



QUORUM QUENCHERS – PAST, PRESENT AND FUTURE OF THIS NOVEL THERAPEUTICS.

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1. INTRODUCTION

One of the significant developments of modern medicine is the discovery and development of antibiotics to treat infectious diseases. The treatments by using antimicrobial agents aim to bring either bacteriostatic or bacteriocidal activity in pathogenic microbes. A major difficulty with this approach is development of multi resistance pathogenic bacterial strains. Hence the development of novel therapeutic approaches to treat bacterial infections constitutes a focal point of modern research. The discovery of quorum-sensing systems, which coordinate important sequential events during the infection

process, has provided a novel opportunity to fight bacterial infection. Compounds which are capable of interfering bacterial signalling processes were discovered in the recent years. Observations on Quorum sensing linked virulence factor production and biofilm formation suggests that many virulent Gram-negative organisms could potentially be rendered non virulent by inhibition of their quorum-sensing systems. Dong *et al* (2001; 2004), Hentzer and Givskov (2003) explored these approaches as feasible ways to prevent and control bacterial infection popularly known as 'quorum quenching' (QQ), 'anti pathogenic' or 'signal interference'. Over the last 15 years, use of quorum quenchers to modulate bacterial sensing systems has attracted significant interest.

2. MOLECULAR MECHANISMS OF SIGNAL INTERFERENCE

AHLs are the most common class of autoinducer used by Gram-negative bacteria; indeed it represents one of the best-understood bacterial systems at the molecular level. Gram negative bacteria control quorum sensing circuits based on Lux I/Lux R gene regulation. As microbial QS systems rely on QS signals and the cognate receptor proteins for gene regulation, any reagent that prevents accumulation of or recognition between QS signals and receptor proteins block bacterial QS-dependent virulence gene expression.

Three different targets were observed for these quenching molecules.

1. The signal production or bio synthesis
2. The signal molecule and
3. The signal receptor.

2.1 Targeting at Signal production or its Biosynthesis

The majority of natural AHLs reported to-date share conserved structural characteristics, a homoserine lactone ring unsubstituted at the β and γ positions, which is N-acylated at the R-position with an acyl group derived from fatty acid biosynthesis (Figure 1). AHL synthesis occurs via a sequentially ordered reaction. Acyl side chain of AHL is synthesized by fatty acid biosynthesis pathway and Homoserine Lactone moiety is synthesized by S-Adenosyl methionine, an amino donor for the formation of homoserine lactone ring moiety which couples with acyl side chain to form Acyl homoserine lactone. Different proteins are involved in this pathway which includes Acyl carrier protein, enoyl-ACP reductase, Fab I and AHL synthase. AHL synthases are highly conserved sequences in Quorum sensing regulating bacteria. Enoyl ACP is reduced by Fab I to produce acyl ACP which reacts with S-Adenosyl methionine in the presence of AHL synthase enzyme to produce AHL.

Another important protein involved in AHL biosynthesis is Lux R type transcription factor which is involved in activating the expression and production of AHL's. Interfering with AHL autoinducer production is a simple method for modulating quorum sensing pathways as no signalling can occur if there is no signal production. AHL synthases are highly conserved sequences in Quorum sensing regulating bacteria. S-adenosyl-homocysteine (SAH), sinefungin, and butyryl SAM are various analogs of SAM which can cause the chemical modulation of AHL synthesis (Figure 2). Hentzer, M.; Givskov, M. (2003) Rasmussen, T. B.(2005 a & b) and Givskov, M (2006) proved that analogs of SAM are potent inhibitors of the *P. aeruginosa* AHL synthase RhlI in vitro.

Cronan, J. E., *et al* (2000) observed that other homologues and analogues of purine nucleotides (e.g thiol derivatives and alkylated thio derivatives) and homoserine lactone derivatives are AHL synthase blocking compounds. Hoang and Schweizer (1999) found that acyl - ACP one of the essential intermediates in AHL biosynthesis, could be inhibited by Triclosan a potent inhibitor of the enoyl-ACP reductase and thus reduces AHL production. Interestingly, Parnham, M. J. (2005) found that certain macrolide antibiotics are repressing the *P. aeruginosa* AHL synthesis when applied at sub minimal growth-inhibitory concentrations. Recently, several crystal structures for Lux I-type proteins have been reported by Pappas, K. *et al* (2004), Gould, T. A. *et al* (2004), Geske, G. D. *et al* (2008), which could potentially be exploited in the rational design of synthetic ligands. Geske, G. D. *et al* (2008), predicted that this field is expected to gain significant interest in the near future.

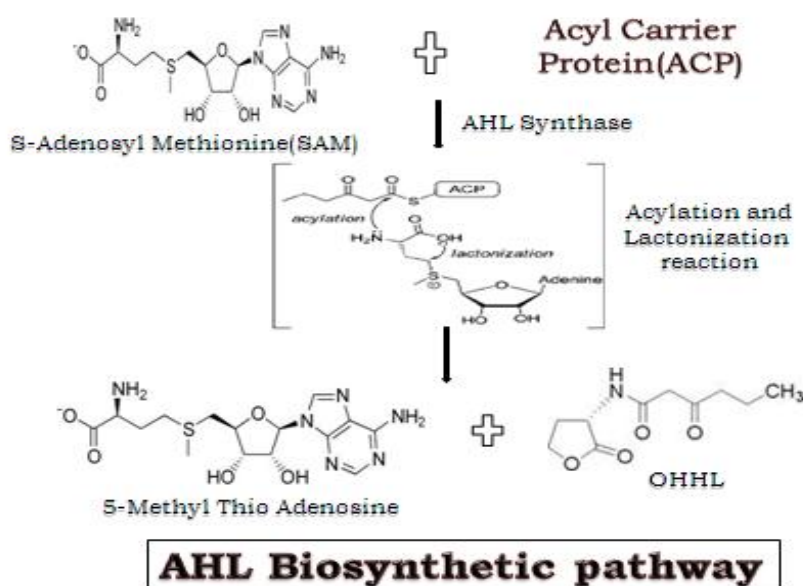


Figure 1: AHL biosynthetic pathway in signalling bacteria.

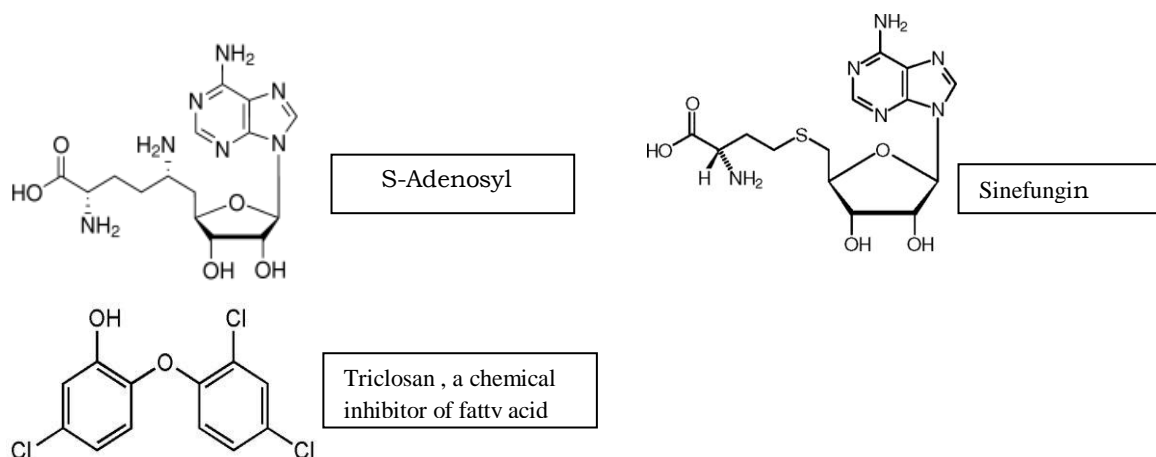
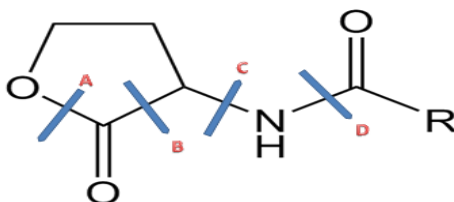


Figure 2: Biological and chemical inhibitors of AHL biosynthetic pathway.

2.2 Target at signal level

Nature is known to have evolved quorum quenching enzymes that are capable of hydrolyzing both the amide and lactone moieties of AHL signaling molecules (Figure 3). These enzymes are involved in defense mechanism of host.

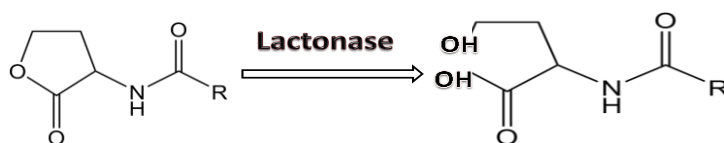


The figure depicts the possible sites of enzymatic cleavage sites

Figure 3: Sites of enzymatic cleavage.

2.2.1 AHL lactonases

Dong *et al.*, (2000; 2001) reported that AHL-lactonase is highly specific against AHLs and hydrolyses the lactone ring to produce corresponding acyl homoserines (Figure 4) and reduces its activity by 1000 fold. AiiA gene encodes the AHL lactonase production. Dong. Y. H., *et al* (2000) first identified in gram positive *Bacillus isolate 240 Bi*.



The above figure illustrates the degradation mechanism of Lactonase enzyme

AHL Lactonase hydrolyses and open the Lactone ring of AHL and reduces its activity by 1000 fold

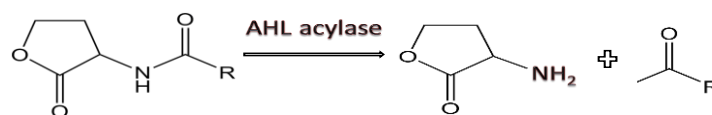
Figure 4: Degradation mechanism of Lactonase enzyme.

Dong *et al* (2000, 2001), Lee *et al* (2002), Wang *et al* (2004) reported the production of AHL lactonase in many *Bacillus* species like *Bacillus mycoides*, *Bacillus thuringensis* and *Bacillus cereus*. When AiiA gene was cloned into *Pseudomonas fluorescens* it was able to prevent soft rot disease in potatoes caused by *Erwinia carotovora* and crown gall disease in tomato plants caused by *Agrobacterium tumifaciens*. Further investigations of Molina *et al.*, (2003) on transgenic tobacco expressing AiiA gene demonstrated the less vulnerability of

plants to infection by *Erwinia carotovora* compared to its wild type. AHL lactonases were also found in gram negative bacteria. Lactonases are thus proved to be potent enzyme in degrading AHL's.

2.2.2 AHL acylases

Lead better and Greenberg (2000) showed that AHL-acylase breaks the amide linkage of AHLs and yields fatty acids and homoserine lactone (Figure 5), which fails to show any biological activity. These were first observed in a gram negative soil bacterium, *Variovorax paradoxus*, which utilizes AHL as sole carbon source and releases homoserine lactone into surrounding medium. These bacteria produce an amino acylase enzyme which acts on peptide bond of signal molecule. Huang *et al* (2003) described that the side chain of the molecule is used as carbon source, the amide bond is used as nitrogen source and the ring part is used as energy source.



The above figure depicts the mode of action of acylase

AHL acylase breaks the amide linkage of AHLs and yield Fatty acids and homoserine Lactone

Figure 5: Mode of action of acylase.

2.2.3 Interfering with signal receptor molecule

Another important approach to interfere with signal molecule is to prevent the signal reception by bacteria thereby blocking the receptor protein or destruction of receptor protein. Significant efforts in recent years were put in towards the identification of new classes of small molecule modulators of LuxR-type proteins. It is convenient to categorize small molecules that modulate LuxR type receptors according to source from where they were identified as synthetic & natural molecules.

3. QSI ACTIVITY REPORTED BY SYNTHETIC ANALOGUES

Compounds that exhibit structural similarity to native AHLs are referred as AHL analogues, that is, compounds that contain a carbon chain (of any functionality or substitution) or aryl group linked to an amide group (or derivative thereof), the nitrogen atom of which is directly

attached to a ring system at a chiral carbon. Utilizing the structure of the known natural AHL signaling molecule as a template, small-molecule agents capable of modulating a given quorum sensing pathway via interaction with the relevant LuxR-type receptor were discovered through a design and synthesis process.

Many of the synthetic compounds proved to be potent competitive agonists by Schaefer *et al* (1996). Smith *et al* (2003) made the most effective analogue 3-oxo-C12 amino cyclohexanone (Figure 6), which was able to reduce Las R controlled expression.

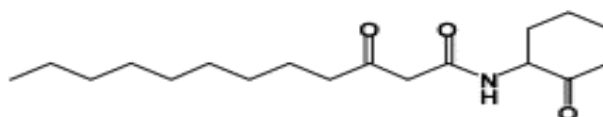


Figure 6: 3-Oxo C12 amino cyclo hexanone

Persson *et al* in 2005 created a synthetic analogue N-(heptyl sulfanyl acetyl) -l-homoserine lactone by replacing C-3 carbon with sulphur group in acyl side chain. This analogue was able to block transcriptional regulators LuxR and Las R by competitive inhibition. Castang *et al* in 2004 reported that QSI activity is enhanced by replacing c-1 carbonyl group of side chain with a sulphonyl group. The research group of Ishida *et al* (2007) has studied the use of N acyl cyclopentylamine (Cn-CPA) derivatives as quorum sensing inhibitors. They synthesized Cn-CPA derivatives with a variety of acyl chain lengths and reported that C10-CPA, the most effective inhibitor of the LasR and RhlR quorum sensing systems in *P.aeruginosa*.

Computational pharmacophore modeling studies of Kim, C. *et al* (2009), Geske, G. D *et al* (2008), Ahumedo, M. *et al* (2010) helped in understanding how different AHL structural features bring about various biological activities. Structure Activity Relationships (SAR) associated with non-native AHLs represent the most extensively studied class of synthetic quorum sensing modulators to date.

Blackwell and co-workers figured out five broad activity trends from their studies:

- (1) The length of the acyl chain was found to be critical.
- (2) Modification at the 3-carbon of the acyl chain (e.g., a carbonyl), was important, but not essential, for activity.
- (3) Natural L-stereoisomer of the lactone ring is needed for activity.

(4) Direct modifications to the lactone ring in some, but not all, systems result in less active compounds.

(5) Analogues with inhibitor activity were obtained by incorporation of aromatic functionality into AHLs, as either lactone ring replacements or substituents inside chains.

Blackwell and co-workers have reported the synthesis and evaluation of quorum sensing modulators using small-molecule macroarrays. Kim *et al.* (2009) reported the development of inhibitors against TraR quorum sensing in *A. tumefaciens*. A series of structural analogues of the native autoinducer OOHl in which the carboxamide bond was replaced with a nicotinamide or a sulphonamide bond (i.e., *N*-nicotinyl and *N*-sulfonyl homoserine lactones) were designed by in silico molecular modeling to exhibit tight binding to the TraR receptor. Frezza *et al* 2006 prepared nine homoserine lactone-derived sulfamide derivatives substituted with either an alkyl chain or a phenyl group. All of these compounds inhibited the action of the *V. fischeri* quorum sensing regulator OHHL, with the aliphatic compounds showing higher levels of activity. Compounds which showed the best antagonist activity in the alkyl and phenyl series, respectively, were selected for molecular modeling in the ligand-binding site of TraR.

Synthetic analogs that function as QSI compounds have potential clinical applications. Despite the progress made, this field is still in its infancy. The most widely used method for the discovery of non-AHL based modulators has been the screening of either natural product isolates or synthetically derived chemical libraries.

4. QUORUM SENSING INHIBITORS FROM NATURALLY OCCURRING COMPOUNDS.

The discovery of quorum sensing signals operating in different pathogenic bacteria species have paved for the way to search on naturally existing quorum quenching compounds among prokaryotes and eukaryotes. QSI compounds have been isolated from natural sources such as bacteria, algae, fungi, and plants as they co-existed with QS bacteria for millions of years

4.1. From Bacteria

Quorum quenching (QQ) activity was subsequently observed in a range of bacteria. QQ activity so far has been demonstrated in more than 10 bacterial species. The identified organisms include species of *Bacillus*, *Arthrobacter*, *Klebsiella*, *Pseudomonas*, *Ralstonia*, *V.Paradox* etc.

The organisms that were identified to produce QQ activity are found to belong to three phyla of bacterial kingdom namely *Actinobacteria* (*Arthrobacter* spp) *Fermicutes* (*Bacillus* spp) and *Proteobacteria* (*Klebsiella pneumonia*, *Ralstonia*, *Pseudomonas* spp and *V. paradoxus* spp). The diverse distribution of QSI activity in different phyla indicates that AHL degradation enzymes might be widely conserved in prokaryotes. Huang, J. J., *et al.* (2003), Carlier, A., *et al* (2003) have identified enzymes that degrade AHLs in bacteria that produce AHLs, and these enzymes could potentially play a role in regulating quorum sensing in *P. aeruginosa* PAO1 and *A. tumefaciens*. Interestingly, Non-AHL-producing bacteria were found to make AHL-degrading enzymes, which have evolved to utilize this signal molecule as a carbon and nitrogen source. Chifiriuc, M. *et al* (2009) observed that sub inhibitory concentrations of phenyl lactic acid, (produced by *Lactobacillus* probiotic strains), could attenuate *P. aeruginosa* virulence and pathogenicity by interfering with different processes regulated by quorum sensing.

4.2 From Algae

Anti QS agents were first characterized in the marine red alga *Delsia puchra*. Halogenated furanones produced by *Delsia puchra* could inhibit QS in a number of bacteria. In a landmark paper, Givskov *et al.* (1996) hypothesized that the biological effects of the furanones may be due to their ability to interfere with AHL-regulated quorum sensing systems. These authors demonstrated that purified samples of the *D. pulchra* furanones inhibit various AHL-controlled processes in prokaryotes without affecting their growth, specifically the swarming motility of *Serratia liquefaciens*. Similar observations were also made by Gram *et al.* (1996) in *Proteus mirabilis*.

Eberl *et al* (1999) hypothesized that furanones are structural analogues of native AHL of prokaryotes (Figure 7). They interfere with the activity by binding to active site on SwrR, a putative regulatory protein. The inhibitory effect was also demonstrated on bioluminescence in a *Vibrio fischeri*. Manefield *et al.* (1999) showed that the furanones are capable of displacing 3-oxo-C6-HSL from an *Escherichia coli* strain over-producing LuxR, thus providing direct evidence for the specific activity of the furanones. They also inhibit pigment production in *C. violaceum* (Martinelli *et al.*, 2004).



A. Halogenated Furanone

B. AHL

Figure 7: Structural similarities between AHL and Halogenated Furanones.

The synthetic derivatives and natural furanones are found to inhibit *Pseudomonas aeruginosa* infection in *in vitro* and studies on murine model. (Hentzer *et al.*, 2003; Wu *et al.*, 2004). Liu, H. B. *et al* (2008), Kim, J. S, *et al* (2007), have reported that extracts of the red alga *Ahnfeltiopsis flabelliformis* inhibited quorum sensing mediated by OHL and the TraR transcriptional activator protein. Using activity-guided fractionation, they isolated an active fraction containing betonicine, floridoside and isethionic acid. When tested individually, none of the three compounds exhibited inhibition activity. In contrast, a complex of floridoside and isethionic acid revealed a dose-dependent inhibition on OHL activity, suggesting that these two compounds are responsible for the inhibition activity of red algae extract.

4.3 From Fungi

In 2005 Rasmussen *et al.* identified the naturally occurring compounds patulin and penicillic acid produced by *Penicillium coprobium* and *P. radicum*, respectively, as inhibitors of quorum sensing in *P. aeruginosa*. Zhu, H.; *et al* and Sun, S.,J., *et al* (2008) reported QSI activity in extracts of *Tremella fuciformis* (white jelly mushroom) against *C. violaceum*. Zhu *et al* (2011) demonstrated that edible and medicinal fungi *A. auricular* were able to inhibit quorum sensing in *Chromobacterium violaceum* 026 strain. Further studies were in progress to isolate the active component from *A.auricular* pigments.

4.4. From Insects

Park *et al.*(2008) have reported that a venom alkaloid from the fire ant *Solenopsis invicta*, solenopsin A - inhibits quorum sensing in *P. aeruginosa*. This compound is structurally similar to OdDHL (the natural AHL of the LasR system) in that both contain a long hydrocarbon chain attached to a nitrogen containing heterocycle, via a chiral carbon. However, both structures contain distinct molecular frameworks. In these studies it was observed that exogenously added BHL, but not OdDHL, restored *P. aeruginosa* quorum sensing signaling, suggesting that solenopsin A actually targets the BHL-dependent *rhl* quorum sensing system. Truchado, P, *et al* (2009) reported that honey had QSI activity against *E. carotovora*, *Yersinia enterocolitica*, *Aeromonas hydrophilia*, and *C. violaceum*.

4.5 From mammals

Xu *et al* (2003) demonstrated that porcine kidney acylase I could deacylate AHLs. The enzyme was shown to inhibit biofilm formation in aquarium water samples. Chun *et al*, (2004) found that 3-oxo C12 HSL was inactivated by lactonases from differentiated human airway epithelia. It was observed that inactivation was selective only for acyl side chains. However, these lactonases did not inactivate C4HSL. They concluded that mammalian airways protect themselves by producing quenching enzymes like lactonases from pathogen attacks. Teiber, J.*et al* (2008) identified a class of enzymes known as paraoxanases in several mammals, which are capable of inactivating OdDHL and thus attenuating *P. aeruginosa* quorum sensing in cell cultures and in vivo.

4.6 From Plants

Over the course of the past decade, a wide range of naturally occurring substances, particularly extracts from plants, have been evaluated for their ability to modulate LuxR-type quorum sensing in Gram-negative bacteria. Plants are known to synthesize numerous metabolites with various structural characteristics. They have evolved sophisticated mechanisms to perceive pathogen attacks. Plants can produce molecules that structurally mimic the AHLs, and such competitive binding is effective to block activation of AHL-mediated QS. The natural substances known to modulate various AHL-mediated quorum sensing systems is reviewed in chronological order.

Teplitski, M. *et al* (2000) observed that multiple signal molecules present in the pea seedlings exudates inhibit quorum sensing in *Chromobacterium violaceum* but activate quorum sensing in *Pseudomonas* and *Serratia*. Study on *Medicago truncatula* by Gao, M. S. *et al* (2003) indicated that it produces at least 15 to 20 compounds that are capable of specifically activating or inhibiting quorum-sensing-regulated behavior in different bacterial strains. The *M. truncatula* extracts activated reporter strains based on the quorum-sensing regulator proteins. In addition, several compounds inhibited gene expression regulated by CviR (*C. violaceum*), LasR, AhyR, and LuxR. Peters, L. *et al* (2003) performed the analysis of the secondary metabolites of the North Sea bryozoan *Flustra foliacea*, which led to the isolation of a variety of brominated alkaloids, two of which were found to specifically caused a reduction in the signal intensities in biosensor strains of *P. putidia* and *E. coli*. Niu *et al* (2004) screened plant essential oil components and isolated cinnamaldehyde which was established as an effective inhibitor of 3-oxo-C6-HSL (OHHL) quorum sensing in *E. coli* and

3-hydroxy-C4-HSL quorum sensing in *V. harveyi* at subinhibitory concentrations. Interestingly, cinnamaldehyde had minimal effect on *lasR* promoter activity, induced by OdDHL, in an *E. coli* strain containing a LasR biosensor (OdDHL inducible).

In vitro studies by Rasmussen, T. B. (2005) on *P. aeruginosa* biofilms demonstrated that garlic extract significantly reduced the tolerance of the bacteria to the antibiotic tobramycin. Bjarnsholt, T (2005) have demonstrated that garlic extract promotes rapid clearing of mice pulmonary *P. aeruginosa* infections in vivo. Six sulfur-containing compounds from the garlic extract were identified by bioassay-guided fractionation, which inhibited quorum sensing in a LuxR monitor system. Choo, J. *et al* (2006) studied on vanilla extracts from Vanilla beans (*Vanilla planifolia Andrews*) on *C. violaceum CV026* culture. Their study revealed that vanilla extract significantly reduced violacein production in a concentration dependent manner, indicating inhibition of quorum sensing. The intake of vanilla-containing food materials might promote human health by inhibiting quorum sensing and preventing bacterial pathogenesis. However specific substances from vanilla extract as QSI was not reported. Recent studies by Ponnusamy *et al*(2009) suggest that vanillin itself may be the active agent. Juan E. Gonza´lez and Neela D. Keshavan (2006) screened compounds from alfalfa (*Medicago sativa*) exudates for QSI activity on violacein production in the indicator strain *Chromobacterium violaceum CV026*. Some compounds had quorum-sensing-inhibitory effects, while others seemed to activate quorum sensing .They identified the structure of one of the quorum-sensing-inhibitory compounds as L-Canavanine. Since L-canavanine was isolated from seeds of *M. sativa*, quorum-sensing system of its bacterial symbiont, *Sinorhizobium meliloti* was analyzed. L-Canavanine is an arginine analog found exclusively in the seeds of legumes.

Similar studies were also made by Vатtem, D., *et al* (2007), who tested the dietary phytochemicals i.e secondary metabolites of plants against *C. violaceum* and *P. aeruginosa*. Their study indicated that various phytochemical extracts which inhibited QS also inhibited swarming of pathogenic bacteria, known to be modulated by QS. The mechanism of inhibition appeared to be combination of interfering with AHL activity and modulating the synthesis of AHL's .The observation that phytochemicals from foods can inhibit QS related processes opens up an exciting new strategy for antimicrobial chemotherapy.

Allison Adonoz *et al* (2008) reported inhibition of Quorum sensing controlled virulence factor production in *Pseudomonas aeruginosa* by South florida plant extracts. Out of the

aqueous extracts of six plants, *Conocarpus erectus*, *Chamaesyce hypericifolia*, *Callistemon viminalis*, *Bucida buceras*, *Tetrazygia bicolor*, and *Quercus virginiana*, only three plant extracts like *C. erectus*, *B. buceras*, and *C. viminalis* caused a significant inhibition of LasA protease, LasB elastase, pyoverdinin production, and biofilm formation. Different mechanisms were responsible for efficacy of each plant extract as they presented a distinct effect profile on the *las* and *rhl* QS genes of *Pseudomonas aeruginosa*.

Feldman, M. *et al* (2009) reported QSI activity of cranberry juice against *V.harveyi*. Bryant, S. *et al* (2008) published that extracts of various medicinal plants from the Indian subcontinent had QSI activity against *P. aeruginosa*. Mathee, K.; *et al* (2009) reported that Ellagitannin, natural product from various medicinal plants have also shown anti-quorum sensing activity against various Gram negative bacteria including *P. aeruginosa*. Jones, S. *et al* (2009) reported that Tannic acid, a plant polyphenol, could inhibit quorum sensing systems in various Gram-negative bacteria (Figure 8). Al-Hussaini, R and Mahasneh (2009) published that Ethanolic extracts of the plants *Sonchus oleraceus* and *Laurus nobilis* have been shown to inhibit quorum sensing in the Gram-negative bacterium *C. violaceum*. Their results indicate the potential of the plant extracts in treating microbial infections through quorum sensing antagonism & validating their medicinal use.

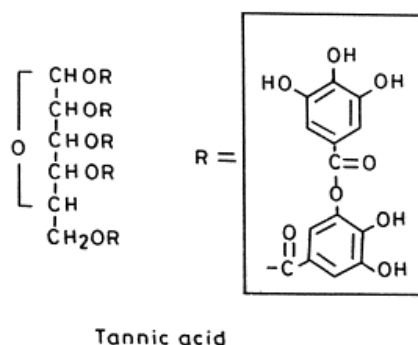


Figure 8: Structure of Tannic acid.

Marayam Zahin, *et al* (2010) screened certain medicinal plants of Indian origin for QSI activity. Ethanolic extracts of 24 medicinal plants were evaluated against biomonitor strain *Chromobacterium violaceum* CV026 and *Pseudomonas aeruginosa* PAO1. Of the 24 medicinal plants screened, *Hemidesmus indicus*, *Schult* (root), *Holarrhena antidysenterica* (root) ADC (bark) *Mangifera* (seed) *Punica granatum* (pericarp) and *Psoralea corylifolia* (seed) demonstrated varying level of inhibition of violacein production in the reporter strain. However bioactive principle is yet to be characterized in these extracts. K. Syed Musthafa et

al (2010) reported anti-quorum-sensing activity of edible plants and fruits through inhibition of the N-Acyl-Homoserine Lactone System in *Chromobacterium violaceum* and *Pseudomonas aeruginosa*. Their study identified the anti-QS activity of *A. comosus*, *M. paradisiaca*, *M. zapota* and *O. sanctum*. An AHL-inactivating compound from these plant sources can be used as an alternative to antibiotic compounds to prevent AHL-mediated bacterial infection in higher organisms.

In a recent study Sandy Siew -Mian Yeo and Foong -Yee Tham (2012) screened twenty Traditional Chinese medicine (TCM) plants commonly used in South-East Asia for QS inhibitors using two biomonitor strains, *Chromobacterium violaceum* CV026 and *Pseudomonas aeruginosa* PAO1. Ten of these selected TCM plants (50%) were found to have QS inhibitory properties: *Angelica sinensis* (Umbelliferae), *Cnidium monnieri* (Umbelliferae), *Astragalus membranaceus* (Leguminosae), *Crataegus cuneata* (Rosaceae), *Dioscorea nipponica* (Dioscoreaceae), *Lilium brownii* (Liliaceae), *Aloe barbadensis* (Liliaceae), *Magnolia officinalis* (Magnoliaceae), *Ephedra sinica* (Ephedraceae) and *Panax pseudoginseng* (Araliaceae). Of these, six (30%) also showed varying antimicrobial activity against *C. violaceum* and *P. aeruginosa*.

Li Ying Tan, Wai-Fong Yin and Kok-Gan Chan (2012) published their findings on anti-quorum sensing properties of edible, endemic plants in Malaysia. Extracts from *Melicope lunu-ankenda* (Gaertn) Malay garden salad, inhibited response of *Chromobacterium violaceum* CV026 to N-hexanoylhomoserine lactone, thus interfering with violacein production; reduced bioluminescence expression of *E. coli* [pSB401], disrupted pyocyanin synthesis, swarming motility and expression of *lecA::lux* of *Pseudomonas aeruginosa* PAO1. In another paper published by Thiba Krishnan, Wai-Fong Yin and Kok-Gan Chan (2012) reported that anti-quorum sensing activity of an Ayurveda spice, clove (*Syzygium aromaticum*). These authors demonstrated that clove extract inhibited quorum sensing-regulated phenotypes in *Pseudomonas aeruginosa* PAO1, including expression of *lecA::lux* (by hexane extract), swarming (maximum inhibition by methanol extract), pyocyanin (maximum inhibition by hexane extract).

Siti Nur Maisarah Norizan *et al* in 2013 tested caffeine as quorum sensing inhibitor by using *Chromobacterium violaceum* CV026 as a biosensor. Caffeine did not degrade the N-acyl homoserine lactones tested but it could inhibit N-acyl homoserine lactone production and swarming of a human opportunistic pathogen *Pseudomonas aeruginosa* PAO1.

Sajal Sarabhai et al (2013) reported that Ellagic Acid derivatives from *Terminalia chebula* Retz downregulate the expression of quorum sensing genes to attenuate *Pseudomonas aeruginosa* PAO1 Virulence. Methanol extract of *T. chebula* Retz. fruit showed anti QS activity using *Agrobacterium tumefaciens* A136. Biofilm formation and alginate were significantly reduced with enhanced susceptibility to tobramycin. LC-ESI-MS revealed the presence of ellagic acid derivatives responsible for anti QS activity in *T. chebula* extract.

Screening of popular food spices for QSI activity by Y.Aparna (2014) revealed that *Syzygium aromaticum* (clove) and *Cinnamomum verum* (Dalchini) had good QSI activity. Recognizing their medicinal value and dietary consumption, an attempt was made to evaluate QSI activity of *Cinnamomum verum* and *Syzygium aromaticum* on bacterial pathogens like *Pseudomonas* and *Serratia* and confirmed by using a bioindicator organism *Chromobacterium violaceum*. Interestingly aqueous spice extracts could reduce swarming nature, in both the organisms, which is one of the important virulent factor regulated by QS. The phytochemical components in the crude extracts were analyzed by qualitative methods. MS analysis of the treated samples revealed the fact that AHL molecule is subjected to lysis or degradation.(Y.Aparna 2014 a;2014 b). Chemical characterization of bioactive components in the crude extract of *Syzygium aromaticum* and *Cinnamomum verum* is under process. The exact mode of action at molecular level has to be further explored with purified bioactive component from spice extracts. Recognition of bioactive components in the crude extracts is under process.

Table: 1. Phytochemicals with proved anti-quorum sensing activity.

Natural QSI compound	Reference
Extracts of pea seedlings - <i>Pisum sativum</i>	Teplitski, M. <i>et al</i> 2000
Chlorogenic acid, vanillic acid, proanthocyanidins	Leach <i>et al.</i> , 2007; Feucht <i>et al.</i> , 2000
Furanones	Manfield <i>et al.</i> , 2002
Extracts of <i>Medicago truncatula</i> seedlings	Gao, M. S. <i>et al</i> 2003
Plant essential oil components - cinnamaldehyde	Niu, C. <i>et al</i> 2004
Ursolic acid	Ren <i>et al.</i> , 2005
Gamma aminobutyric acid (GABA)	Chevrot <i>et al.</i> , 2006 ; Zhang <i>et al.</i> , 2004
Vanillin extracts	Choo, J. <i>et al</i> 2006
Epigallocatechin gallate	Riedel <i>et al.</i> , 2006
Rosmarinic acid	Vattem <i>et al.</i> , 2007
Salicylic acid	Yuan <i>et al.</i> , 2007
Dietary phytochemicals	Vattem, D., <i>et al</i> 2007
Extracts of various South Florida plants	Adonizio, A. <i>et al</i> 2008

Pyrogallol	Ni <i>et al.</i> , 2008
Curcumin	Rudrappa and Bais, 2008
Cinnamaldehyde	Brackman <i>et al.</i> , 2008 ; Niu <i>et al.</i> , 2006
Furocoumarins	Girenavar <i>et al.</i> , 2008
Ellagitannin from medicinal plants	Adonizio, A. <i>et al</i> 2008, Mathee, K. <i>et al</i> 2009
Honeys	Truchado, P, <i>et al</i> 2009
Cranberry juice	Feldman, M. <i>et al</i> 2009
Clove oil	Khan, M. S., <i>et al</i> 2009
Tannic acid, a plant polyphenol	Jones, S. M. <i>et al</i> 2009
Ethanolic extracts of the plants <i>Sonchus oleraceus</i> and <i>Laurus nobilis</i>	Al-Hussaini <i>et al</i> 2009
<i>p</i> -coumaric acid	Bodini, S. F.; <i>et al</i> 2009
Tannic acid	Larrosa <i>et al.</i> , 2010a
4,5- <i>O</i> -dicaffeoyl quinic acid	Fiamegos <i>et al.</i> , 2011
Urolithin A and B	Larrosa <i>et al.</i> , 2010b; Gimenez-Bastia <i>et al.</i> , 2012
Ellagic acid	Sarabhai <i>et al.</i> , 2013
Flavanones, flavonoids, flavonols	Truchado <i>et al.</i> , 2012 ; Vandeputte <i>et al.</i> , 2011 Vikram <i>et al.</i> , 2010 ; Vandeputte <i>et al.</i> , 2010 Rasamiravaka <i>et al.</i> , 2013 ; Leach <i>et al.</i> , 2007
Traditional Chinese medicinal plant extracts	Sandy Siew -Mian Yeo and Foong -Yee Tham (2012)
Extracts from <i>Melicope lunu-ankenda</i>	Li Ying Tan, Wai-Fong Yin and Kok-Gan Chan (2012)
Caffeine	Siti Nur Maisarah Norizan <i>et al</i> , 2013
<i>Cinnamomum verum</i>	Y.Aparna <i>et al</i> , 2014
<i>Syzygium aromaticum</i>	Y.Aparna <i>et al</i> , 2014

Table 1 gives list of some of the plants exhibiting quorum quenching ability. Since these plants can be consumed by humans, the active compounds that are having QS inhibitory activities from the plants should be safe and should not cause toxicity towards human cells, but toxicity studies on these compounds are still necessary. This projected wealth of information will give the medicinal chemist a powerful platform for developing the next generation antibiotics that aim to curb bacterial virulence instead of killing bacteria. Most of the anti-QS agents reported are experimental, and comprehensive pharmacokinetic data on these molecules are lacking. More anti-QS drugs that do not have *in vivo* instability issues and are drug like are desired.

It is important to establish the *mode of action* of the QSI in the pathogens in order to establish whether they are narrow or broad spectrum. Most QSI agents are reported with narrow spectrum activity which may be used as a shield or sword. A narrow spectrum QSI compound will only target specific pathogens where this may be useful to specifically targeting a type of pathogen in a polymicrobial environment, but with limited clinical value. Many QS inhibitors

can also affect the integrity of biofilms and thus, will make the bacteria more susceptible to conventional antibiotics. This serves as an advantage as it can help to minimize the possibility of the bacteria from becoming resistant. Thus Phytochemical QSI compounds may serve as the next generation “magic bullets”, if they are not bactericidal. Perhaps, a combined therapy involving both antibiotics and QSI may provide synergistic effects.

This novel approach of quorum quenchers gain importance in present context which involves novel drug design and development for specific bacterial infections which cannot be cured by antibiotics. This strategy is also applicable to plant pathogenic bacteria in agriculture, industrial organisms of commercial importance and pharmaceutical arrears in treating clinical pathogens. The knowledge and expertise generated will undoubtedly stimulate further research and biotechnological innovation in formulating better practical ways to control bacterial diseases.

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