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Review Article

PLANT BASED QUORUM SENSING INHIBITORS OF *PSEUDOMONAS AERUGINOSA*

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ABSTRACT

Phytochemical research has gained a lot of momentum in the medical field for the discovery of new, safe, and effective remedies. In the context of antimicrobial research, various plant sources have been discovered with the potential to disrupt bacterial quorum sensing (QS), which plays a key role in the regulation of virulence in many Gram positive and Gram negative bacteria. *Pseudomonas aeruginosa*, a Gram negative bacterium, is known to produce multiple QS systems that control the expression of virulence determinants and biofilm development in this pathogen. Hence, the inhibition of QS has been pursued as a promising therapy for treatment of drug resistant *Pseudomonas* infections. A comprehensive review of the research data available for plant products as QS inhibitors of the organism has been presented here, while further suggesting the future prospects for these inhibitors.

Keywords: *Pseudomonas aeruginosa*, Phytochemicals, Quorum sensing, Anti-quorum sensing, Anti-virulence

INTRODUCTION

Quorum sensing (QS), the cell-to–cell signalling system in bacteria, has now been well recognized as an antimicrobial target. It is understood that inhibition of QS, also called as quorum quenching (QQ), results in the subsequent reduction of bacterial virulence, so that the infection is controlled and the host immune system can further clear out the bacteria. It also presents an advantage of lower risks for resistance development owing to the absence of selective pressure, since bacterial growth is not affected. The QS pathway monitors the population density of bacteria in a confined environment by the production of small signals called as autoinducers. At the sensing of a *quorate* population level, it induces expression of specific genes associated with secondary phenotypes in bacteria, such as bioluminescence, conjugation, antibiotic production, virulence, biofilm maturation, sporulation, pigment production, etc [1]. Different types of signal molecules are produced in Gram positive and Gram negative bacteria. The most common signalling molecules include acyl homoserine lactones (AHLs), auto inducing peptides (AIPs) and AI-2. Other less characterized signal molecules have been detected in some bacteria, such as the cyclic dipeptides, diffusible signal factor, AI-3, etc, which appear to regulate motility, virulence and biofilm formation [2].

AHL – LuxR/I system in Gram negative bacteria

AHLs are the autoinducers commonly produced in Gram negative bacteria and generally follow the LuxR/I mechanism of the bioluminescent bacterium, *Vibrio fischeri*. Each bacterial cell produces a small amount of AHL, which is catalyzed by the synthase protein, LuxI. The AHLs diffuse in and out of the cell. With increase in bacterial number, the AHL accumulates in the environment. At a threshold concentration of AHLs (*quorate* level), it binds and activates transcriptional regulator, LuxR. The AHL-LuxR complex further binds to specific promoter regions, regulating the expression of QS controlled genes, including the LuxI gene. The LuxR/I homologous proteins have been observed in many Gram negative bacteria, such as *Aeromonas hydrophila*, *Acinetobacter baumannii*, *Agrobacterium tumefaciens*, *Chromobacterium violaceum*, *Pseudomonas aeruginosa*, *Serratia marcescens*, etc [1]. An additional AHL system has been identified in *V. harveyi*, called as the LuxM/N system, which regulates the production of luminescence in this marine bacterium [3].

AIP system in Gram positive bacteria

The peptide pheromones or autoinducing peptides produced in Gram positive bacteria, are small peptides (5-34 aa long), that are produced from the post-translational modification of large precursor peptides (40-65 amino acids (aa) long). The AIPs produced by each cell are secreted into the environment by an ATP-binding cassette (ABC) exporter. At a threshold concentration, the AIPs bind to the external domain of a cognate membrane bound sensor (histidine kinase), resulting in the phosphorylation of its cytoplasmic domain. This activated sensor further phosphorylates and activates a second cytoplasmic response regulator protein, which is directly responsible for regulating the expression of QS controlled genes. The Gram positive bacteria known to produce AIPs, include *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Enterococcus faecalis*, *Streptococcus pneuomoniae*, etc [1].

LuxS/AI-2 system in Gram positive and Gram negative bacteria

AI-2 forms the common autoinducer produced by both Gram negative and Gram positive bacteria. It was initially discovered in *V. harveyi*, where it regulates the production of bioluminescence. AI-2, in general, is a furanosyl borate diester and the regulating mechanism involves a two component signalling system. The specific AI-2 produced by *V. harveyi* is a boron diester of (2*R*,4*S*)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran (S-THMF), which is synthesized by the enzyme, LuxS. At a low cell density, a small amount of AI-2 is produced, which accumulates in the environment with an increase in cell density. At a threshold concentration, it binds to the periplasmic receptor protein LuxP. This protein then activates the sensor kinase LuxQ which further regulates expression of luciferase genes in *V. harveyi* [2].

The LuxS/AI-2 system has been detected in many Gram negative bacteria such as *Vibrio cholerae, Vibrio vulnifucus*, *Salmonella typhimurium*, *Escherichia coli*, etc. and Gram positive bacteria such as *Streptococcus pyogenes*, *Streptococcus mutans, Staphylococcus aureus*, *Staphylococcus epidermidis*, etc [4]. Further, AI-2 has been implicated in interspecies communication in bacteria, wherein the AI-2 produced by one bacterium induces gene expression in another bacterium. When the resident oropharyneal bacteria of cystic fibrosis (CF) patients were isolated and introduced to the lungs of rat model, along with *P. aeruginosa*, the AI-2 produced by oropharyngeal bacteria was found to enhance the pathogenicity of *P. aeruginosa* [5]. Co-culturing the human oral commensal organisms, *Actinomyces naeslundii* and *Streptococcus oralis*, resulted in an abundant, mixed species biofilm; however, a *luxS* mutant of *S. oralis* failed to display such effects, suggesting the role of AI-2 in mixed species biofilm formation, via interspecies communication [6].

The human pathogen, *Pseudomonas aeruginosa*

The Gram negative bacterium, *P. aeruginosa* is an aerobic bacillus that is able to survive easily in minimal nutrient condition, and exists abundantly in the environment. The organism is able to cause infection in humans, animals, as well as plants. In humans, it acts as an opportunistic pathogen, commonly associated with immunocompromised patients, burn victims, ICU patients, and patients on longer antibiotic treatment. It is commonly detected in the sputum of CF individuals who are generally affected by the chronic pulmonary infection of *P. aeruginosa*. The decreased mucus clearance in the lungs of CF patients provides a suitable niche for the growth of many bacteria, and further the CF specific alteration in the receptor for the adhesins on pili of *P. aeruginosa*, facilitates *Pseudomonas* infection in CF lung [7]. It has been one of the most common nosocomial pathogens, prominently causing ventilator associated pneumonia, surgical site infections or wound infections, catheter associated urinary tract infections and bloodstream infections. Almost all the clinical strains of *P. aeruginosa*. have evolved as multidrug resistant, and have been commonly associated with low susceptibility to the fluoroquinolones, cephalosporins and carbapenems [8–10].

The capability to form biofilms (communities of cells encased in a slimy matrix) and its adaptive nature, allows the organism to thrive on the medical devices for longer periods, while being unharmed with the detergents and disinfectants, and such devices often forms the source of entry into the host. The organism establishes its infection by numerous virulence factors that are responsible for invading the host tissue, inducing inflammation, damaging host tissue, inactivating host defense components, consequently leading to tissue destruction and further dissemination to other tissues by invading the blood vessels. The cell associated virulence factors such as pili, flagella, lectins, etc. assist in bacterial adherence to the host tissue, facilitating bacterial colonization within the host. Then, colonization may proceed to acute infection or chronic infection, by the aid of its extracellular virulence factors. Chronic infection is characterized by a low production of virulence factors, biofilm formation and tissue damage, mainly caused by chronic inflammation. Acute infections involve extensive tissue damage, bloodstream invasion and dissemination, achieved by the production of several extracellular virulence factors such as elastases (LasA and LasB), exotoxin A, alkaline protease, exoenzyme S, rhamnolipids, phospholipase, pyocyanin, etc [11]. In the current scenario, very few treatment options are available for the management of *Pseudomonas* infections, and hence, there is an urgent need to develop new drugs with stronger efficacy and lower risks of resistance. Besides the development of new antibiotics or antibacterial drugs, medical research has been targeted towards the discovery of QQ compounds as anti-virulence drugs to fight the infections caused by *P. aeruginosa*.

QS in *P. aeruginosa*

The QS systems of *P. aeruginosa* are very well studied, and it is also considered a model system for AHL signalling [12]. The organism produces two types of AHL systems, LasR/I and RhlR/I, which follow the LuxR/I mechanism. The LasI produces N-3-oxo-dodecanoyl-L-homoserine lactone (3O-C12-HSL), which at the threshold concentration, binds to the LasR protein, further regulating the expression of LasI, virulence factors and biofilm maturation. Apart from virulence regulation, the 3O-C12-HSL molecule also contributes to the *P. aeruginosa* infection with its immunomodulating properties that assist the organism in protecting itself from the host immune reactions [13,14]. In the Rhl system, the RhlI synthase produces N-butyryl-L-homoserine lactone (C4-HSL), which binds to the RhlR protein and the activated C4-HSL-RhlR complex then stimulates the expression of RhlI, virulence genes and biofilm associated genes. A third QS system produced by the organism operates via quinolone signal molecule. 2-heptyl-3-hydroxy-4(1H)-quinoline, called as *Pseudomonas* quinolone signal (PQS). It is produced by the products of the *pqs* (*pqsABCDE*) operon and *pqsH* gene, wherein the *pqsABCDE* operon products synthesize 2-heptyl-4-hydroxyquinoline (HHQ), and the PqsH converts HHQ into PQS. PQS diffuses in and out of the cell, accumulates in the environment, and at a threshold concentration, binds to the regulator protein, PqsR, which then modulates gene expression. PQS system has been found to regulate genes encoding virulence factors and the synthesis of PQS itself, resulting in autoinduction [15]. In addition to the virulence and biofilm regulation, the QS systems have been associated with other functions, involved in cellular metabolism, stress responses, etc. Some of the important functions of the Las, Rhl and PQS systems are depicted in Figure 1a. These three main signalling systems of *P. aeruginosa* are interlinked with each other, wherein one system controls the functioning of the other system (Figure 1b), and further all the systems are embedded in a global complex regulatory network [12].

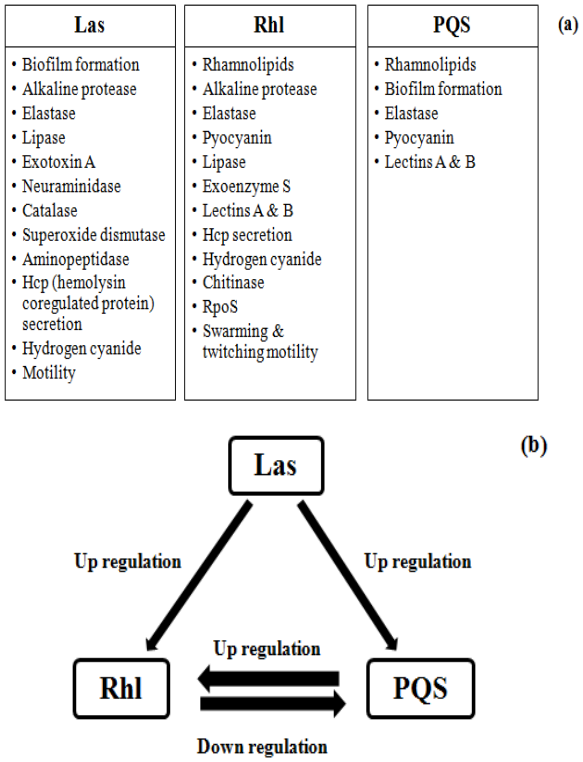
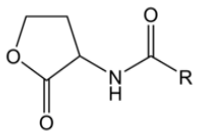


Fig. 1: The regulatory functions of Las, Rhl and PQS systems of *P. aeruginosa* (a) and the interlinked regulatory circuit of Las, Rhl, and PQS (b)

The diverse mechanisms for disruption of AHL systems

Among the QS autoinducers in bacteria, AHLs are the most well studied family. Structurally, they are made up of conserved homoserine lactone ring, which is fused to a variable acyl chain by an amide bond (Figure 2). The variability in the acyl chain, in terms of length, unsaturation and oxo- or hydroxy- substitutions provide AHL specificity to a particular bacterial species, and on the basis of its length, AHLs are classified as short (C4-C8) and long chain AHLs (C10-C18). The important role of AHL in the pathogenicity of many Gram negative bacteria has rendered it as a significant target for anti-pathogenic drugs. Thus, various natural and synthetic sources have been elucidated for the inhibition of AHLs, exhibiting varied mechanistic properties such as AHL inactivation, hindrance in AHL synthesis and interference in the AHL-LuxR binding or LuxR activity. AHL degrading enzymes such as AHL lactonases have been discovered in some bacteria, *Bacillus* spp. *A. tumefaciens*, etc. AHL lactonases act by hydrolyzing the ester bond in the lactone ring of the molecule, resulting in AHL inactivation. AHL acylases, that target the amide bond between the homoserine lactone and acyl side chain, have also been discovered in some bacterial species, such as *Variovorax paradoxus*, *Rhodococcus erythropolis*, etc., which use AHL degradation products as nutrient sources. Antibody disruption of AHLs is another mechanism of quorum quenching in bacteria, that

has demonstrated effectiveness in infected mice, that were previously vaccinated with AHL-carrier protein conjugates [16].



Homoserine lactone **Acyl chain**

Short chain: R = C4-C8

Long chain: R = C10-C18

Fig. 2: General structure of AHL molecule

The second mechanism of AHL inhibition involves disrupting its synthesis, which can be achieved either by affecting the expression of LuxI (AHL synthase), or by interfering with the normal functioning of enzyme catalysis. In a study by Tateda *et al.* [17], the sub-MIC concentration of azithromycin was found to disrupt QS in *P. aeruginosa*, by affecting the expression of LasI, and it was further capable of attenuating the organism's virulence *in vitro* as well as *in vivo* [18]. Moreover, azithromycin was also found to be effective in a randomized clinical trial as a preventive measure against ventilator associated pneumonia [19]. An AHL analog, called as J8-C8, exhibited inhibition of AHL synthesis by the LuxI homolog, TofI synthase of the bacterium, *Burkholderia glumae*. *In silico* studies were applied to understand the mode of action, wherein J8-C8 was found to bind strongly at the acyl chain binding site of TofI, suggesting a competitive inhibition of the enzyme [20].

Most of the QQ studies have focused on the inhibition of LuxR, as an effective means to disrupt the QS system in bacterial pathogens. LuxR is the transcriptional regulator protein, which gets activated by the binding of AHLs. It possesses separate binding sites for the AHL molecule, and the promoter sequences in the bacterial DNA [21]. Inhibition of LuxR can be achieved by preventing the binding of its cognate AHL, or by affecting gene expression. The halogenated furanone, the first natural QQ compound to be discovered, has been found to operate by the competitive inhibition of the LuxR protein [22]. Various synthetic furanones, AHL analogs or other dissimilar compounds such as triphenyl compounds, salicylic acid, nifuroxazide, etc. have been identified as identified as LuxR inhibitors, and have been found to attenuate QS and its regulated phenotypes in Gram negative bacteria such as *A. tumefaciens*, *P. aeruginosa*, *V. fischeri,* etc [23–27].

Plant products as anti-microbial and anti-QS agents

It is very well known that plants have been traditionally used in ancient civilization for the treatment of various human diseases, and they also form the major principle of current medicinal practices, such as Ayurveda, Homeopathy, Naturopathy, etc. Plant powders, decoctions, extracts are commercially available and have been effective not only for specific diseased states, but also for the general well being of human health. Furthermore, plants have firm stand in the pharmaceutical sector since many phytochemicals have guided the development of successful and effective drugs. The well-known examples of plant derived drugs include the pain killer, aspirin which is a simplified form of the compound, salicin (*Salix alba*), the anti-malarial quinine (*Cinchona officinalis*), anti-cancer drug paclitaxel (*Taxus brevifolia*) etc [28]. Plants are the sources of varied bioactive metabolites such as the non polar hydrocarbons and their derivatives, such as terpenes, aromatic compounds such as phenolics and nitrogen containing alkaloids. Many of these have been identified with potential anti-microbial properties, suggesting their prospective future in infection therapy. Common phenolic compounds such as catechol, eugenol, caffeic acid, catechin, phloretin, warfarin, etc. have been reported with antimicrobial potential. The essential oils (monoterpenoids) occurring in plant species such as *S. alba*, *Valeriana officinalis*, *Rosmarinus officinalis*, *Cinnamomum verum*, exhibited. exhibited potential antimicrobial activity [29]. It is not surprising that the capabilities of phytochemicals extend beyond the inhibition of microbial growth, that is, towards the bacterial quorum quenching. Various plants have demonstrated the ability to interfere with bacterial QS systems and further control the associated virulence and biofilm formation. The Gram negative bacterium *C. violaceum* is known to produce a short chain AHL, C6-HSL, which regulates the production of the purple pigment, violacein.

This organism has been commonly used as a biosensor to discover compounds that can inhibit short chain AHL system in bacteria, since decreased violacein production is indicative of AHL inhibition. Genetically engineered strains of *A. tumefaciens* are often used for the detection of both short and long chain AHLs, wherein decreased AHL production results in the reduced activity of the enzyme, β-galactosidase [30]. With the help of such biomonitor organisms, the AHL antagonistic activity has been discovered in various plants such as *Zingiber officinale*, *Brassica oleracea*, *Ocimum basilicum*, *Acacia nilotica*, *Laurus nobilis, Mangifera indica*, *Punica granatum*, *Hemidesmus indicus*, *Vanilla planifolia*, *Tremella fuciformis*, etc [31].

QS inhibition of *P. aeruginosa* by plants

The Las and Rhl systems of *P. aeruginosa* are well known to control the production of extracellular virulence factors; hence, interfering with any of these systems will hamper the organism's virulence. With such opinion, many plants that have been able to affect short chain AHL regulated violacein in *C. violaceum*, were tested for inhibition of Rhl regulated swarming motility, pyocyanin and biofilm development in *P. aeruginosa.* Most of these plants were capable of affecting the above virulence factors in the organism; their inhibitory effects have been presented in Table 1.

Table 1: Plants capable of inhibiting short chain AHL regulated phenotypes in bacteria

Plant	QS regulated phenotypes inhibited	References
Ocimum basilica, Thymus spp., *Brassica oleracea & Zingiber officinale*	Violacein production in *C. violaceum,* swarming motility in *P. aeruginosa*	[32]
Hemidesmus indicus, *Holarrhena antidysentrica, Mangifera indica*, *Punica granatum* & *Psoralea corylifolia*	Violacein production in *C. violaceum,* swarming motility in *P. aeruginosa*	[33]
Capparis spinosa	Violacein production in *C. violaceum*, virulence, motility and biofilm formation in *P. aeruginosa*, *S. marcescens*	[34]
Myristica cinnamomea	Violacein production in *C. violaceum,* virulence and biofilm formation in *P. aeruginosa*	[35]
Syzygium aromaticum	Violacein production in *C. violaceum,* virulence and motility in *P. aeruginosa*	[36]
Melicope lunu-ankenda	Violacein production in *C. violaceum,* virulence and motility in *P. aeruginosa*	[37]
Phyllanthus amarus	QS regulated violacein production in *C. violaceum*, virulence in *P. aeruginosa*	[38]
Cecropia pachystachya	QS regulated violacein production in *C. violaceum*	[39]

As per the available knowledge, the Las system is responsible for inducing the expression of RhlI and RhlR. Thus, disruption of the Las system will prevent the activation of the Rhl system; and it can be thought to be more effective in controlling the organism's virulence. In a study conducted by Delden *et al*. [40], *lasR*, *lasIrhlI* and *lasRrhlR* deletion mutants were subjected to starvation, and checked for the appearance of mutants with restored elastase and rhamnolipid production, which is generally directed by the functional *las* and *rhl* systems, respectively. The *lasR* mutant showed restoration of its elastolytic activity and rhamnolipid production, in spite of a *lasR* deletion, while the *las rhl* double mutants did not show the expression of either of the above virulence factors. This suggested that the Rhl system was responsible for the expression of virulence factors in the absence of the Las system. Hence, it becomes necessary to inhibit both the Las (long acyl-HSL) and Rhl (short acyl-HSL) systems of *P. aeruginosa* to develop a promising therapy against the pathogen.

Some of the QQ studies have discovered plant sources with the potential to inhibit both Las and Rhl systems, providing prospective candidates to control *P. aeruginosa* infections. One of the most cited study, conducted by Adonizio [41], reported significant inhibition of short and long chain AHLs by six plants of South Florida, *Conocarpus erectus*, *Bucida buceras*, *Callistemon viminalis*, *Tetrazygia bicolor* and *Chamaesyce hypericifolia*. The plants also inhibited multiple virulence factors (LasA & LasB elastase, pyoverdin) and biofilm formation in *P. aeruginosa* PA01. The extracts of *C. erectus*, *B. buceras* and *T. bicolor* were found to significantly reduce infection associated mortality in the nematode model, *Caenorhabditis elegans* and further, the anti-QS components in the aqueous extract of *C. erectus* were identified to be ellagitannins, such as vescalagin and castalagin. The toluene extract of garlic was found to be effective in controlling the QS genes, its regulated virulence and biofilm formation in *P. aeruginosa in vitro* as well as *in vivo*. By employing bioassay guided fractionation, the primary QS inhibitor in the crude extract of garlic was determined to be Ajoene, an unsaturated disulphide compound. This compound, in at lower than its bactericidal concentration (25 µg/ml), exhibited significant reduction of few virulence factors, including rhamnolipids. It further reduced the susceptibility of biofilms to tobramycin, and also prevented the biofilm killing of human polymorphonuclear leukocytes. Furthermore, it exhibited enhanced bacterial clearance in infected mouse model at 3 days post infection, when compared with the control group [42–44]. Song *et al*. [45] reported the QS inhibitory potential of *Panax ginseng* roots, which exhibited significant reduction of the virulence factors, LasA and LasB in *P. aeruginosa* PAO1 and alginate production in the mucoid variant PAO*mucA22*. Further, the concentration of C4-HSL and 3O-C12-HSL was significantly reduced in the presence of *P. ginseng* powder. In previous studies, *P. ginseng* was found to stimulate defense mechanisms of infected rat models, assisting bacterial clearance from the lungs infected with *P. aeruginosa* [46,47]. It is thought that the anti-QS and immunomodulatory properties of Ginseng might have contributed to its effectiveness in treating *Pseudomonas* infection in rats.

Flavonoids such as catechin isolated from the bark of *Combretum albiflorum*, was found to reduce the expression of QS genes, *lasI, lasR, rhlI* and *rhlR*, and further attenuate virulence factors such as pyocyanin, elastase and biofilm formation in *P. aeruginosa* [48]. Based on this research, further work was conducted to evaluate the effect of commercially available flavonoids on the QS systems and virulence of the organism. Out of these, three flavonones, viz. naringenin, eriodictyol, and taxifolin, exhibited significant reduction of pyocyanin and elastase, without affecting bacterial growth. Further, naringenin and taxifolin reduced the expression of QS-controlled genes in *P. aeruginosa* PAO1 and naringenin also dramatically reduced the production of C4-HSL and 3-oxo-C12-HSL [49]. Similarly, phenolic compounds in different species of the genus *Terminalia*, viz. *T. catappa* (tannins) and *T. chebula* (ellagic acid derivatives) have been attributed for the inhibition of AHL systems and the regulated phenotypes in *P. aeruginosa* [50,51].

In an interesting study presented by Jakobsen, Bragason, *et al*. [52], iberin, an isothiocyanate isolated from the plant, *Armoracia rusticana*, exhibited significant QSI activity by blocked the expression of QS genes in *P. aeruginosa*. However, the compound iberin, failed to affect bacterial clearance in infected mouse model. The plausible explanation for the results implied the reactive nature of the isothiocyanate group of the compound as being responsible for losing its activity *in vivo*. Natural isothiocyanates, sulforaphane and erucin, isolated from *Brassica oleracea*, were also shown to significantly inhibit AHL regulated virulence and biofilm formation in *P. aeruginosa* [53]. With respect to the results obtained for iberin by Jakobsen, Bragason, *et al*., it is recommended to determine the effects of sulforaphane and erucin on *in vivo P. aeruginosa* infection.

Additional studies on QQ in *P. aeruginosa* unveiled novel plant sources such as *Lagerstroemia speciosa*, *Rhizophora* spp., *Dalbergia* spp., *Ananas comosus*, *Musa paradiciaca*, *Manilkara zapota*, *Ocimum sanctum,* showing the capability to interfere with the organism's AHL systems and virulence [54–57]. Also, in an anti-QS screening study by Gala *et al*. [58]; the cold and hot ethyl acetate extracts of *Tinospora cordifolia* were found to be effective in disrupting both short and long chain AHL production.

Future scope

Several plant based inhibitors of the AHL systems of *P. aeruginosa* have been discovered in the last decade, and some of these (garlic, *C. erectus*, *P. ginseng*) have also exhibited significant *in vivo* potential in treating *P. aeruginosa* infections. Further investigation for these plant sources may focus on the evaluation of the synergistic effects of the QSI compounds or crude extracts along with the antibiotics. Alternatively, a combination of two or more QSI compounds on bacterial growth, QS signalling, and its virulence may also be tested. Some reports on the *P. aeruginosa* clinical isolates have implied clinical variations in terms of drug resistance, biofilm formation, QS genes, virulence factors, etc [59–61]. Hence, it is also important to study the effect of the QSI sources on the clinical isolates, before these can be considered for drug development. The isolated phytochemicals such as ellagitannins, ellagic acid derivatives, flavonones, must be evaluated further for their *in vivo* efficacy. Additionally, research investigations based on the isolation of active components from the anti-QS plant extracts and their effectiveness *in vivo* is recommended is recommended, as this will help to build a library of QSI phytochemicals for *P. aeruginosa*. This library can be then screened for the production of hits and leads in the process of drug discovery.

Apart from the AHL systems, disruption of the quinolone signalling in *P. aeruginosa* also presents an important anti-virulence target. Few studies have been directed towards quenching of the quinolone signalling system in *P. aeruginosa*. In a study by Pustelny *et al*. [62], the enzyme 1H-3-Hydroxy-4-oxoquinaldine 2,4-dioxygenase, abbreviated as Hod, produced by the bacterium, *Arthrobacter nitroguajacolicus* Rü61a, was found to convert PQS into N-octanoylanthranilic acid and carbon monoxide. Hod is a part of the quinaldine utilization pathway, wherein it acts on 3-hydroxy-2-methyl-4(1H)-quinolone and cleaves it into N-acetylanthranilic acid and carbon monoxide. Thus, when Hod was added externally to *P. aeruginosa* PAO1 culture, by the virtue of its catalysis, it was able to reduce the expression of the *pqsA* gene, and the PQS regulated virulence factors such as lectin A, rhamnolipids and pyocyanin. However, due to the cleavage of Hod protein by the extracellular proteases of *P. aeruginosa*, the enzyme was ultimately found to be ineffective as a QQ agent. In another study, first of its kind, HHQ analogs were synthesized and their agonistic and antagonistic effects on PqsR were evaluated using the reporter strain, *Escherichia coli* containing the plasmid pEAL08-2. The activation of PqsR by the HHQ analogs resulted in the induction of β-galactosidase activity in the reporter strain. Out of 30 HHQ analogs, three potent HHQ antagonists were obtained, which were also effective in reducing the PQS regulated pyocyanin production in *P. aeruginosa* PA14 cultures [63]. These studies have incited the opinion that PQS inhibitors may serve as important anti-virulence drugs for controlling *Pseudomonas* infections. Plants have been found to secrete AHL mimics that act as competitive inhibitors of Lux homologous proteins. These compounds constitute the plant defense system against the phytopathogenic bacteria [64]. This study also suggests the

probability for the existence of quinolone inhibitors in plants as their protection from bacterial infection. Hence, exploring the plant resources to identify potent PQS inhibitors is greatly encouraged.

CONCLUSION

The organism, *P. aeruginosa*, is emerging as a dreadful nosocomial pathogen, with its evolving drug resistance and the amazing adaptability to diverse environments. Hence, it is indispensable to develop new drugs, not only to control the infections, but also to counteract the resistance phenomena. It can be observed that an enormous resource of active metabolites has been contained in the huge diversity of plants on earth and only a small portion has been discovered with the quorum quenching potential. Not only is it necessary to explore more plant sources for the anti-QS activity, it is also very important to promote the known phytochemical QS inhibitors as anti-pathogenic drugs, before the pathogen evolves to defeat all the available antibiotics.

CONFLICT OF INTERESTS

Declared None

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