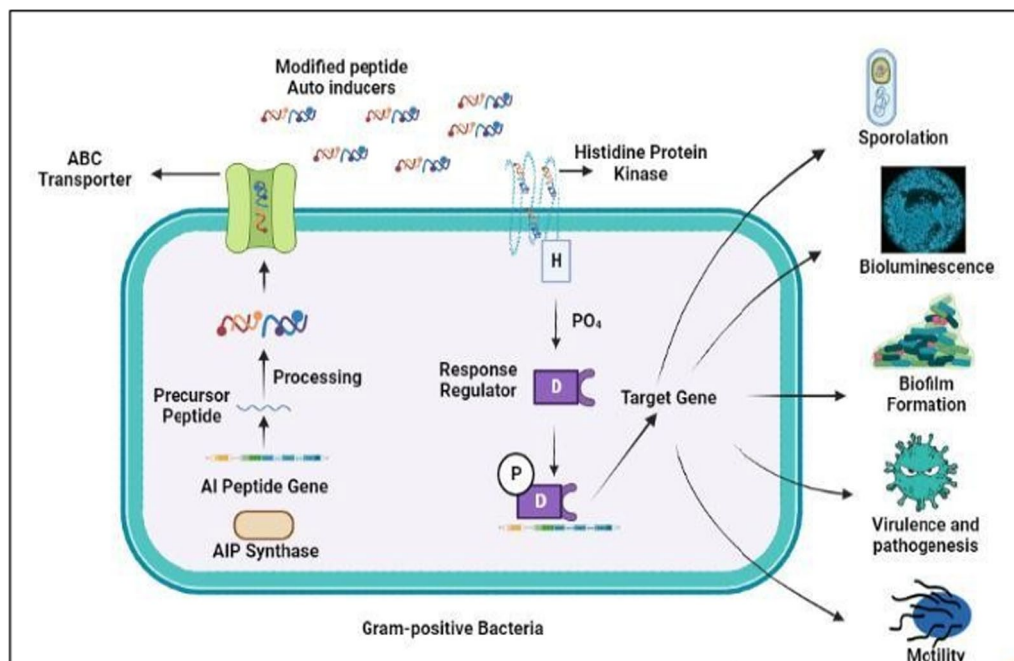

Fig. 3 Quorum sensing mechanism in Gram-positive bacteria includes the production of oligopeptides (AIP) which are transported into the bacterial cell through the ABC transporter and bind to histidine sensor kinase segment, once activated it auto-phosphorylates and activates targeted gene expression

Plants act as QSI by exhibiting chemicals like those of QS signals and additionally their ability to degrade the signalling receptor. In *Agrobacterium tumefaciens*, GABA promotes the AHL signal OHC8HSL degradation by the enzyme lactonase [27].

Synthetic QSIs outwit the limitation of lower concentration of signalling molecules in natural QSIs by chemically synthesizing them. A synthetic QSI mainly aims at the biosynthetic pathway that steers the production of signalling molecules, by changing the chain length, by substituting the signalling molecules, modifying the AHL ring moiety, and many more.

Synthetic compounds

Ibrahim et al., synthesized and evaluated 5-acetyl-4-methyl-2-(3-pyridyl) thiazole (AMPT) (Fig. 4) for the anti-quorum sensing activity against the MRSA strain. *Chromobacterium violaceum* produces a violet pigment called violacein through quorum sensing. Compounds that inhibit quorum sensing of the bacteria, reduce the violet colour. *C. violaceum* is used as a reference standard for quorum sensing inhibition sensing. It was found that AMPT can interfere with the quorum-sensing mechanism in *Staphylococcus aureus* and *C. violaceum*. The effect of AMPT on violacein of *C. violaceum* was observed based on the diameter of the yellowish opaque zone The plate with 4 mg/l concentration of AMPT showed the best anti-QS activity followed by 2, 1, and 0.5 mg/l concentration [29–31].

Rajkumari et al., evaluated 5-hydroxymethylfurfural (5-HMF) against *P. aeruginosa* PAO1. The QS boosts bacterial virulence by stimulating biofilm formation in *P. aeruginosa*. The anti-QS activity was also detected against *C. violaceum* using the agar well diffusion method, resulting in a non-pigmented zone of *C. violaceum* around a well-containing 5-HMF. 5-HMF decreased the production of *C. violaceum* by 98.12% at a conc. of 1.25 μL/mL. 5-HMF showed an effect against the biofilm formation of PAO1 with a MIC and sub-MIC concentration of 2.5 μL/mL and 1.25 μL/mL, respectively. 5-HMF inhibited the formation of biofilm by 29.80%. In the presence of 5-HMF, there was an inhibition of pyocyanin pigment up to 65.34%, a reduction of LasA staphylolytic activity by 77.92%, LasB elastase activity reduction by 36.48% and 57.71% reduction of chitinase activity. There was a decrease in cell
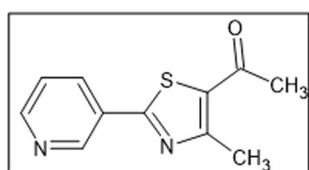

Fig. 4 5-Acetyl-4-methyl-2-(3-pyridyl) thiazole

surface hydrophobicity and production of eDNA by 16.01% and 17.57%, respectively. *Caenorhabditi elegans* infected with the PAO1 strain showed an improved mortality rate of 78.33% resulting from the effect of 5-HMF on killing *C. elegans* by PAO1. On treatment with sub-MIC of 5-HMF, there was a significant down-regulation by 92.79% and 90.15%, respectively, on the expression of lasI and lasR. On the exposure of 5-HMF, there was also an influence on lasB and rhlA by 59.66% and 66.20% inhibition, respectively [32–34].

Seleem et al., describe whether the analgesic drugs, indomethacin, and paracetamol act as quorum sensing inhibitors. In this study, *P. aeruginosa* O1 (PAO1) a QS positive strain and a violacein-negative mutant strain *C. violaceum* 026 (CV026) in the presence of AHL, the violacein purple pigment is induced. About 20 *Acinetobacter baumannii* isolates were used in this study. During the screening of the analgesic drugs for suppression of QS activity, it was seen that paracetamol inhibits the QS in CV026 and there was an inhibition of violacein pigment due to the halo formation around the well on purple background. Inhibition in CV026 was in presence of 1/8 MIC of paracetamol with an optical density (OD) value of 0.138 and optical density value of 0.332 in control CV026 therefore, leading to 58.4% violacein pigment inhibition. In the presence and absence of paracetamol, the virulence factors for PAO1 were determined. The MIC value of paracetamol against PAO1 was 256 μg/mL. In the presence of 1/8 MI of paracetamol, with a significant decrease in OD value ($p < 0.05$)from 0.723 to 0.243 at 600 nm an inhibition of biofilm formation by $66.4 \pm 0.4\%$ was found. The absorbance of culture supernatant in the absence was 0.128 and in the presence of paracetamol, it was found to be 0.085 thus showing an inhibition of pyocyanin production by $33.1 \pm 0.3\%$. Paracetamol also inhibited the swarming motility of PAO1 by $57.1 \pm 0.5\%$. In the presence of sub-MIC concentration of paracetamol, the twitching motility of PAO1 was inhibited by $7.7 \pm 0.2\%$. Gelatinase production was inhibited by $17.5 \pm 06\%$ which was noted in the Gz value of 0.40 in the presence of paracetamol and the absence of paracetamol showed a Gz value of 0.33. Protease production and Rhamnolipid (in the presence of sub-MIC of paracetamol) production were inhibited in the PAO1 strain by $8.7 \pm 0.4\%$ and $33.3 \pm 0.74\%$, respectively. Inhibition of sensitivity to hydrogen peroxide was by $9.1 \pm 0.2\%$. The virulence factor of clinical *A. baumannii* isolates in the presence and absence of paracetamol were studied. Out of 20, 13, and 7 clinical isolates of *A. baumannii* showed the MIC of paracetamol of 512 μg/mL for MIC_{50} and 1024 μg/mL for MIC_{100}, respectively. Biofilm formation was inhibited in the range of 39.7% to 93% in various isolates indicating that $\geq 50\%$ of inhibition was observed in 75% of *A.*

baumannii. In the presence of 1/8 MIC of paracetamol, there was an inhibition of twitching motility in the range of 6.7–82.5% with 40% isolate showing ≥ 50% inhibition of twitching motility and surface motility inhibition ranging from 7.7–29.4% with ≥ 20% inhibition of surface motility in 4 isolates. In the presence of 1/8 MIC of paracetamol, the sensitivity to hydrogen peroxide inhibition was in the range of 3.3–36.4% with 2 isolates inhibiting > 20%. The production of phospholipase in 8 isolates was detected, moderate in 2 isolates and weak in 6 isolates, the inhibition of phospholipase activity observed was in the range of 8.7–100% with 4 isolates ≥ 50% [35–37].

Vila-Sanjurjo et al., depicted the crosslinking of a category of nanoparticles of Chitosan with sodium tripolyphosphate (TPP) and Genipin (GNP) for its quorum quenching activity. In this study, the chitosan is first crosslinked with Genipin (as it is more compatible) and further an ionotropic formation of nanoparticles in the presence of TPP which leads to quorum quenching. The GNP Pre-Crosslinked Nanoparticles (PC-NPs) can effectively reduce the AHL-interfered quorum sensing of fluorescent biosensors in *Escherichia coli*. Two types of prototypes, Prototype A (PC-A) and Prototype B (PC-B) were prepared for the evaluation of the inhibition of quorum sensing activity of PC-NPs. PC-A and PC-B both showed a remarkable decrease in the fluorescence response of biosensor FI/OD600 but while comparing the two prototypes PC-B was more active than PC-A, with a decrease in the endpoint FI/OD600 in the range ~ 50–92% and ~ 35–85%, respectively. Along with the fluorescence reduction, their growth inhibition was also significantly minimized but the growth impairment magnitude by the PC-NPs was lesser than the fluorescence. The study also provided the data of standard deviation and mean in the experiment with replicates providing a *p* value of $p \leq 0.01$, $p \leq 0.001$, and $p \leq 0.0001$ [38–40].

Molnar et al., have come up with an ingenious approach for Cyclodextrin (CD) mediated inhibition of quorum sensing. The bioluminescence of *A. fischeri* was investigated. Various native cyclodextrin was considered in this article such as α-, ß- and γ-cyclodextrins (ACD),(BCD) and (GCD), 2-hydroxypropyl (HPACD, HPBCD) trimethylaminopropyl (QAACD), random methylated (RAMEA, RAMEB, RAMEG), sulfobutyl ether (SBEBCD) derivatives and their epichlorohydrin-crosslinked polymer (ACDPS and BCDPS). There was

a significant reduction in the bioluminescence with the highest inhibition observed was ~ 64% after 120 min obtained by 10 mM ACD; it was also low as 0.625 mM ACD concentration after 120 min. Inhibition of enzyme activity by the addition of ACD by 24% for 5 mM and 29% for 10 mM. BCD correspondingly to ACD showed an inhibitory effect with an increase in time and concentration but comparatively, the effect of BCD was less than ACD. Out of the studied BCDs, the SBECD revealed high QS inhibition ~ 30% at 5 mM concentration at 120 min. RAMEB and BCD exhibited a minute stimulatory effect < 20% on the bioluminescence at 30 min and 60 min at low concentration, whereas no effects showed at 120 min. With this, it was concluded that there were no significant antibacterial effects offered by BCD.

The data obtained Table 1 summarizes the efficiency order of cyclodextrins are: BCDPS < ACDPS, SBEBCD < RAMEB < QAACD < HPACD < BCD < RAMEA < ACD.

Efficiency order of cyclodextrin based on the MIC given in Table 1 are as follows: ACDPS, QAACD, RAMEB < ACD, BCDPS, HPACD, RAMEA, SBEBCD < HPBCD, BCD.

The low MIC of BCD and HPBCD indicates it can be considered for its effective quorum quenching activity. Based on the correlation coefficient(r) the ACD showed a well-built correlation between ACD concentration and bioluminescence at *r* > 0.85 at both time intervals depicting the great QQ effect of ACD [41–43].

Liu et al., had evaluated the quorum-quenching effects of aromatic furanones and brominated pyrrolones. They additionally designed and synthesized a new series of aryl-substituted pyrrolones. Based on biofilm formation and pyocyanin production, the QS inhibition was evaluated. In this study, there is a structural optimization of brominated furanone and pyrrolidones. First, the aromatic furanones were designed and synthesized, and then the aryl-substituted pyrrolidone derivatives were designed and synthesized for their QQ activity based on the series of furanones and pyrrolidones. The aromatic furanone didn't show antibacterial activity in the case of both Gram-positive and Gram-negative bacteria, they also had no effect against the metabolic activity, whereas a slight effect on its growth. In comparison with it, the brominated pyrrolidone derivatives showed a fair inhibitory effect on Gram-positive bacteria including *S. aureus* ATCC25923 and *S. aureus* ATCC43300, and *Bacillus subtilis* ATCC9372. It didn't show any effect against

Table 1 EC_{20} and MIC values for ACD, BCD, and their derivatives at 120 min

	ACD	RAMEA	HPACD	QAACD	ACDPS	BCD	RAMEB	HPBCD	SBEBCD	BCDPS
EC_{20}	2.300	3.200	3.500	6.000	7.390	3.240	6.790	4.080	7.390	10.000
MIC	0.625	0.625	0.625	2.500	2.500	0.156	2.500	0.156	0.625	0.625

Table 2 MIC values (µg/mL)

Compound	*B. subtilis* ATCC9372	*S. aureus* ATCC25923	*E. coli* ATCC25922	*P. aeruginosa* PAO1	*S. aureus* ATCC43300	*S. pneumoniae*	*S. epidermidis*
1	>128	>128	>128	>128	>128	>128	>128
2	>128	>128	>128	>128	128	>128	>128
3	>128	>128	>128	>128	>128	>128	>128
4	>128	>128	>128	>128	>128	>128	>128
CLA (clarithromycin)	<0.25	<0.25	32	64	16	128	128
CIP (ciprofloxacin)	2	8	>128	4	4	2	2

Gram-negative bacteria including *E. coli* ATCC25922 and *P. aeruginosa* PAO1. Since the bacterial cell wall of Gram-negative bacteria has a protective action against the antibacterial agents, the pyrrolidone derivatives exhibited a little inhibitory effect against Gram-negative bacteria such as *E. coli* and *P. aeruginosa* (Table 2).

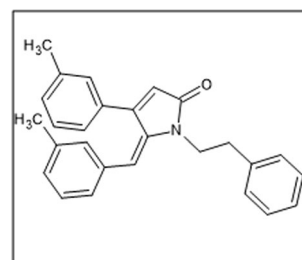### Inhibition of pyocyanin production

Table 3 provides inhibition of pyocyanin production and protease activity of Compound 1 and Compound 3. Out of 6 compounds that exhibited potent inhibition about 80% of pyocyanin production with an OD_{520} value of 0.0082. Compounds 2, 3, and, 4 were inhibited by 43.3%, 52.6% and 43.1%, respectively. All six compounds showed protease inhibitory effect, in which compound 1 exhibited as the most potent inhibitor with 78.5% followed by compound 4 with 75.6%, the remaining 4 compounds were in ratios around 65%. As the inhibitory effect of compound 1 (Fig. 5) and 3 (Fig. 6) was more than 50% their IC_{50} values were also determined.

Based on the dose–response curve, it was observed that at high concentrations there was an increase in the inhibition rate of protease activity while at low concentrations there was an increase in the inhibition of pyocyanin production. Compound 1 showed better inhibition of biofilm formation with IC_{50} value 0.260 ± 0.035 mM and the coefficient of determination i.e., R^2 of 0.885 whereas compound 4 didn't exhibit any inhibitory effect against biofilm formation [44–46].

Bueloni et al., designed a complex of wide spectrum antibiotics that is nalidixic acid (NA) and oxidovanadium (IV) ion, and its incorporation into hybrid nanoparticles


Fig. 5 Compound 1: an aryl-substituted pyrrolidone derivative
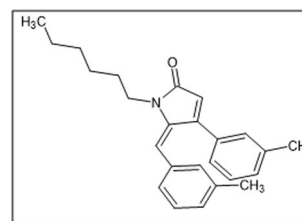
Fig. 6 Compound 3: an aryl-substituted pyrrolidone derivative

were also inspected. In this study, an encapsulation of vanadium and nalidixic acid (V-NA) is done. The swarming ability of Gram-positive bacteria and the quorum sensing of Gram-negative bacteria are evaluated. Chi-coated and Eudragit nanoparticles and myristyl myristate NLCs were suggested as good bio carriers for encapsulation. The complex V-NA showed better antibacterial results than the NA ligand with twofold decrease in the MIC and IC_{50} values (Table 4). There was a great inhibition observed in *E. coli* and *B. cereus* in both strains whereas lesser antibacterial effect concerning biofilm formation in *P. aeruginosa* and *S. aureus.*

An antibiogram assay was also done, which provided a more enhanced result compared to the MIC values. At the conc. of 10 mM an increase in the inhibition of halo formation was detected against *P. aeruginosa* and *S. aureus*. A halo increase ranging from 46 to 65% was observed for V-NA in contrast with NA indicating the effectiveness of the antibacterial activity with complexation. When treated with sub-inhibitory conc. of V-NA

Table 3 Inhibition of pyocyanin production and protease activity of Compound 1 and Compound 3

Compound	Pyocyanin production		Protease activity	
	IC_{50} (mM)	R^2	IC_{50} (mM)	R^2
1	0.2897	0.9464	0.4364	0.9540
3	0.7492	0.9020	0.7060	0.9390

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Table 4 MIC and IC_{50} values of NA and V-NA complex using different ATCC strains showing its antibacterial property:

Organism	NA		V-NA		Reduction ratio (NA/V-NA)	
	MIC (µM)	IC_{50} (µM)	MIC (µM)	IC_{50} (µM)	MIC	IC_{50}
E. coli	20.0	11.5	10.0	5.3	2.0	2.2
P. aeruginosa	1250.0	730.0	625.0	430.0	2.0	1.7
S. aureus	250.0	53.0	125.0	40.0	2.0	1.1
B. cereus	20.0	7.8	10.0	3.1	2.0	2.5
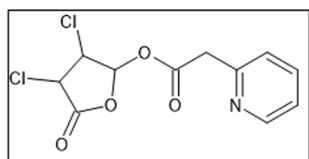
Fig. 7 Compound 5: 3,4-Dichloro-5-oxo-2,5-dihydrofuran-2-yl 2-(pyridin-2-yl)acetate
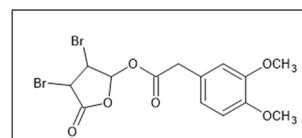
Fig. 9 Compound 7: 3,4-Dibromo-5-oxo-2,5-dihydrofuran-2-yl 2-(3,4-dimethoxyphenyl) acetate
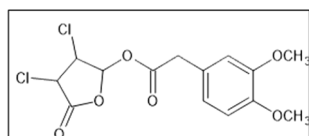
Fig. 8 Compound 6: 3,4-Dichloro-5-oxo-2,5-dihydrofuran-2-yl 2-(3,4-dimethoxyphenyl) acetate
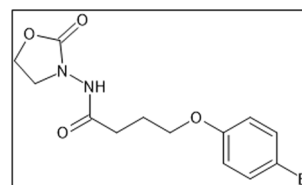
Fig. 10 Compound 8: 4-(4-bromophenoxy)-N-(2-oxooxazolidin-3-yl) butanamide

there was a significant reduction in swarming motility seen in *S. aureus* and *P. aeruginosa*, 2.5 to 10 times lesser compared to CIM value at 1,2, and 7 days. Quorum quenching when V-NA was encapsulated with NLCs and EuNPs, was evaluated based on the production of violacein pigment by *C. violaceum.* From the obtained results, it showed that the encapsulated formulation with nanoparticles showed better loss of pigment of *C. violaceum* whereas the free V-NA didn't exhibit a loss of pigment [47–49].

Chang et al., designed, synthesized various derivatives of halogenated furanones with 5-hydroxyl-3,4-halogenated-5H-furan-2-ones as a precursor and substituting with alkyl chains, vinyl bromide or aromatic rings. An evaluation of its biofilm formation in *P. aeruginosa* was done on the synthesized compounds. Compound 5 (Fig. 7) and 6 (Fig. 8) showed a MIC value of 32 µg/mL for strain ATCC9027.

There was a remarkable inhibition of pyocyanin production of *P. aeruginosa* when treated with phenolic compounds and their derivatives. Compound 7 ((Fig. 9) exhibited the best results in the depletion of pyocyanin production along with the inhibition of biofilm formation. There was a reduction in the GFP fluorescence that indicates the presence of 3-oxo-C12-HSL antagonist and thereby inhibition of lasB gene. With GFP values with OD_{600}, it was proved that compound 7 was effective in a dose-dependent manner for the inhibition of lasB expression [50–52].

Jiang et al., have designed and synthesized eighteen oxazolidinone derivatives with 3-amino-2-oxazolidinone compounds using ZS-12 as the lead compound. The compounds synthesized were evaluated against the quorum quenching activities on the CV026 strain of *C. violaceum*. An evaluation was done on the synthesized compounds there was an inhibition of violacein production observed in CV026 by considering C10-HSL as a positive control. Compound 8 (Fig. 10) showed the maximum inhibitory effect in *C. violaceum* with IC_{50} 1⁄4 3.686 ± 0.5790 mM. The inhibition of biofilm formation in *P. aeruginosa* was found in the range of 42.98%—17.67%. The inhibition was concentration-dependent, for the compound 8 at 162.5 mM ($p < 0.05$) was 40.39%. With 162.5 mM as the concentration of compound 8, the suppression observed was 22.53% ($p < 0.01$) for pyocyanin, 16.25% ($p < 0.01$) for elastase, 57.91% ($p < 0.01$) for rhamnolipid and 14.91% ($p < 0.01$) for protease. There was also a decrease in the

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swarming motility when treated with the compound concerning the control [53–55].

Marjani et al., has detected the inhibition of biofilm formation in *A. baumannii* using 1,3-oxazole and imidazole-5-one. The evaluation of biofilm formation was done on 127 *A. baumannii* out of which 119 that is 93.7% were biofilm producers. 5 isolates were taken and the test and control isolates were evaluated. The difference between the rate of biofilm formation of test and control isolates was estimated at a significant level of $p > 0.01$. From the results obtained the lowest biofilm formation was observed in compound 9 with a value of 0.06350 ± 0.003536 and compound 10 with a value of 0.06350 ± 0.000707 against *A. baumannii* 114 and it was evaluated to the rate of control with a value 0.143 [56–58].

Yang et al., have synthesized 22 quorum quenching derivatives of 3-Hydroxy-2,3-dihydroquinazolin-4(1H)-one that mimics the structure autoinducer and acinetobactin. To understand the effect of inhibition of biofilm formation 3-Hydroxy-2,3-dihydroquinazolin-4(1H)-one was modified at positions R and R^1 (Table 5).

Biofilm inhibition was evaluated using crystal violet stain and at four different concentrations of 500 μM, 250 μM, 50 μM and 10 μM. Inhibition of biofilm formation of compound 11 and Compound 12 was evaluated against *A. baumannii*, *E. coli*, and *P. aeruginosa considering* (S)-2-(3-bromophenyl)-N-(2-oxotetrahydrofran-3-yl) acetamide(3-BrBAHSL) as the positive control (Table 6). Among the derivatives obtained, compound 11 and 12 (Fig. 11) showed significant suppression of biofilm formation by 31% and 34%, respectively, proving to be the best quorum-quenching inhibitors. These two compounds (11 and 12) were then evaluated against Gentamycin sulfate. The MIC value, FIC (Fractional Inhibitory Concentration), and FICI value were established, which showed a synergistic effect (Table 7).

Hence 2-hydroxyphenyl at 2nd position is critical for inhibition of biofilm formation. And there will be a synergistic effect with Gentamycin [56, 59, 60].

El-Khouly et al., designed and synthesized various benzofuran-based derivatives. The quorum quenching activity of the Compounds 13, 15, 16, 17, 18, 19, 20, and 21 (Fig. 12) against both Gram-positive and Gram-negative

Table 5 R and R^1 substitution of Compound 11 and Compound 12

Compound	R	R^1
11	n-C10H21	2-OHPh
12	3-ClBn	2-OHPh

Table 6 Inhibition of biofilm formation at 50 μM

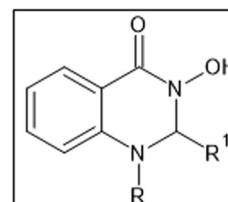|	*A. baumannii* (%)	*Escherichia coli* (%)	*P. aeruginosa* (%)
3-BrBAHSL (positive control)	28.22 ± 6.47	50.10 ± 3.26	-1.72 ± 2.16
11	43.38 ± 4.74	40.83 ± 5.25	44.51 ± 9.57
12	38.20 ± 9.43	27.46 ± 3.90	55.35 ± 4.29

Fig. 11 Compound 11: 1-decyl-3-hydroxy-2-(2-hydroxyphenyl)-2,3-dihydro quinazolin-4(1H)-one and Compound 12: 1-(3-chlorobenzyl)-3-hydroxy-2-(2-hydroxyphenyl)-2,3-dihydroquinazolin-4(1H)-one

Table 7 MIC, FIC, and FICI value of Gentamycin sulfate, Compound 11, and Compound 12

	MIC alone (μg/mL)	MIC combination (μg/mL)	FIC (MIC comb/MIC alone)	FICI
Gentamycin sulfate	0.25	0.0625	0.25	0.252
11	1024	2	0.002	
Gentamycin sulfate	0.25	0.0625	0.25	0.252
12	1024	2	0.002	

bacteria with ampicillin as the reference drug was evaluated (Table 8). In the Gram-positive bacteria, the compounds 15, 16, 17, 18, 19 and 20 with respect to ampicillin exhibited a good inhibition zone in *S. aureus*, likewise compound 13, 15, 16, 17, 18 and 19 showed inhibition zone with respect to ampicillin in *Bacillus cereus.* In Gram-negative bacteria, compounds 17, 19, 20 and 21 showed the inhibition zone in *P. aeruginosa.* The quorum sensing inhibitions were evaluated based on the violacein production of *C. violaceum* and from the compounds synthesized, compound 13 and 14 exhibited quorum quenching effect.

The MIC of compounds synthesized were evaluated in comparison with ampicillin (Table 9). The compounds 19, m-Cl substituted, 18, p-NO3 substituted showed the best inhibition against *S. aureus* with a MIC value of 8 μg/mL [61–63].
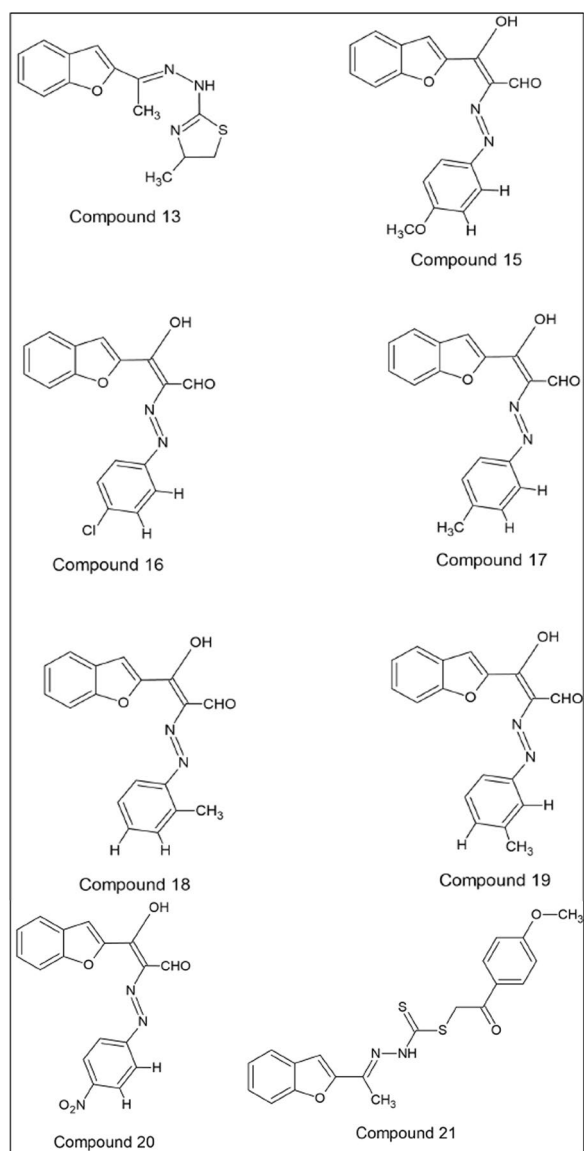
Fig. 12 Compound 13: (E)-2-(2-(1-(benzofuran-2-yl)ethylidene) hydrazineyl)-4-methylthiazole, Compound 15: (E)-3-(benz ofuran-2-yl)-3-hydroxy-2-((E)-(4-methoxyphenyl) diazenyl) acrylaldehyde, Compound 16: (E)-3-(benzofuran-2-yl)-3-hydrox y-2-((E)-(4-cholorophenyl) diazenyl)acrylaldehyde, Compound 17: (E)-3-(benzofuran-2-yl)-3-hydroxy-2-((E)-(4-methylphenyl) diazenyl) acrylaldehyde, Compound 18: (E)-3-(benzofuran-2-yl)-3-hydrox y-2-((E)-(3-methylphenyl) diazenyl)acrylaldehyde, Compound 19: (E)-3-(benzofuran-2-yl)-3-hydroxy-2-((E)-(2-methylphenyl) diazenyl) acrylaldehyde, Compound 20: (E)-3-(benzofuran-2-yl)-3-hydrox y-2-((E)-(4-nitrolphenyl) diazenyl)acrylaldehyde, and Compound 21: 2-(4-methoxyphenyl)-2-oxoethyl (E)-2-(1-(benzofuran-2-yl)ethylidene) hydrazine-1-carbodithioate

Nagasundaram et al., synthesized a series of azo fused 2,3-dihydro-1H-perimidine scaffolds and its antibacterial activity were evaluated further the compound that showed the best results were evaluated for its various bacterial functions that is governed by the quorum sensing activity like biofilm formation, swimming and swarming motility activity. The quorum quenching activity was evaluated against *C. violaceum.* The synthesized compound was (aryldiazenyl)−2-(2,3-dihydro-1H-perimidin-2-yl) phenol and its derivatives. The antibacterial activity of the derived compound was evaluated based on the inhibition of the zone (Table 10), tested against various Gram-positive and Gram-negative bacteria and was examined using 50 µL.

Based on the antibacterial activity, it was observed that the Compound 22 and 23 (Fig. 13) showed the better activity among others against *P. aeruginosa.* Hence the two azo fused compounds were tested for antibiofilm formation. From the evaluation of biofilm formation against *P. aeruginosa*, the result obtained were showing a significant reduction in the biofilm production with MIC value 1.56 µg/mL for compound 22 and 3.13 µg/mL for compound 23 and the percentage obtained gives a comparison between 22 and 23, 22 is a p-nitro substituted compound with 37.08 and 23 is a m-nitro compound with 30.23% and Rifamycin that is taken as the standard positive control shows 52.75% of inhibiting biofilm formation (Table 11).

The compounds 22 and 23 showed a significant decrease in the swimming and swarming motility of *P. aeruginosa.* The quorum quenching was also evaluated against *C. violaceum* CV026, the compound 22 and 23 at a concentration of 50 µL, showed a remarkable reduction in the violacein production with Rifamycin taken as a control (Table 12).

From the results obtained (Table 12), it is evident that 22 offers a stronger quorum quenching, anti-swimming, and anti-swarming activity than 23 [64–66].

Hopa et al., has synthesized novel mixed-ligand two Co (II) complexes, Compound 24-[Co(btmpp)(NCO)$_2$] and Compound 25-[Co(btmpp)(NCSe)$_2$] in which btmpp is 2,6-bis(3,4,5-trimethylpyrazolyl)pyridine). These complexes were tested against both Gram-positive and Gram-negative bacteria. The quorum sensing behavior in bacteria like swarming motility and biofilm production were evaluated. The MIC values expressed in mM, were evaluated by comparing the compound 24 and 25 with the standard drug (Table 13). From the values obtained compound 25 showed better results against *P. aeruginosa and S. sonnei* whereas compound 24 showed better results when it was evaluated against *Yersinia enterocolitica* in comparison with azithromycin and ampicillin which was taken as the standard drug. Antibacterial activity was evaluated based on the MIC values against various bacterial strains for both compound 24: [Co(btmpp)(NCO)$_2$]

Table 8 Zone of Inhibition and Quorum-sensing inhibition of synthesized compounds

Compound	Inhibition zone diameter (mm)				QS inhibition (mm)
	Gram-positive bacteria		**Gram-negative bacteria**		*C. violaceum*
	S. aureus	*B. cereus*	*E. coli*	*P. aeruginosa*	
13	7	11	–	–	2
15	7	5	–	–	–
16	9	6	–	–	–
17	6	6	–	10	–
18	7	8	–	–	–
19	7	6	–	10	–
20	6	–	–	10	–
21	–	–	–	8	–
Ampicillin	6	10	10	–	NA

Table 9 MIC values of compounds synthesized (µg/mL)

Compound	Minimal inhibitory concentration (MIC µg/mL)			
	S. aureus	*B. cereus*	*P. aeruginosa*	*C. albicans*
13	64	128	–	128
14	–	–	–	256
15	128	256	–	–
16	128	256	–	–
17	128	256	512	–
18	64	128	–	–
19	8	256	512	512
20	8	–	512	256
21	–	–	512	256
Ampicillin	128	128	>5000	–

and compound 25: [Co(btmpp)(NCSe)$_2$]. The bacterial strains were more sensitive to compound 25 than for 24, also the inhibition of compound 25 was evaluated in contrast to *S. sonnei* with 30 mm and compound 25 was evaluated in contrast to *P. aeruginosa* with 21 mm. Hence compound 25 exhibited better antibacterial activity than

compound 24 and this might be due to the selenocyanate group present in compound 25 (Table 14).

There was a complete inhibition of the bacterial strain with the treatment of compound 25 whereas there was an increase in the activity against swarming of bacterial strains when treated with compound 24. As the rhl system is the key contributor to the swarming ability in *P. aeruginosa*, the results obtained indicate that the rhl inhibitor is present in compound 25 (Table 15).

When the activity of the complex against the biofilm formation was evaluated compound 25 showed significant results in the range of 1–0.001 mM in contrast to it, compound 24 in the range of 10–0.01 mM, didn't show significant results against *S. sonnei* and *P. aeruginosa* [67–69].

Qin et al., synthesized and characterized compounds that are structurally related to homoserine γ-lactone, these compounds are known as TGK-series and its quorum quenching activity was evaluated against *E. coli.* Hence in this study, the TGK series of compounds hinder the potential of the bacteria to produce GFP at 50 µM concentration. The compound TGK series were evaluated against *E. coli* top 10 strains for its potential to interfere

Table 10 Zone of Inhibition (mm)

Compound		Diameter of zone of inhibition (mm)					
		Gram-positive bacteria			**Gram-negative bacteria**		
		B. cereus	*S. aureus*	*S. epidermidis*	*K. pneumoniae*	*E. coli*	*P. aeruginosa*
22	4-NO$_2$	15	–	–	13	10	16
23	3-NO$_2$	13	-	–	11	8	14
Positive control	Rifamycin	22	–	–	17	11	16
Negative control	DMSO	–	–	–	–	–	–
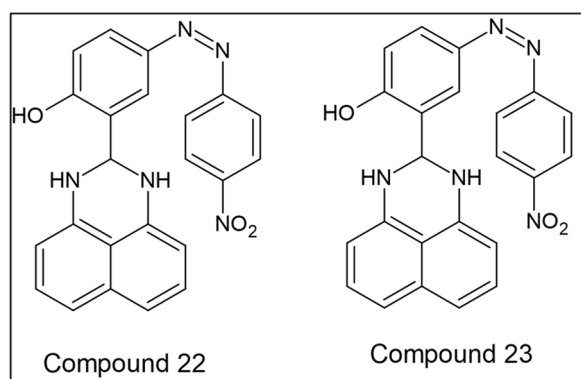
Fig. 13 Compound 22 and 23: Azo fused 2,3-dihydro-1Hperimidine derivatives

Table 12 Anti-QS and Violacein Inhibition

Compound	Anti-QS (in mm)	Violacein inhibition (%)
22	8	50.25
23	4	27.02
Standard (Rifamycin)	12	69.72

with the GFP fluorescence produced by 3OC6HSL. The compounds TGK2, TGK3, and TGK4 suppressed the quorum sensing activity by 53.5% ($p \leq 0.01$), 56.9% ($p \leq 0.01$) and 57.9% ($p \leq 0.01$), respectively, as these compounds didn't have any substituents added with it whereas TGK1 compound had two benzene rings attached with methyl group at the para position with respect to methylene connected to nitrogen in that molecule hence there was a stronger quorum sensing inhibition by 47.9 ($p \leq 0.001$) observed in compound TGK1 [70–72].

Maruthupandy et al., had prepared Nickel oxide nanoparticles (NiO NPs) and its quorum quenching and antibiofilm activity were evaluated. The violacein inhibition of C. *violaceum* was also evaluated. The antibacterial activity was evaluated at 50 μg/mL to check the zone of inhibition. Ceftazidime which was taken as the positive control exhibited a zone of inhibition of 6 mm. The Nickel oxide nanoparticles exhibited a zone of inhibition of 7 mm at 10 μg/mL concentration and 12 mm at 5 μg/mL concentration hence indicating that the antibacterial effect of the NiO NPs is concentration-dependent and with an increase in concentration the antibacterial effect also increases. The introduction of NiO NPs with C6-HSL to C. *violaceum* CV026 showed an excellent inhibition of violacein up to 90% at a concentration of 60 μg/mL. The NiO NPs at concentration of 60 μg/mL showed 94% of inhibition of biofilm formation against P. *aeruginosa* with

respect to the control which indicates activity against biofilm formation also increases with increase in the concentration of NiO NPs [73–75].

Li et al., describes the effect of Methyl anthranilate at sub-MIC30 for evaluating the phenotypes that are regulated by quorum sensing against *Aeromonas sobria*. At a concentration ranging from 0.125μL/mL to 5 μL/mL the MIC value of methyl anthranilate was determined. The MIC value of *C. violaceum* CV026 was 2.0 μL/mL and *A. sobria* was 5.0 μL/mL. The inhibitory effect of methyl anthranilate to produce violacein was determined on *C. violaceum* CV026, it was observed that there was concentration-dependent inhibition of violacein. At a concentration of 0.5 μL/mL of methyl anthranilate, the maximum inhibition rate of violacein production was 41.01% with no growth inhibition. The determination of the effect of methyl anthranilate on the biofilm formation was done by treating methyl anthranilate with different sub-MICs against *A. sobria.* A remarkable suppression of biofilm formation was observed to the level of 31.67%, 34.01%, 41.27% and 51.44% when treated with 0.5μL/mL, 0.25 μL/mL, 0.125 μL/mL and 0.0625 μL/mL values of sub-MIC, respectively, against *A. sobria.* The swimming and swarming motility of *A. sobria* at a concentration of 0.5μL/mL of methyl anthranilate showed a gradual reduction with the maximum inhibitory rate of 74.84% and 71.63%, respectively. The protease activity of *A. sobria* was also evaluated. At a concentration of 0.5 μL/mL of methyl anthranilate there was a reduction of up to 43.08% of *A. sobria.* The incorporation of C4-HSL the protease activity enhances. With the treatment of 0.5 μL/mL methyl anthranilate the minimal concentration of C4-HSL was reduced to 0.865 μL/mL [76–78].

Table 11 MIC values of compounds synthesized (μg/mL)

Compound	Minimum inhibitory concentration (MIC) (μg/mL)					
	Gram-positive bacteria			Gram-negative bacteria		
	B. cereus	*S. aureus*	*S. epidermidis*	*K. pneumoniae*	*E. coli*	*P. aeruginosa*
22	3.13	–	–	6.25	6.25	1.56
23	6.25	–	–	6.25	12.5	3.13

Table 13 MIC values (mM) of compounds synthesized and Standards (Ampicillin and Azithromycin)

Compounds	Bacteria strains (MIC values)				
	Bacillus subtilis ATCC 6633	*P. aeruginosa* ATCC 35032	*Shigella sonnei* ATCC 25931	*Salmonella typhimurium* ATCC 14028	*Yersinia enterocolitica* ATCC 9610
24	1	10–1	100–10	10–1	1
25	1	1	1–0.1	10–1	100–10
Ampicillin	0.1	100–10	100–10	1–0.1	10
Azithromycin	0.1	10	10–1	10–1	100–10

Table 14 Zone of Inhibition (mm)

Bacteria strains	Antibacterial activity zone of Inhibition (mm)	
	24	25
B. subtilis ATCC 6633	17±2	29±3
P. aeruginosa ATCC 35032	15±5	21±3
S. sonnei ATCC 25931	16±2	30±1
S. typhimurium ATCC 14028	15±4	23±3
Y. enterocolitica ATCC 9610	17±3	28±1

Table 15 Anti-swarming activity

Bacteria strains	Anti-swarming activity swarming zone (mm)		
	24	25	Control
B. subtilis ATCC 6633	52±6	Inhibited	9±4
P. aeruginosa ATCC 35032	69±5	Inhibited	11±1
S. sonnei ATCC 25931	75±6	Inhibited	11±4
S. typhimurium ATCC 14028	50±4	Inhibited	14±2
Y. enterocolitica ATCC 9610	60±7	Inhibited	12±3

Salini et al., evaluates the biofilm formation of *Vibrio harveyi* when treated with undecanoic acid (UDA), the auxins such as Indole-3-Acetic Acid (IAA) and Indole-3-butyric acid (IBA) individually, and in combination. The MIC values of UDA was 20 µg/mL and IAA or IBB was 200 µg/mL, respectively (Table 16). The biofilm formation of *V. harveyi* was evaluated based on FICI value which was found to be 0.65 when UDA and auxins were combined (Table 16).

The biofilm formation was evaluated both individually and in combination. With a maximum conc. of 20 µg/mL the compounds undecanoic acid, Indole-3-Acetic Acid, and Indole-3-butyric acid, suppressed the biofilm formation up to 18.9%, 14.5%, and 8.6%, respectively, whereas on combination the reduction of biofilm formation was concentration dependent of UDA with IAA or IBA in the range of 21–79.7% and 25.8–60.1%, respectively. It was also observed that there was a concentration-dependent reduction during the evaluation of bioluminescence on *V. harveyi.* The reduction in bioluminescence with the combination of the UDA (at a conc. of 10 µg/mL) with IAA or IBA (at a conc. of 10, 20 and 30 µg/mL) observed were at a range of 75.9–93.6 and 13.2–76.7%, respectively [79–81].

Saqr et al., describes Allopurinol as a quorum quenching agent when evaluated for the various virulence factors that are regulated by the QS system. The determination of MIC values of Allopurinol against *P. aeruginosa* was done and the least concentration that hindered the growth of *P. aeruginosa* PAO1 was 2 mg/mL. There was a reduction of violacein production by 60% observed on *C. violaceum* CV026 when treated with allopurinol at 1/10 MIC. There was an inhibition of biofilm formation by Allopurinol against *P. aeruginosa* PAO1 by 61%. On the treatment with Allopurinol at sub-MIC, the ability of *P. aeruginosa to* swim was reduced to 92%, the twitching ability was reduced to 87%, and swarming motility was reduced to 85%. Other virulence factors such as hemolytic activity, elastolytic activity, pyocyanin production, and rhamnolipid production (which is determined by clearance zone) was significantly reduced by Allopurinol against *P. aeruginosa* by 95%, 93%, 74% and 74%, respectively. The expression of QS genes was also tested

Table 16 Individual and combined MIC and FICI values of UDA and auxins

Conc. of UDA in combination (µL/mL)	MIC of UDA (µL/mL)	Conc. of IAA in combination (µL/mL)	MIC of IAA (µL/mL)	FICI for UDA+IAA	Conc. of IBA in combination (µL/mL)	MIC of IBA (µL/mL)	FICI for UDA+IBA
10	20	30	200	0.65	30	200	0.65

and showed a decrease in the expression of *P. aeruginosa* genes by Allopurinol at sub-MIC [82–84].

Natural compounds

Zhu et al., explored the effect of exudates taken from the root of *Sedum alfredii* regulated by quorum sensing on the bacteria *P. aeruginosa*. The quorum sensing ability of *S. alfredii* root extracts were evaluated against *P. aeruginosa* PAO1(WT), green fluorescence reporter (gfp-lasI), and the rhl QS mutant strain (ΔrhlI). When the root extracts were evaluated for its quorum quenching activity, it was observed that the protease synthesis of WT and ΔrhlI were inhibited by 51.4% and 30.4%, respectively, and the protease concentration of WT was reduced to 0.36 mg/L and of ΔrhlI was reduced to 0.32 mg/L. The screening of quorum sensing inhibition was analyzed, and several substances were found like leucine, serine, threonine, aspartic acid, glycerol, sorbitol,2-piperidine carboxylic acid, squalene, tropone, methyl oleate, monolinolein and thymol. Out of these substances obtained thymol showed the best inhibition among other substances. At a concentration of 50 μmol/L of thymol there was a reduction of protease activation by 44.7% and 33.7% in WT and ΔrhlI, respectively, in comparison with the control and rate of inhibition approximately 24.3% when evaluated on *P. aeruginosa*. The fluorescence expression of gfp-lasI represents the gene expression of 3-oxo-C12. The resulting gene avg. gfp-las expressions obtained were 1.64×10^6, 1.71×10^6, and 1.72×10^6/OD$_{600}$ for untreated, $1 \times$ root exudates, and for thymol at a concentration of 50 μmol/L. It was also observed that there is an inhibition of expression of lasR gene and transcription of elastase by treating it with thymol on *P. aeruginosa*. In order to understand the transcriptional expression of lasR and lasB genes a qRT-PCR test was done and lasB gene showed significant reduction in the gene expression rate by 36.0% for $1 \times$ root exudates and 73.0% for 50 μmol/L thymol, but no result was found by lasB gene [85–87].

Ya Fei Geng et al., describes luteolin the ability to inhibit quorum sensing when evaluated against *P. aeruginosa*. The Minimum Inhibitory Concentration (MIC) values obtained was 1 mM for luteolin whereas the value obtained for positive control (quercetin) was greater than 1 mM. At sub-MICs 50, 100 and 200 μM, 100 μM luteolin had the maximum inhibition of biofilm formation ($p < 0.01$). At the sub-MICs 50 μM, 100 μM and 200 μM, 200 μM luteolin showed a remarkable reduction in pyocyanin production ($p < 0.01$). The elastase activity was significantly reduced by luteolin. Likewise, it was observed that there was a great decrease in the rhamnolipid of 100 μM luteolin that showed a similar reduction effect as that of quercetin 100 μM. The swimming ability was significantly suppressed with 200 μM luteolin showing the maximum inhibition ($p < 0.01$) and swarming motility showed intermediate inhibition at 200 μM, in comparison with the positive control ($p < 0.05$) [88–90].

Shukla et al., describes gingerol as an effective quorum sensing inhibitor when evaluated against *P. aeruginosa*. At 30 μg/mL of gingerol, there was a decrease by 30%, 40%, 40%, and 20% for biofilm formation, Extracellular polymeric substance (EPS) production, pyocyanin production and rhamnolipid production, respectively. The reduction in LasR led to the suppression of biofilm formation and EPS. PhzR and RhlR is controlled by LasR and the suppression of PhzR has an effect on pyocyanin and RhlR on rhamnolipid. The potency of ciprofloxacin, an antibiotic, was increased with gingerol by 20% with a minimal conc. of 0.5 μg/mL and at 1.0 μg/mL of ciprofloxacin the effect was doubled [91–93].

Abdulrahman et al., used curcumin-mediated antimicrobial photodynamic therapy (APDT) for quorum quenching in *P. aeruginosa*. APDT is a type of treatment that involves a combination of visible light and photosensitizer(PS) in presence of oxygen. Cell death occurs when this photosensitizer absorbs light of a particular wavelength that leads to the formation of reactive oxygen species. Curcumin was evaluated after the APDT and there was an inhibition in the biofilm formation by 23% without light, 40% and 70% inhibition by 5 J/cm^2 and 10 J/cm^2 light, respectively. With laser light 10 J/cm^2 showed more effectiveness than 5 J/cm^2 and curcumin alone. It was observed that there was a major downregulation of genes with 10 J/cm^2 light doses [94–96].

Prateeksha et al., presented isolated ELF from lichen and *Usnea longissima Ach* to determine its effect on quorum sensing inhibition. The fungal extract known as metabolites extract (MELF) was evaluated for its quorum quenching activity. The decrease in the violacein production in *C. violaceum* ATCC 12472 was evaluated and it showed a concentration dependent inhibition. At 6 mg/ml of MELF violacein inhibition was by $\sim$81% whereas naringenin which was taken as a positive control was inhibited by $\sim$72% at 0.5 mg/ml concentration of violacein production. There was an inhibition of pyocyanin production by $\sim$75% at 6 mg ml concentration whereas naringenin showed 70% at 0.5 mg ml concentration. At 6 mg/ml of MELF showed 77% proteolytic inhibition wherein the positive control showed 79% at 0.5 mg/ml. There was 16%, 42% and 69% reduction of elastolytic activity at 2, 4 and 6 mg/ml of MELF and the positive control showed inhibition of $\sim$61% in elastase activity. MELF at 6 mg/ml showed 79% and 72% decrease in rhamnolipid and extracellular polysaccharides, respectively [97–99].

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Chang et al., describes the quorum quenching ability of chrysin which was isolated from *Penicillium chrysogenum* DXY-1. Tyrosol is also an active molecule obtained from the extract which was evaluated along with chrysin. The inhibition of violacein production in *C. violaceum* was found to be concentration dependent. At a concentration of 20 μg/mL chrysin inhibited violacein production by 31.6% in comparison with the negative control i.e., DMSO, whereas the inhibition of violacein production of DXY-1 metabolite at 20 μg/mL was found to be 21.5% and 17% violacein inhibition by tyrosol at a very high concentration of 100 μg/mL. 41.4% of pyocyanin inhibition of 40 μg/mL of chrysin was obtained, 13.8% of suppression in the elastase activity and 8.3% of proteolytic activity when compared to the negative control. At 100 μg/mL of tyrosol only 8.5% of inhibition of pyocyanin production was observed. The positive control AZM with a concentration of 50 μg/mL dose, 77.0%, 79.1% and 75.9% inhibition of pyocyanin production, elastase activity and proteolytic activity, respectively, was observed. At 40 μg/mL concentration of chrysin there was a suppression of 42.4% of biofilm formation whereas AZM which is taken as a positive control inhibited by 76.7%. The resulting value shows that there is concentration dependent decrease overall [100–102].

Ghoreishi et al., have investigated the effectiveness of the extracts from *Halobacillus karajensis* of their pyocyanin and biofilm formation inhibition against *S. aureus and P. aeruginosa.* From the results obtained the crude supernatant extract, the middle phase of Methanol/Chloroform Supernatant extract (MMS) from *H. karajensis* showed the best reduction in biofilm formation in *S. aureus* by 74%, and *P. aeruginosa* by 27%. The protein profile of MMS was evaluated for its pyocyanin inhibition and the most effective protein detected was the fragment with the lightest molecular weight of 25 kD with 60% of pyocyanin reduction [103–105].

Molina et al., have evaluated different *Laurus nobilis* extracts based on their polarity such as n-hexane (HE), Chloroform (CE), ethyl acetate (EAE), methanol (ME) and total methanol extract (TME). At a concentration of 100 μg/mL the *Escherichia coli* strains showed an inhibition of biofilm formation below 40%. *S. aureus* strains inhibited biofilm formation up to 76% and 55% by the strains ATCC 6538 and ATCC 25904, respectively. The only extract capable of inhibiting biofilm formation against all the strains was HE at a concentration of 100 μg/mL. A significant inhibition was also observed in CE and EAE extracts against *E. coli, P. aeruginosa and S. aureus.* HE and CE were evaluated if it could protect the surface against biofilm formation hence it was covered with 10 μg/mL and 100 μg/mL of polystyrene fragments. There was a decrease detected in biofilm biomass and activity between 40 and 60%. The swimming motility was determined against *P. aeruginosa* PAO1 against the extracts EAE, HE, CE and TME all were above 30%. At 100 μg/mL, there was a decrease in pyocyanin by 23%, 35%, 50% and 54% for HE, TME, CE and EAE, respectively, and 43% and 45% reduction in the elastase activity by 29%, 43% and 45% for TME, HE and CE, respectively [106–108].

Ahmed et al., investigated the inhibition of virulence factor of *P. aeruginosa* by isolated plant compounds such as trans-cinnamaldehyde (CA) and salicylic acid (SA). The decrease in biofilm formation was better detected in SA than CA. The reduction in biofilm formation was 54% and 26% of SA and CA, respectively, but when CA and SA were combined, they showed an efficient reduction of 62%. The lasA protease activities of SA and CA were evaluated, the OD440 dropped from 0.3 to 0.1 for 31% reduction ($p < 0.05$) in absorbance reading in the presence of SA whereas in the presence of CA a better reduction of 65% ($p < 0.01$) was observed. The combined effect of CA and SA showed the best reduction of up to 80% ($p < 0.001$) in comparison with the untreated PAO1. LasB elastase activity in presence of CA showed a decrease of OD495 from 0.08 to 0.06 with reduction of 22% ($p < 0.01$) whereas SA showed OD495 reduction with 28% and when combined exhibited better reduction of up to 46%. The pyocyanin production in presence of CA and SA were decreased from 3.1 μg/ml to 2.1 μg/ml and 0.922 μg/ml, respectively, and in combination reduced up to 1.1 μg/ml with an yield of 64%. The decrease in the yield of rhamnolipid in the presence of CA and SA was approx. from 1.72 g/l and 0.7 g/l [109–111].

Mu et al., investigated the quorum sensing inhibitory activity on violacein production of *Coreopsis tinctoria* Nutt. When the concentration of the crude extract of *Coreopsis tinctoria* Nutt is ≥ 0.25 mg/mL there was a reduction of violet production in *C. violaceum* ATCC12472 by ≥ 50% and at 4 mg/mL it decreases up to ≥ 90%. The violacein production was also detected by using different fractions and hence 50% and 95% MeOH fractions of the crude extract possessing the highest inhibition on violacein production. These were separated and identified and out of which the Okanin separated from 95% MeOH played an important role in inhibiting the violacein production. There was an inverse correlation between okanin concentration and the intensity of purple pigment, at a concentration of 7.81 μg/mL of okanin was found to decrease significantly ($p < 0.05$) in comparison with untreated control whereas with at least 15.63 μg/mL of okanin developed turbid yellowish suspensions with no hint of purple depicting the bacterial growth in absence if violacein production. The effect of violacein production by Okanin via influencing the expression of

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vioABCDE operon, founded that at a concentration of 62.5 µg/mL of *C. violaceum* ATCC12472 showed a downregulation of vioD and vioE by two-fold and vioB by four-fold in comparison with the untreated control. The best result was obtained by vioA by at least 15-fold of Okanin treatment [112–114].

Wei et al., investigated the effect of Phloretin an antibacterial on *Listeria monocytogenes* biofilm. At a concentration of 20 µg/mL of Phloretin, *L. monocytogenes* showed an inhibition of biofilm production up to 60% at the maturation stage and there was a decrease in the biofilm thickness by 2 µm approximately. At the same concentration, there was a decrease by 50% in the transcription level of the agrosystem [115–117].

Guzman et al., explored the effect of *Piper betle L.* leaf extract such as rude ethanolic extract (CE) and crude alkaloids (CA) were investigated for the ability to inhibit the quorum sensing activity of Shrimp pathogen *V. harveyi.* The inhibition of biofilm formation without inhibiting their growth was found to be in a concentration dependent manner, at 100 µg/mL concentration CE ($p < 0.05$) inhibited biofilm formation on *V. harveyi* VH0, *V. harveyi* VH1 and *V. harveyi* BAA-1116 by 58.88%, 56.42% and 57.89%, respectively. At 50 µg/mL concentration of CA ($p < 0.05$), the inhibition of biofilm formation on. harveyi VH0, *V. harveyi* VH1 and *V. harveyi* were by 6.77%, 54.41% and 55.05%, respectively. At 100 µg/mL concentration of CE ($p < 0.05$) the bioluminescence of in *V. harveyi* BAA-1116 strain was reduced up to 64% and significant reduction in lower concentrations. Considering the CA at 50 µg/mL, 25 µg/mL, and 12 µg/mL the bioluminescence inhibition ($p < 0.05$) was found to be 50%, 30%, and 10%, respectively. *Piper betle* CE and CA also inhibited the QS via AI pathways in a concentration dependent manner. At 100 µg/mL concentration of CE AI-2 affected by 97% whereas AI-1 by 83%, similarly 88% by AI-1 and 87% by AI-2 was observed with 50 µg/mL concentration of CA [118–120].

Wang et al., investigated the inhibition of quorum sensing activity of *Pseudomonas fluorescens* and *Shewanella baltica* in seafood products and the main QS regulators such as LuxR/I family were evaluated. The MIC of 5′-CMP and 5′-AMP inhibiting *P. fluorescens* PF08 were 0.155 mmol/L and 0.3 mmol/L, respectively, and those inhibiting *S. baltica* OS155 were 0.139 mmol/L and 0.130 mmol/L. 5′-CMP and 5-AMP could inhibit AHL and DKP production in *P. fluorescens* PF08 and inhibition of DKP production in *S. baltica* OS155. C4-HSL is an QS signalling molecule that was reduced by 58.4% and 55% when *P. fluorescens* PF08 was treated with 5′-CMP and 5′-AMP [121–123].

Danaraj et al., have reported the QS inhibitory activity of the active constituents present in the seagrass *Halodule*

pinifolia against *P. aeruginosa* PAO1. The leaves were extracted using chloroform and methanol and the biofilm formation was obtained maximum by the methanolic extract at 35 µg/mL in comparison with Cephalosporin at 25 µg/mL taken as the positive control. Eight compounds were extracted from the *H. pinifolia* leaves out of which 4-methoxybenzoic acid (4-MBA) at 100 µg/mL showed the maximum inhibition of cell density. 62.5 µg/mL was found to be the maximum biofilm inhibitory concentration of 4-MBA in contrast to which the positive control showed inhibition at 31.2 µg/mL concentration and hence 4-MBA was taken for further evaluation. 4-MBA at 62.5 µg/mL has showed an inhibition of various virulence factors such as inhibition of lasB, protease, pyocyanin formation, rhamnolipid, alginate and chitinase by 87.5%, 83.29%, 91.46%, 79.38%, 86% and 72.09%, respectively. The expression of virulence genes was downregulated the transcript levels by 1.9, 1.63, 2.38 and 1.06 for lasI, lasR, rhlI and rhlR, respectively [124–126].

Liu et al., investigated the quorum sensing inhibition and virulence effect of Tea polyphenols (TPs) of *Klebsiella pneumoniae.* The sub-MICs TPs ($p < 0.05$) at 100 µg/mL showed 26.28% of violacein inhibition and at 200 µg/mL 56.73% inhibition in comparison with the positive control. The proteolytic activity in untreated bacteria reduced till 100% whereas in TPs treated K4 culture were 31.24% in 200 µg/mL and 16.73% in 400 µg/mL. The EPS production was reduced by 29.24% in 200 µg/mL and 36.79% in 400 µg/mL. The biofilm formation was reduced by 23.7% in 200 µg/mL and 44.4% in 400 µg/mL. This shows that TPs activity is based on its concentration [127–130].

Chang et al., reported the Anti-quorum sensing activity of marine fungal strain *P. chrysogenum* DXY-1. There was a reduction in violacein production of *C. violaceum* CV026 by 53.5% when treated with 0.5 mg/mL of tyrosol. The other virulence factors also showed an inhibition of pyocyanin by 63.3%, elastase activity by 57.8% and proteolytic activity by 9.9% and also showed a significant decrease in the biofilm formation when evaluated against *P. aeruginosa* PAO1 [101, 131–133].

Conclusion

Quorum sensing is a fundamental mechanism in order to regulate various functions, this has also become one of the causes leading to antimicrobial resistance. Quorum Sensing Inhibitors interfere by reducing or suppressing this bacterial communication by various mechanisms hence hinder the bacterial infections. This review summarized different synthetic and natural quorum sensing inhibiting compounds against both Gram-positive and gram-negative bacteria and thus inhibiting different virulence factor of the organism. The different technique

depicts broad spectrum of strategies to inhibit the quorum sensing between bacterial cells. The compounds also exhibited their concentration dependent activity against the quorum sensing system of bacteria. Hence discovering newer quorum quenching compounds can be considered as one of the future approaches to tackle the antimicrobial resistance.

Abbreviations

QS	Quorum Sensing
QSI	Quorum Sensing Inhibitors
AIs	Auto-Inducers
QQ	Quorum Quenching
LCD	Low Cell Density
HCD	High Cell Density
AIPs	Autoinducing Peptides
AHL	N-Acyl-homoserine Lactone
ABC	ATP-binding cassette
GABA	Gamma-aminobutyric acid
AMPT	5-Acetyl-4-methyl-2-(3-pyridyl) thiazole
MRSA	Methicillin-resistant *Staphylococcus aureus*
C. violaceum	*Chromobacterium violaceum*
S. aureus	*Staphylococcus aureus*
P. aeruginosa	*Pseudomonas aeruginosa*
5-hydroxymethylfurfural	5-HMF
3-oxo-C12-AHL	3-Oxododecanoyl-L-homoserine
C4-HSL	Lactone N-butanoyl homoserine lactone
C. elegans	*Caenorhabditis elegans*
MIC	Minimum Inhibitory Concentration
A. baumannii	*Acinetobacter baumannii*
PAO1	*Pseudomonas aeruginosa* O1
CV026	*Chromobacterium violaceum* 026
OD	Optical density
TPP	Sodium tripolyphosphate
GNP	Genipin
PC-NPs	Pre-Crosslinked Nanoparticles
PC-A	Prototype A
PC-B	Prototype B
E. coli	*Escherichia coli*
CD	Cyclodextrin
A. fischeri	*Aliivibrio fischeri*
ACD	α-Cyclodextrins
BCD	ß-cyclodextrins
GCD	γ-Cyclodextrins
HPACD	2-Hydroxypropyl α-cyclodextrins
HPBCD	2-Hydroxypropyl ß-cyclodextrins
QAACD	Trimethylaminopropyl α-cyclodextrins
RAMEA	Random methylated α-cyclodextrins
RAMEB	Random methylated ß-cyclodextrins
RAMEG	Random methylated γ-cyclodextrins
SBEBCD	Sulfobutyl ether ß-cyclodextrins
ACDPS	Epichlorohydrin-crosslinked polymer of α-cyclodextrins
BCDPS	Epichlorohydrin-crosslinked polymer of ß-cyclodextrins
EC	Effective concentration
A. tumefaciens	*Agrobacterium tumefaciens*
B. subtilis	*Bacillus subtilis*
S. pneumoniae	*Streptococcus pneumoniae*
S. epidermidis	*Staphylococcus epidermidis*
CLA	Clarithromycin
CIP	Ciprofloxacin
IC50	Half maximal Inhibitory Concentration
R^2	Correlation Coefficient
NA	Nalidixic acid
IV	Oxidovanadium
V-NA	Vanadium and Nalidixic acid
EuNPs	Eudragit nanoparticles
NLCs	Nanostructured lipid carriers
B. cereus	*Bacillus cereus*
GFP	Green Fluorescent Protein
FIC	Fractional Inhibitory Concentration
FICI	Fractional Inhibitory Concentration Index
C. albicans	*Candida albicans*
K. pneumoniae	*Klebsiella pneumoniae*
DMSO	Dimethylsulfoxide
btmpp	2,6-Bis(3,4,5-trimethylpyrazolyl)pyridine)
[Co(btmpp)(NCO)$_2$]	Complex I
[Co(btmpp)(NCSe)$_2$]	Complex II
S. sonnei	*Shigella sonnei*
Y. enterocolitica	*Yersinia enterocolitica*
S. typhimurium	*Salmonella typhimurium*
TGK	Substituted γ-lactams
NiO NPs	Nickel oxide nanoparticles
A. sobria	*Aeromonas sobria*
V. harveyi	*Vibrio harveyi*
UDA	Undecanoic Acid
IAA	Indole-3-Acetic Acid
IBA	Indole-3-butyric acid
S. alfredii	*Sedum alfredii*
gfp-lasl	Green fluorescence reporter
Δrhll	Rhl QS mutant strain
μM	Micrometre
EPS	Extracellular polymeric substance
APDT	Antimicrobial Photodynamic Therapy
PS	Photosensitizer
J/cm^2	Joule per cm square
ELF	Endolichenic Fungi
MELF	Metabolites Extract
AZM	Azithromycin
H. karajensis	*Halobacillus karajensis*
MMS	Methanol/Chloroform Supernatant extract
kD	Killodalton
L. nobilis	*Laurus nobilis*
HE	N-hexane
CE	Chloroform
EAE	Ethyl acetate
ME	Methanol
TME	Total methanol extract
CA	Trans-cinnamaldehyde
SA	Salicylic acid
C. tinctoria	*Coreopsis tinctoria*
MeOH	Methanol
L. monocytogenes	*Listeria monocytogenes*
P. betle	*Piper betle*
CE	Ethanolic Extract
CA	Crude Alkaloids
P. fluorescens	*Pseudomonas fluorescens*
S. baltica	*Shewanella baltica*
CMP	Cytosine monophosphate
AMP	Adenosine monophosphate
DKP	Diketopiperazine
H. pinifolia	*Halodule pinifolia*
4-MBA	4-Methoxybenzoic acid
TPs	Tea polyphenols
K4	Cultured K. pneumoniae
3-BrBAHSL	(S)-2-(3-bromophenyl)-N-(2-oxotetrahydrofran-3-yl)acetamide

Author contributions
SN conceptualized the work, TB wrote the manuscript and ST did the critiqued the manuscript.

Declarations

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