CHAPTER - 1

Background and significance of the study
CHAPTER 1: BACKGROUND AND SIGNIFICANCE OF THE STUDY

1.1 Introduction

*Pseudomonas aeruginosa* is a Gram-negative bacterium, recognized as an important opportunistic human pathogen. It normally lives in moist environments and has the ability to colonize ecological niches, soil, water, plant and animals. *P. aeruginosa* can infect and proliferate inside host cells such as plants, animals, humans and nematodes. *P. aeruginosa* is normally found in the human body as a normal microflora. Colonization rates for specific sites in humans are 0 to 2% for skin, 0 to 6.6% for the throat, 0 to 3.3% for the nasal mucosa and 2.6% to 24% for fecal samples (Morrison and Wenzel, 1984). It can cause a wide range of severe infections in patients with serious underlying medical conditions (Sadikot et al. 2005). In the hospital environment, *P. aeruginosa* can easily infect patients who are immunocompromised and it rarely infects healthy people as well as hospital coworkers (Zawacki et al. 2004). However, healthy people can also develop mild infections especially after exposure to water, contaminated hot tubs and swimming pools (Kiska and Gilligans, 2003).

In the health care setting, *P. aeruginosa* is one among the leading causes of nosocomial infections in humans and it is associated with a significant morbidity and mortality rate, particularly in people with weakened immune system (Kang et al. 2003; Khan et al. 2015). This bacterium is reported to be the second most frequently recovered pathogen from intensive care unit (ICU) patients and it is mainly transmitted through the hospital coworkers and colonized patients (Chastre and Fagon, 2002). *P. aeruginosa* is well adapted and adhered to the respiratory tract, particularly in patients with chronic bronchitis disease or patients who admitted to ICU ward (Bonten et al. 1996). *P. aeruginosa* can infect patients suffering from cystic fibrosis, severe burns and acquired immune diseases
syndrome (AIDS) (Sadikot et al. 2005). In addition, *P. aeruginosa* is also the third leading cause (12%) of urinary tract infections in hospital environments and the infection causing route via insertion of urinary catheters, instrumentation and surgery (Nicolle, 2014). This organism causes bloodstream infections in human and the mortality rate is reported to be greater than 20% as the patients receive inappropriate antimicrobial chemotherapy (Micek et al. 2005).

![Diagram of various infections caused by *P. aeruginosa*](image)

**Figure 1.1:** *P. aeruginosa* infection occurs in various parts of the human body

*P. aeruginosa* meningitis is a rare infection, but the organisms are able to infect patients who are immunocompromised or have undergone neurological surgery (Fong and Tomkins, 1985). *P. aeruginosa* has the ability to cause meningitis and brain abscesses as it invades through the central nervous system (CNS) from a contiguous structure such as paranasal sinus or inner ear (Dando et al. 2014). Another route of infection is directly by mean of head trauma, head injury and neurological surgery (Dando et al. 2014). *P. aeruginosa* infection is major problem in patients who undergoing external ventricular drainage (EVD) and external cerebrospinal fluid (CSF) drainage
catheters are used to manage critically ill patients with elevated intracranial pressure (Juhi et al. 2009). External ventricular drainage (EVD) is the main source for entry of *P. aeruginosa* and has been associated with a risk of infection (Figure 1.1) (Juhi et al. 2009).

*P. aeruginosa* causes overwhelming infection in the human eye. *P. aeruginosa* can colonize the ocular epithelium and it can proliferate rapidly through the vast production of enzymes such as elastase, alkaline protease and exotoxin A which cause a rapidly destructive infection that can lead to permanent loss of vision such as dacroyystitis, endophthalmitis, conjunctivitis, infection of corneal ulcers and keratitis (Henry et al. 2012) (Table 1.1).

**Table 1.1:** Common *P. aeruginosa* infection and risk factors

<table>
<thead>
<tr>
<th>S. No</th>
<th>Infection</th>
<th>High Risk factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Central nervous system infections (brain abscess, meningsitis)</td>
<td>Brain surgery, head injury, neurological surgery and trauma.</td>
</tr>
<tr>
<td>2</td>
<td>Bacteremia</td>
<td>Immunocompromised patients, malignacies, patients who are neutopenic (chemotherapy) and acquired immuno diseases syndrome (AIDS).</td>
</tr>
<tr>
<td>3</td>
<td>Endocarditis</td>
<td>Patients who have undergone haemodialysis.</td>
</tr>
<tr>
<td>4</td>
<td>Urinary tract infection</td>
<td>Insertion of urinary catheter prolonged time, Indwelling medical devices etc.</td>
</tr>
<tr>
<td>5</td>
<td>Respiratory / bronchitis</td>
<td>Chronic obstructive pulmonary disease, cystic fibrosis, mechanical ventilation.</td>
</tr>
<tr>
<td>6</td>
<td>Otitis externa (swimmer's ear)</td>
<td>Tissue injury, water blockage in ear canal</td>
</tr>
<tr>
<td>7</td>
<td>Skin, muscle-skeletal and burn wound</td>
<td>Cut the skin, malignancies, AIDS patients, burns wound, diabetics, indwelling urinary catheter etc.</td>
</tr>
</tbody>
</table>
"P. aeruginosa" has the ability to colonize medical and surgical devices, including catheters, dental implants, endotracheal tubes, needless connectors, central venous catheters tympanostomy tubes, prosthetic joints and peritoneal dialysis catheters (Donlan, 2001a). The primary contamination of the medical devices mainly occurs by inoculation with few microorganisms from the patient’s skin or mucus membranes during implantation. Another way of the pathogens may acquire from the surgical instrument by clinical staff and healthcare workers (Donlan, 2001a).

"P. aeruginosa" rarely infects healthy tissues, but when externally cut it can easily invade the tissues and proliferate to damage the tissue. "P. aeruginosa" is usually an extracellular pathogen but is also known to invade epithelial cell during infection. After invading, "P. aeruginosa" is able to proliferate and adhere onto the host tissue. It releases a variety of virulence factors and toxins which are mainly involved in the progression of the disease through enforcing the adhesion, modifying the immune response, evading from phagocytosis and destroying the host tissue (Moghaddam et al. 2014).

"P. aeruginosa" is notorious for intrinsic resistance to many antibiotics due to low outer membrane permeability and adaptive resistance mechanisms. Moreover, the emergence of the clinical strains of "P. aeruginosa" with modified virulent factors makes the treatment difficulties (Fernandez and Hancock, 2012). A number of virulence factors, toxins produced by "P. aeruginosa" play important roles in caused infection in human beings. The production of these virulence factors and toxins are mediated by small signaling molecules (autoinducer) called quorum sensing (QS) (Bjarnsholt and Givskov, 2007).
1.2 Bacterial quorum sensing

The bacterial pathogens are normally associated with human and animals. Even though the bacterial pathogens continue contact with the host cells, a successful infection is rare, because the host cell possesses a potential immune system that prevents the cause of infection in such way. The first line order of immune defence mechanisms such as macrophages prevent the entry of bacterial pathogens into the host cells. Similarly, the second line order of immune mechanisms such as an antibody which bind to the bacterial receptors and destroy it (Janeway et al. 2001). Once the host immune system compromised, the bacterial pathogens are free to cause the infection to the host cells. On the other side, the bacterial pathogens have been a counter attack to the host cells; the pathogen develops strategies to overcome host defence mechanisms by using quorum sensing signalling molecules called autoinducers (AIs) (Van Delden and Iglewski, 1998; Zhang, 2003). These molecules play an important role in the production of virulence factors, toxins and also involved in the formation of biofilms in the host cells (Van Delden and Iglewski, 1998).

Quorum sensing (QS) is a phenomenon in which bacteria communicate and coordinate with the other bacterial cells via a small diffusible signalling molecule called autoinducers (AIs) (LaSarre and Federle, 2013). The signaling molecules of Gram-positive bacteria are usually polypeptides which regulate control the bacterial cell density, whereas acyl-homo serine lactones (AHLs) are signaling molecules secreted by the Gram-negative bacteria (LaSarre and Federle, 2013). Gram- positive bacteria, once threshold concentration level reached the organism able to release and segregate the peptide molecules, which bind to a cognate membrane - bound histidine kinase receptor (Ng and Bassler, 2009) (Figure 1.2). Similarly, Gram- negative bacteria of quorum sensing is mediated by small
signaling molecules such as \( N \)-acyl homoserine lactones (AHLs) which in turn regulate and coordinate the expression of certain genes (Ng and Bassler, 2009) (Figure 1.2).

The first QS system was characterized in the 1970s in the luminescent marine species of \textit{Vibrio} sp (Nealson et al. 1970). In Gram-negative bacteria, once population density reached its ability to release QS molecules in the surrounding area which helps them to communicate with each other bacterial cells (LaSarre and Federle, 2013). Although, there have been a number of different QS systems are involved in controlling the population density, virulence factors and toxin production in the Gram-negative bacteria (Deep et al. 2011). The archetype QS system in marine bacteria of \textit{Vibrio fisheri} and \textit{Vibrio harveyi} are controlled by homologs of regulatory proteins, LuxI and LuxR and represent the most extensively studied types of QS mechanisms (Water and Bassler, 2006).

**Figure 1.2:** Schematic diagram of Gram-positive and Gram-negative bacterial quorum sensing system.

Acyl-homoserine lactone (AHL) is the main signaling molecule in Gram-negative bacteria which controls the QS system. AHL-mediate QS system is the counterpart in marine bacteria of \textit{V. fisheri} of luciferase system. The emission of light is tightly associated
with the population density of the bacterial cells in the organ, and this phenomenon is controlled by QS system (Miyashiro and Ruby, 2012). QS system mediates bioluminescence, and are products of genes regulated by lux operon (Miyashiro and Ruby, 2012). The emission of light in marine bacteria of *V. fischeri* is mainly controlled by two regulatory proteins such as LuxI and LuxR. LuxI is an autoinducer synthase, which is responsible for the synthesis of acyl-homoserine lactone (S-adenyosyl methionine) autoinducer (Miyashiro and Ruby, 2012). LuxR is a transcriptional regulatory protein, which binds to autoinducer and promotes transcription of the luciferase structural operon luxCDABE (Engebrecht et al. 1983). Homologues of LuxI and LuxR systems are present in proteobacteria and the main function is to control the production of virulence factors and biofilms formation (Engebrecht et al. 1983). The prototypes of QS system have been also characterized in human pathogens such as *Yersinia pseudotuberculosis, Escherichia coli* and *Pseudomonas aeruginosa* as well as plant associated bacteria such as *Rhizobium leguminosarum, Erwinia carotovora* and *Ralstonia solanacearum* (Fugua et al. 1994; Cha et al. 1998; Atkinson et al. 1999; Sperandio et al. 2002).

### 1.3 Quorum sensing in *Pseudomonas aeruginosa*

*P. aeruginosa* possesses two QS systems namely *las* and *rhl* which utilize N-acyl-homoserine lactones (AHLs) as signaling molecules (Pesci et al. 1997). These signaling molecules, synthesized by bacterial cells, diffuse out of the cells and bind to transcriptional regulators. There are two QS systems in *P. aeruginosa* (LasR/I and RhlI/R) which have been identified to regulate the expression of virulence factors (Gambello and Iglewski, 1991). The LasI is essential for the production of the AHL molecule, N-(3-oxododecanoyl)-L-homoserine lactone (3O-C12-HSL) and lasR is the transcriptional regulator (Pearson et al. 1994). The second QS system comprises RhlI and RhlR proteins. The RhlI
synthase mediates the synthesis of the signal molecule, N-butyryl-homoserine lactone (C4-HSL) and RhlR is the transcriptional regulator (Pearson et al. 1995). The las system controls virulence factors such as LasB elastase, LasA protease, alkaline protease, exotoxin A and biofilm formation (Pesci et al. 1997; Pearson et al. 1997). Similarly, rhl system controls the production of pyocyanin pigment, rhamnolipids, LasB elastase and hydrogen cyanide (Brint and Ohman, 1995; Pearson et al. 1995). These virulence factors are involved in cellular toxicity and acute infection (Sawa et al. 1998). Las and Rhl QS systems are interdependent to each other. *P. aeruginosa* is an ability to release many Las and Rhl dependent virulence factors such as secretion of exotoxin, protease, serine protease, elastase and biofilm formation. These virulence factors are involved into facilitates and the establishment of infections (Van Delden and Iglewski, 1998). Treatment strategies of *Pseudomonas* infections are greatly challenged by the emergence of drug-resistant strains and also on account of biofilm formation by this bacterium (Tenover, 2006).

1.4 Production of virulence factors and toxins in *Pseudomonas aeruginosa*

The pathogenesis of *P. aeruginosa* involves the ability to secrete numerous toxic compounds and extracellular virulence factors help to cause diseases and evade the host defenses (Beceiro et al. 2013). The production of extra cellular virulence factors and toxins such as pyocyanin, pyoverdin, LasA protease, LasB elastase, phospholipase C, rhamnolipid, superoxide dismutase, hydrogen cyanide (HCN), exotoxin A, exoenzyme and colony morphology, swarming motility and biofilms formation are regulated by bacterial QS and it plays an important role in pathogenesis and progression of human infections (Balasubramanian et al. 2013). Similarly, these virulence factors are also involved in cellular toxicity and acute infection in the host cells (Sawa et al. 1998). Pyocyanin and precursor molecules inhibit the beating of respiratory cilia in the lungs and alter the
expression of immunomodulatory proteins, thus allowing the pathogen to evade host’s innate and acquired immunity (Rada and Leo, 2013). Elastase is one of the prototype virulence factors of *P. aeruginosa* regulated by the quorum-sensing cascade and it degrades the infected tissue, promoting bacterial invasions (Hoge et al. 2010). Lipopolysaccharide endotoxin is a complex glycolipid present in the outer membrane of *P. aeruginosa*. It is responsible for antigenicity, inflammatory response and mediates interactions with conventional antibiotics (Nau and Eiffert, 2002). In acute *P. aeruginosa* infection, an increased production of all these extracellular virulence factors is involved and contributes to considerable damages host tissue. It is also facilitating their dissemination to distinct sites of infection in blood vessels. The production of these virulence factors limit the treatment options which are currently available as broad-spectrum antibiotics (Wagner et al. 2016).

### 1.5 Biofilm formation in *Pseudomonas aeruginosa*

Biofilms are conglomerate of microbes enclosed in a self-secreted exopolymeric substance (EPS) that hold microbial cells together onto the surface and environs the bacterial population (Donlan, 2002). It is mainly composed of polysaccharides, proteins, DNAs, lipids and other macromolecules that contribute to the structural scaffold providing the bacterial cell attachment and form a biofilm formation in the host cells (Vu et al. 2009). The EPS is an essential component in the formation of the biofilm matrix. It is mainly involved in the shaping of the cell structure, promoting attachment to surfaces, maintaining the biofilm structure, evading host immune responses and it providing the resistance to antibiotics (Davies, 2003; Orgad et al. 2011). Biofilm shields the bacterial population from clearance by the immune system and contributes to the pathogenesis of acute infections such as cystic fibrosis and other pulmonary illnesses (Høiby et al. 2010). Biofilm formation and maintenance of its architecture are QS-dependent phenomena. Similarly, the production
of biosurfactants, such as rhamnolipid, swimming and swarming motility is also associated with the establishment of biofilm formation on the host cell (Vu et al. 2009; Wang et al. 2014). *P. aeruginosa* produces three polysaccharides such as alginate, *Pel* and *Psl* that are used for the stability of the biofilm structure (Ryder et al. 2007). Alginate is a linear unbranched polymer which contributes to the structural stability and protection of biofilm formation (Ryder et al. 2007). Swarming motility is also associated with the establishment of biofilm formation (Ryder et al. 2007). Alginate is a linear unbranched polymer which contributes to the structural stability and protection of biofilm formation (Ryder et al. 2007). Swarming motility is also regulated by *rhl* system. Swarming motility is organized surface translocation, depends on extensive cell-to-cell contact (Ramasamiravaka et al. 2015). The production of rhamnolipids is under controlled by QS molecules. Rhamnolipids is one of the biosurfactants which facilitating three-dimensional mushroom-like structure formation in *P. aeruginosa* biofilms (Pamp et al. 2007). The production of these virulence factors results in bacterial persistence and reduced sensitivity to antimicrobials (Wagner et al. 2016).

### 1.6 Emergence of antibiotics resistance in *Pseudomonas aeruginosa*

Bacteria are naturally resistant to antimicrobial agents, because do not possess molecular target of the antibiotics or acquired resistance occurs through mutation, mobile genetic elements such as plasmids and transposons (Alekshun and Levy, 2007). There are four major mechanisms contributing to antibiotics resistance in *P. aeruginosa* includes acquired resistance includes drug inactivation, target modification, reduced permeability and efflux pumps (Sun et al. 2015). *P. aeruginosa* is intrinsically resistant to many antimicrobial agents and continuous to exposure to many antibiotics often leads to multidrug resistant *P. aeruginosa* strains (Morita et al. 2013). It can develop resistance to all conventional antibiotics via different mechanisms (Table 1. 2).
Table 1.2: Antibiotics resistance mechanisms in *Pseudomonas aeruginosa*

<table>
<thead>
<tr>
<th>Resistance Mechanism</th>
<th>Class of Resistance</th>
<th>Antibiotics</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efflux pumps</td>
<td>Intrinsic</td>
<td>MexAB–OprM, MexCD–OprJ, MexEF–OprN, MexXY–OprM (cephalosporins, carbapenems, aminoglycosides, quinolones, ureidopenicillins)</td>
<td>Hocquet et al. 2006</td>
</tr>
<tr>
<td>β-lactamases</td>
<td>Intrinsic</td>
<td>AmpC (penicillins)</td>
<td>Moya et al. 2009</td>
</tr>
<tr>
<td>Targeted mutation</td>
<td>Acquired</td>
<td>DNA gyrase, DNA topoisomerase (quinolones) MexZ (quinolones, cefapimes, aminoglycosides)</td>
<td>Ruppe et al. 2015</td>
</tr>
<tr>
<td>Horizontal transfer</td>
<td>Acquired</td>
<td>Metallo-β-lactamases, ESBLs (penicillins, cephalosporins, carbapenems)</td>
<td>Shaikh et al. 2015</td>
</tr>
<tr>
<td>Membrane changes</td>
<td>Adaptive</td>
<td>Lipid A modification (aminoglycosides, polymyxins)</td>
<td>Lee et al. 2016</td>
</tr>
</tbody>
</table>

*P. aeruginosa* is intrinsically resistant to a wide verity of antibiotics (eg. aminoglycosides, β-lactams and fluoroquinoles) because of the low permeability of its outer membrane, which prevents the entry of conventional antimicrobial drugs, due to the presence of porins in this bacterium (Lister et al. 2009). *P. aeruginosa* typically expresses a number of multidrug efflux systems such as MexAB-OprM, MexXY-OprM, MexCD-OprJ,
MexEF-OprN and MexJK-OprM which mainly contributing to antibiotic resistance (Hocquet et al. 2006). This multidrug efflux systems effectively resistances to different classes of antimicrobial drugs includes β-lactams (and β-lactamase inhibitors), sulfonamides, chloramphenicol, fluoroquinolones, trimethoprim, quinolones, macrolides, tetracycline, fourth-generation cephalosporins, cefpirome, aminoglycosides, and tetracyclines (Nikaido, 2010).

1.7 Mechanism of *P. aeruginosa* quorum sensing (QS) inhibition

*P. aeruginosa* has two QS system, which has been extensively studied. AHLs signaling molecules are required for *P. aeruginosa* to cause disease (Schuster and Greenberg, 2006). The treatment of *P. aeruginosa* infection usually fails due to the production of virulence factors controlled by QS system (Bonte et al. 2007). Since *P. aeruginosa* QS system is involved in the regulation of QS-depended virulence factors and the formation of biofilms, interfering with QS system may be a promising strategy in the treatment of severe chronic infections (Bonte et al. 2007).

The disruption of QS genes or elimination of QS-controlled virulence factors in *P. aeruginosa* which indicates the less severe and easy to treat the bacterial infections (Smith and Iglewski, 2003). It is possible that the QS system would be an ideal target for the inhibition of *Pseudomonas* infections (Smith and Iglewski, 2003). The current therapeutics for nosocomial and cystic fibrosis are unable to cure the *P. aeruginosa* infection. Therefore, alternative mechanisms for inhibition of *P. aeruginosa* QS system (cell-to-cell communication) are more appreciable to treat the infections.

The main target of QS inhibition in *P. aeruginosa* LasR or RhlR gene activation is the use of AHL analogues that act as antagonists for 3O-C₁₂-HSL and C₄-HSL (Smith and
Iglewski, 2003). There have been a number of ways to inhibit the QS signaling molecules and virulence factors such as signal binding, degradation of the signaling molecules, competitive inhibition and genetic regulation systems. Catalytic antibodies are hydrolysis of AHLs molecules (QS system) through signal binding. Antibodies are capable of hydrolyzing AHL molecule of 3O-C\textsubscript{12}-HSL and it inhibiting the production of pyocyanin in \textit{P. aeruginosa} (Marin et al. 2007).

The first QS inhibitor is a furanone which was isolated from marine algae. Furanones derivatives have successfully inhabited QS regulatory system and cause a reduction in LasR activity (Manefield et al. 1999). LasI is responsible for synthesis elastase virulence factors in \textit{P. aeruginosa}. S-adenosyl methione is necessary to synthesize AHLs leads to increase the production of virulence factors. If the QS inhibitors are able to suppress S-adenosyl methione (SAM), which leads to decreased the production of C12-AHL by LasI (Hoang et al. 2002).

Genetic modification of upstream regulators such as Vfr and GacA are involved in the production of many virulence factors (Albus et al. 1997; Reimmann et al. 1997). QS Inhibitor involved genetic modification in upstream regulators, which leads to reducing subsequent production of virulence factors (Smith and Iglewski, 2003). Similarly, Lactonases enzymes have significantly decreased production of virulence factors expression in \textit{P. aeruginosa} (Rajesh and Rai, 2014) (Figure 1.3).
Figure 1.3: Targets of different types of anti-QS compounds. The *P. aeruginosa* QS system (A). Competitive inhibition by AHL (B), sequestration of the AHL signal (C), signal degradation by lactonases (D), inhibition of AHL synthesis (E), blocking of upstream regulation (F), interference by antisense RNA (G).

1.8 Medicinal plants and Essential oils

Medicinal plants are widely distributed in the environment. They have their own immune system which is self-protective and prevents the bacterial and fungal diseases. Plants produce numerous bioactive constituents that protect against the herbivores, bacterial and fungal pathogens attack (Kant et al. 2015). In the ancient time, people routinely used plants and herbs extract for the treatment of various ailments and illnesses (Petrovska, 2012). Plants are used as traditional and folklore medicines throughout the world because plant-based medicines are more reliable, safety and more efficacies. It is less
side effect compares to modern and conventional medicines (Petrovska, 2012). The plants release secondary metabolites which have been implicated in therapeutic activities. Medicinal plants contain an enormous amount of phyto-constituents such as phenols, tannin, alkaloids, sterol, flavonoids, terpenoids and saponins which can be exploited for therapeutic uses (Krishnaiah et al. 2009).

Similarly, plant-derived essential oils and culinary herbs contain highly volatile substances that are used for their aromatic values as flavourings in foods, fragrances in pharmaceutical industry and medicines (Burt, 2004). Essential oils are extracted from different parts of plant materials including the leaves, bark, roots, flowers, resin and peels. The essential oils are commonly used for therapeutic application for the treatment of various ailments. Most of the essential oils have antioxidant, antimicrobial and anti-inflammatory properties (Burt, 2004).

1.8.1 Diversity of medicinal plants in India and usages of medicinal plants

Medicinal plants have been widely distributed in India particularly in the region of Himalaya, Northeast India, Mangroves and the Western Ghas. Over the centuries, people in India have been fascination and traditional plant ethics and used to conserve it in different ways. Medicinal plants play important roles in the health care of about 80% of the world population (Hosseinzadeh et al. 2015).

A number of medicinal plants are indigenous and estimated around 3000 to 3, 500 species of higher plants. Similarly, around 2,500 plants have been reported to be used in ethano-pharmacology. Almost 25% of the compounds were derived from plant sources which are used to treat various ailments.
The Indian system of medicine has formulated the drugs from the 387 plants and also the Unani system of medicine describes around 440 medicinal plants which are commonly used in the country (Patwardhan et al. 2004). In India, an estimated 25,000 flowering plant species are used for medical application. Out of that only about 10% plants have been scientifically proved for their medicinal values and still many more medicinal plants constituents await discovery (Patwardhan et al. 2004).

1.8.2 Medicinal plants in the Western Ghats

The Western Ghats are a well covered dense forests area and is a chain of highlands running along the Western border of the Indian sub-continent (Rodgers and Panwar, 1988). The Western Ghats are covering an estimated area of 159,000 sq. km and have outstanding biological diversity and one of the major tropical evergreen forest regions in India (Rodgers and Panwar, 1988). The Western Ghats are also known as the Sahyadri Hills and well known for their unique assemblage of flora and fauna.

Biodiversity hotspots of Western Ghats are associated with the use of wild plants as medicinal herbs. In this region, most of the common medicinal plants are found in the dense forest area. The medicinal plants and herbs are important herbal and folk medicines are used in the treatment of various diseases (Shiddamallayya et al 2010). More than 100 common medicinal plants are used day to day life by tribal people in the Western Ghats region of Karnataka. These medicinal plants are used for the relief of various ailments (Shiddamallayya et al. 2010). Similarly, some of the medicinal plants have potentially inhibited the virulence factors of \textit{P. aeruginosa} and number of medicinal plants are needed to be identified the anti-QS properties.
1.9 Selected plants for the study of anti-QS and antibiofilm activity

1.9.1 Murraya koenigii (L.) Spreng

*Murraya koenigii* is a small aromatic tree or perennial shrub belonging to the family Rutaceae. The plant is known for its medicinal values which are exploited in the preparation of tonics and medicine to treat dysentery (Schmelzer and Gurib-Fakim, 2013). The plant also possesses antidiarrhoeal (Mandal et al. 2010), antidiabetic (Yadav et al. 2002), antioxidant (Sasidharan and Menon, 2011), anticancer (Noolu et al. 2013), antiviral (Shah et al. 2008) and wound healing properties (Nagappan et al. 2012).

1.9.2 Terminalia bellerica (Gaertn.) Roxb

*Terminalia bellerica* belongs to the family of ‘Combretaceae’ commonly known as bastard myrobalan. *T. bellerica* is a large deciduous tree. It grows up to 30 m and leaves are about 8-20 cm long present towards the end of the branches. *T. bellerica* is a traditional folk medicine which has been used to treat various ailments (Elizabeth, 2005). There are various reports indicative of a wide range of pharmacological activities of *T. bellerica* such as antimicrobial, antidiarrhoeal, antidiabetics, antioxidant, antianalgesic, anti-inflammatory and antifibrotic (Elizabeth, 2005; Sabu and Kuttan, 2009). It is also used for treating heart diseases and bronchitis.

1.9.3 Stahlia monosperma (Tul.) Urb.

*Stahlia monosperma* belongs to the family of ‘Aesalpiniaceae’. *S. monosperma* is a medium sized evergreen tree 7-15 meters tall and 1-1 feet in trunk diameter. The leaves are 4-7 inches long, with yellow-brown axis. This plant is used only for construction and furniture (Perkins, 1907).
1.9.4 *Diospyros malabarica* (Desr.)

*Diospyros malabarica* is a small sized evergreen tree. Leaves are 10-28 cm long, oblong or oblong-lanceolate, obtuse (Mondal et al. 2006). It belongs to the family of ‘Ebenaceae’. The leaves and bark are mainly used for to cure many diseases such as anti-inflammatory, febrifuge, depurative, constipating, acrid, astringent, cooling and are used in antimicrobial, antioxidant, dyspepsia, leprosy, diarrhoea, dysentery, haemorrhages, skin burning, diabetes, spermatorrhea, vaginal diseases, wounds, flatulence, prolepsis, scabies and as carminative, laxative and tonic (Dhar et al. 1968; Mondal et al. 2006).

1.9.5 *Kingiodendron pinnatum* (DC.) Harms

*Kingiodendron pinnatum* is a legume species and endangered medicinal plant belongs to the family of ‘Fabaceae’. This plant found only in India (Karnataka, Kerala and Tamil Nadu) (Kumar et al. 2011). Photochemical such as phenols, tannins, flavonoids, glycosides and terpenes were present in *K. pinnatum* plant (Sheik et al. 2014). This endangered medicinal plant is used in gonorrhoea, genitourinary tract infection and respiratory tract infection. The plant-derived compound offers numerous sources of antimicrobial, antioxidant and antidiabetic agents (Sheik et al. 2014).

1.10 Model organisms for study of anti-quorum sensing

1.10.1 *Pseudomonas aeruginosa PAO1*

*P. aeruginosa* is a versatile Gram-negative organism, which grows in soil, coastal marine habitats as well as plant and animal tissues (Hardalo and Edberg, 1997; Costerton et al. 1999). It is aerobic bacillus, motile (single polar flagella) organism, with length and width ranging from 1.5 – 3.0 μm and 0.5-0.8 μm respectively (Bergey, 2001).
Biochemical tests is an oxidative positive, nonsporulating and non-fermentative species. It produces pigments such as pyocyanin (blue-green), pyoverdin (yellow-green) and pyorubin (red-brown) which are under the control of QS system (Lee and Zhang, 2015). *P. aeruginosa* is a clinically important organism that ability to causes various diseases such as cystic fibrosis, respiratory tract infection, meningitis and other infections (Sadikot et al. 2005).

**1.10.2 Infection model: Caenorhabditis elegans**

*Caenorhabditis elegans* is a free-living non-parasitic soil nematode and is one of the simplest invertebrate worms normally found in soil (Figure 1. 4). It is small and transparent nematodes (Brenner, 1974; Blaxter, 2011). *C. elegans* is an infectious model organism and has been used research laboratory, particularly in developmental biology and neurobiology (Corsi et al. 2015). The 1 mm long nematode is a self-reproducing hermaphrodite. The approximately life-cycle of *C. elegans* is 3.5 days. *C. elegans* are easily propagated in a Petri dish containing *Escherichia coli* OP50 (as a food source) and hundreds of progeny can be generated rapidly in Petri dishes (Brenner, 1974; Corsi et al. 2015).

![Schematic diagram of Caenorhabditis elegans N2 infection model](image)

**Figure 1.4:** Schematic diagram of *Caenorhabditis elegans* N2 infection model
The current researcher used *C. elegans* for host-pathogen interaction and bacterial virulence as well as pharmaceutical drug delivery (Kaletta and Hengartner, 2006). Two major models of infection such as paralysis and fast killing methods have been developed for exploring the effect of *P. aeruginosa* on *C. elegans* (Gallagher and Manoil, 2001). In rich, high salt medium, *P. aeruginosa* can kill the nematodes within a few hours (fast killing). The fast killing is mediated by the synthesis of phenazines (pyocyanin pigment) (Gallagher and Manoil, 2001). In paralytic killing, *C. elegans* has grown on brain heart infusion medium with *P. aeruginosa* become paralyzed and subsequently die (Gallagher and Manoil, 2001). The *P. aeruginosa* mediated killing of *C. elegans* mainly depends on QS-controlled virulence factors (Gallagher and Manoil, 2001). Since virulence factors have been shown both *in vitro* and *in vivo* in a nematode model is mediated by the bacterial QS system. In addition that the essential oils, plant extracts and purified compounds that attenuate *P. aeruginosa* QS system, therefore reduce the production of virulence factors, toxins and subsequent death of *C. elegans* (Adonizio et al. 2008; Yu et al. 2014).

### 1.11 Main hypotheses and aims of this project

**Hypothesis**

Anti-quorum sensing and antibiofilm compounds from selected medicinal plants such as *Murraya koenigii* essential oil (EO), *Terminalia bellerica* and *Stahlia monosperma* have not been reported earlier. The selected medicinal plants are known to be used in traditional medicine and folk medicine in the biodiversity hotspots. The anti-QS compounds may contribute to the anti-pathogenic properties of medicinal plants and these compounds can inhibit the virulence factors as well as biofilms formation in *P. aeruginosa*. Furthermore, the extracts from medicinal plants with anti-QS properties should prevent the
infection and death in a live animal model system. The main objective of this study is to discover potential QS inhibitors from medicinal plants for developing anti-pathogenic drug principles and to validate the usage of traditional medicines.

**Objective and scope of the present study**

1. Isolation, identification and determination of anti-biogram of clinical strains of *Pseudomonas aeruginosa*.

2. Screening of medicinal plants for anti-quorum sensing activities against biosensor strains and *P. aeruginosa* strains.

3. Characterization of anti-QS compounds from medicinal plant extracts.

4. *In vitro* and *in vivo* studies on inhibition of *P. aeruginosa* virulence factors by an anti-QS compound.