CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Identification of Quorum Sensing Inhibition and Anti-Biofilm Activity of *Reynoutria japonica* with Reference to *P. aeruginosa*

by

Sundas Ishtiaq

A thesis submitted in partial fulfillment for the degree of Master of Science

in the

Faculty of Health and Life Sciences
Department of Bioinformatics and Biosciences

2022

Every challenging work needs self-efforts as well as the guidance of elders. I dedicate this thesis to my parents whose affection makes me able to get such success and to my teachers whose encouragement has always been my source of inspiration.

CERTIFICATE OF APPROVAL

Identification of Quorum Sensing Inhibition and Anti-Biofilm Activity of *Reynoutria japonica* with Reference to *P.aeruginosa*

by

Sundas Ishtiaq

(MBS203027)

THESIS EXAMINING COMMITTEE

S. No.	Examiner	Name	Organization
(a)	External Examiner	Dr. Waseem Sajjad	NUMS, Rawalpindi
(b)	Internal Examiner	Dr. Erum Dilshad	CUST, Islamabad
(c)	Supervisor	Dr. Arshia Amin Butt	CUST, Islamabad

Dr. Arshia Amin Butt
Thesis Supervisor
September, 2022

Dr. Syeda Marriam Bakhtiar
Head
Dept. of Bioinformatics & Biosciences
September, 2022

Prof. Dr. Sahar Fazal
Dean
Faculty of Health & Life Sciences
September, 2022

Author's Declaration

I, **Sundas Ishtiaq** hereby state that my MS thesis titled "**Identification of Quorum Sensing Inhibition and Anti-Biofilm Activity of *Reynoutria japonica* with Reference to *P.aeruginosa***" is my own work and has not been submitted previously by me for taking any degree from Capital University of Science and Technology, Islamabad or anywhere else in the country/abroad.

At any time if my statement is found to be incorrect even after my graduation, the University has the right to withdraw my MS Degree.

(Sundas Ishtiaq)

Registration No: MBS203027

Plagiarism Undertaking

I solemnly declare that research work presented in this thesis titled "**Identification of Quorum Sensing Inhibition and Anti-Biofilm Activity of *Reynoutria japonica* with Reference to *P.aeruginosa***" is solely my research work with no significant contribution from any other person. Small contribution/help wherever taken has been dully acknowledged and that complete thesis has been written by me.

I understand the zero tolerance policy of the HEC and Capital University of Science and Technology towards plagiarism. Therefore, I as an author of the above titled thesis declare that no portion of my thesis has been plagiarized and any material used as reference is properly referred/cited.

I undertake that if I am found guilty of any formal plagiarism in the above titled thesis even after award of MS Degree, the University reserves the right to withdraw/revoke my MS degree and that HEC and the University have the right to publish my name on the HEC/University website on which names of students are placed who submitted plagiarized work.

(Sundas Ishtiaq)

Registration No: MBS203027

Acknowledgement

All the praises are to be for Almighty **ALLAH** and **Prophet MUHAMMAD (SAW)**. I would like to express my wholehearted thanks to my family for the generous support throughout of pursuing the MS degree. I am heartily grateful to my supervisor Dr. Arshia Amin Butt (Assistant professor, Department of Bioinformatics and Biosciences, CUST) for her kind support, guidelines and arrangement of tutorial classes. I especially say thanks to Dr. Shaukat Iqbal (Professor, Department of Bioinformatics and Biosciences, CUST) for his assistance on computational approaches. Thanks to all.

(Sundas Ishtiaq)

Abstract

Current lifestyles, stress and toxic wastes have extremely increased the incidence of various bacterial infections in humans. Bacterial infections account for a major cause of deaths throughout the developing world. It is estimated that by the end of 2025, 226 million people worldwide will be affected with infectious diseases. Due to massive use of antibiotics in clinical practices caused bacteria to become resistant against certain antibiotics. Multidrug-resistance bacteria commonly use cell-to-cell communication which is called quorum sensing which leads to the regulation of numerous virulence factors in bacterial biofilm for developing antibiotic resistance. Bacterial infections such as pneumonia, nosocomial infections, inflammation and respiratory infections especially with multidrug resistant opportunistic pathogens such as *Pseudomonas aeruginosa* are hard to treat due to their high resistance profile and potential to produce biofilm. Currently, there are no effective drugs against these bacteria. The global rise of anti-microbial resistance combined with rapid rate of microbial evolution and the slower development of novel antibiotics focuses the urgent need of development of innovative therapeutics and new strategies to fight emerging infections. One of these strategies is to disturb the quorum sensing mechanism of bacteria which inhibit the regulation of numerous virulence factors in bacterial biofilm by natural means is called quorum quenching. The present study was undertaken to evaluate protein-ligand interactions of all *P. aeruginosa* proteins with natural compounds from a *Reynoutria japonica* in order to identify potential inhibitors of respective bacteria. Ten ligands from respective plant were selected which act as potential inhibitors for quorum sensing mechanism e.g., Juglone, Emodin, Emodin 8-o-b-glucoside, Polydatin, Resveratrol, Juglone derivatives, Physcion and Chrysophanol. These bioactive compounds were taken as ligands and docked with *P. aeruginosa quorum* sensing proteins such as LasI, LasR, RhlI, RhlR and MvfR. The 3D structure of compounds and target proteins was docked. The best ligand was selected on the basis of docking score, absorption, distribution, metabolism and excretion (ADME) screening, toxicity analysis and Lipinski rule of 5. By considering all these parameters Chrysophanol was seen obeying all drug-like properties with docking score -10.1 against LasR

protein. It fulfilled standard criteria and selected as lead compound. To check further effectiveness of Chrysophanol, it was compared with commercially available antibiotic drug Meropenem. A comparison of all drug-like characteristics showed that Chrysophanol was much better in many aspects over Meropenem. Meropenem showed docking score -8.00 while Chrysophanol has -10.1, other pharmacokinetic properties of Chrysophanol were also good than Meropenem. So, it is concluded here that Chrysophanol can prove itself as anti-quorum sensing agent in future drug discovery.

Contents

List of Figures

List of Tables

Abbreviations

ADMET:- Absorption Distribution Metabolism Excretion and Toxicity

AHL:- Autoinducer Homoserine Lactones

AI:- Auto Inducers

AI:- Aliphatic Index

AI-2:- Autoinducer 2

AIP:- Autoinducer Peptides

AMR:- Anti Microbial Resistance

BBB:- Blood brain barrier

BHL:- N-butanoyl-L-Homoserine Lactone

CASTp: - Computer Atlas of Surface Topography of Proteins

CB Dock:- Cavity-detection guided Blind Docking

CF:- Cystic Fibrosis

CNS:- Central Nervous System

CYP2D6:- Cytochrome P450 2D6

Cyst:- Cysteine

EPS:- Extracellular Polymeric Substance

FDA:- Food and Drug Administration

GRAVY:- Grand average of hydropathicity

HAQs:- Hydroxy-Alkyl-Quinolines

HBA:- Hydrogen Bond Acceptor

HBD:- Hydrogen Bond Donor

hERG:- Human Ether-a-go-go-Related Gene

HSL:- Homoserine Lactone

HIV:- Human Immunodeficiency Virus

II:- Instability Index

KEGG:- Kyoto Encyclopedia of genes and genomes

LasI:- Las Inducer

LasR:- Las Receptor

MAM:- 2-Methoxy-6-acetyl-7-methyljuglone

Mbp:- Mega base pair

MDR:- Multiple Drug Resistance

MTD: - Maximum Tolerable Dose

MvfR:- Multi-virulence factor Regulator

MW:- Molecular weight

NFR2:- Nuclear factor erythroid 2-related factor 2

NR:- Total number of negatively charged residues (Asp + Glu)

OdHL:- 3-oxo-dodecanoyl-L-Homoserine Lactone

P.aeruginosa:- *Pseudomonas aeruginosa*

PQS:- *Pseudomonas* Quinolone Signal

PqsR:- Pseudomonas quinolone signal Regulator

PR:- Total number of positively charged residues (Lys + Arg)

QQ:- Quorum Quenching

QS:- Quorum Sensing

RCSB PDB:- Research Collaboratory for Structural Bioinformatics Protein data bank

RhlI:- Rhl Inducer

RhlR:- Rhl Receptor

TTSS:- Type III secretion system

VDss:- Volume of Distribution at steady state

Chapter 1

Introduction

Bacteria interact with each other through signals. As number of bacteria increase signals threshold concentration is reached which results in change in gene regulation of bacteria. This type of communication system which is based upon signaling and bacterial density is called quorum sensing [1]. The quorum sensing system operates through the release of special signals termed as autoinducers, whose accumulation depends upon cell density. Quorum sensing regulates bacteria's important behaviors e.g. attachment to surface, biofilm formation, bioluminescence, secretion of different type of chemicals, motility, virulence and pathogenicity [2].

Quorum sensing is also required for biofilm formation. A biofilm is well organized form of bacterial population. Biofilm protects bacteria from host defense systems, unsuitable environmental conditions and antibiotics approach. The main characteristic of biofilm forming bacteria is the release of extracellular polymeric substances. These polymers have sufficient amount of polysaccharides along with protein and extracellular DNA which helps in formation of matrix in which bacterial cells are embedded [3]. Quorum sensing process is dependent upon generation, release and detection of signals. Different bacterial species produce different type of signals. N-homoserine lactones (AHLs), furanosyl borates (AI-2) and autoinducer peptides (AIP) are among some of the common quorum sensing signals. Among most well studied signals are

- AHLs, which are produced by more than 70 gram- negative bacterial species and diffuse across the cell membrane to bind to regulatory proteins within the cell, and
- Peptide-based quorum sensing systems in gram-positive bacteria, which works through membrane-bound receptor histidine kinases [4].

Pseudomonas aeruginosa is rod-shaped, aerobic, Gram-negative bacterium belongs to *Pseudomonadaceae* family and it has ability to survive in wide variety of environmental conditions such as soil, fresh water and sea water. Its genome is about 5.5–7 Mbp which is considered larger than of the other sequenced bacteria like *Bacillussubtilis, Escherichia coli*, and *Mycobacterium tuberculosis* and it has number of regulatory enzymes which play an important role in metabolism and transportation of organic compounds. That is the reason which makes its genome extremely adaptable to environmental changes. Recently, infections with *Pseudomonas aeruginosa* has spread widely and affect millions of people worldwide [5]. It is an opportunistic pathogen which attaches itself to medical equipment's or medical devices and other hospital surfaces as a result healthcare-associated infections develop such as nosocomial infections, extensive inflammation and sepsis. This type of infections is most common in immune-compromised patients similarly burn victims and those who suffer from malignant tumor or HIV. It rarely affects healthy individuals. It may also most frequent cause of persistent infection of the lungs in cystic fibrosis patients. It may also develop microbial keratitis in those individuals who wear their lenses for a long time. Extensive research on this bacterium has provided detailed information on the subject of quorum sensing and the formation of biofilm. Application of numerous quorum sensing mechanisms facilitate it to survive on harsh surfaces or inside the host, in addition to cause disease by bypassing the host immune system [6].

Studies on *Pseudomonas aeruginosa* shows that quorum sensing cause the expression of virulence and pathogenicity causing factors. In some instances, severe bacterial infections cause life-threatening diseases. Their quorum sensing systems rely upon an autoinducer synthase and its transcriptional regulator protein which

leads to the production and activation of wide variety of diffusible autoinducers in the environment. Based on their chemistry, they can be divided into two groups. The first group of diffusible autoinducers is acyl-homoserine lactones also known as AHLs, and the second group is *Pseudomonas quinolone* signal also known as PQS. OdHL and BHL are two forms of AHLs. PqsR/MvfR is a LysR-type transcriptional regulator protein which is a key component of alkyl-quinolone dependent quorum sensing [7]. The following quorum sensing systems is required to produce and sense these signals. A quorum sensing system such as LasI/R and RhlI/R is required for the synthesis of acyl-homoserine lactones / AHLs and MvFR is required for the synthesis of *Pseudomonas quinolone* signal / PQS. Autoinducers such as OdHL and BHL are synthesized by proteins such as LasI and RhlI respectively outside the cell, when these autoinducers increase in concentration as a function of cell density they will get back to the cell and then bind with their respective regulators such as LasR and Rh1R.

While *Pseudomonas quinolone* signals are synthesized by transcriptional regulatory protein such as MvfR and its bind with LysR-like receptor and then cause some virulence genes like exotoxins and proteases to be expressed which leads to the certain bacterial associated infections in host cell [8]. Hence, quorum sensing systems have important target proteins for potential anti-bacterial.

Until now, there are certain antibiotics which are used to treat related bacterial infections. But repeated and overuse of antibiotics has made bacteria resistant resulting in emergence of multiple drug resistant species. It created urge to look for alternative methods. Soon it was observed that control over virulence factors and biofilm formation can be made possible by controlling its quorum sensing mechanism. Disrupting the quorum system mechanism is a new and promising technique for bacterial infectious disease prevention. The mechanism causing the suppression of quorum sensing communication systems is termed as quorum quenching or QQ [9]. Quorum quenching inhibits group activities of micro-organisms such as the synthesis of virulence factors and biofilm formation by interfering with their bacterial communication pathways. Currently, quorum quenching is thought to be effective in inhibiting or degrading the signaling molecules. Recent studies have

shown that it can be used for inhibiting the activation of the AHL based quorum sensing system of *Pseudomonas aeruginosa*, thus leading towards decrease in virulence factors expression and biofilm formation [10]. Hence, blocking AHL-mediated quorum sensing mechanisms can be done in one of following ways. It can be blocked at the signal, signal receptor, or signal transduction levels. In addition, quorum quenching is performed by using quorum sensing inhibitors which can be obtained from different sources e.g., natural, synthetic or antibody based [11].

Studies on anti-quorum sensing inhibitors showed that they are likely to be found naturally, in legumes or traditional medicinal plants [12]. Recent studies have shown that *Reynoutria japonica*, a medicinal herb/Knotweed alone has significant pharmacological effects. There are many bioactive compounds such as quinones, stilbenes, flavonoids, and phenolic compounds which act as potential inhibitors for quorum sensing systems of harmful bacteria found in this plant. There are certain other bioactive compounds from this plant which act as potential inhibitors for quorum sensing e.g., Juglone, Emodin, Emodin 8-o-b glucoside, Polydatin, Resveratrol, Juglone derivatives, Physcion and Chrysophanol [13].

Therefore, these chemical compounds act as ligands and can be docked with *Pseudomonas aeruginosa* quorum sensing proteins such as LasI/R proteins, RhlI/R proteins and the LysR-type transcriptional regulator (LTTR) protein such as MvfR to check interactions between ligands and target proteins which will be useful in future drug designing purpose [14]. Drug designing is a process of finding new medications based on the knowledge of biological target.

However, during the past ten years, it has been seen an increase in the development and use of computational or Insilico approaches for developing and evaluating pharmacological hypothesis by use of most popular method called docking. Docking is an in-silico method for determining the ligand's proper structure within the target binding site and estimating the strength of a bond between a ligand and a target protein using a special scoring function. The input for docking is the three-dimensional structure of the target proteins and ligands [15]. It is recognized that these novel small molecular compounds have important properties, such as a high

interaction between target binding to target proteins, as well as proper absorption, distribution, metabolism, and excretion and toxicity (ADMET) to help in target lead selection [16]. Molecular docking is the process of molecular identification of target protein and its ligands.

It also focuses on achieving the system's minimum independent energy, which includes properly aligned proteins and ligands. Small ligands, protein peptides, protein proteins, and protein nucleotides can all be performed in the molecular docking of proteins mechanism. Algorithm, receptor flexibility, and ligand flexibility are some of the docking mechanisms. Most frequent software used for docking are Auto Dock vina, Auto Dock, CB Dock and ICM etc. [17]. In computational approaches, docking is main step to determine the binding affinity of certain compound against target proteins. In this case number of ligands is taken because of their potential role in controlling certain diseases [18]. Certain bioactive compounds of *Reynoutria japonica* showed positive results against resistant strains of *Helicobacter pylori* infections [19].

Therefore, it is hypothesized that bioactive compounds from natural source could be effective against infectious *Pseudomonas aeruginosa* strains as it has notorious record worldwide in spread of infections. Multiple in-silico approaches could be helpful in determination of bioactive compounds as best anti-quorum sensing agents on the basis of their binding affinity with target proteins.

In the present study, we performed an in-silico analysis of protein-ligand interactions of all *Pseudomonas aeruginosa* proteins by using natural compounds from *Reynoutria japonica* to predict potential anti-biofilm compounds for emerging bacterial infections. Our analysis predicted ten compounds such as Juglone, Emodin, Emodin 8-o-b glucoside, Polydatin, Resveratrol, Juglone derivatives, Physcion and Chrysophanol that interacted with bacterial proteins such as LasI, LasR, RhlI, RhlR and transcriptional regulator protein MvfR (four proteins from AHL based quorum sensing system, and one protein MvfR from PQS based quorum sensing system) making them potential anti-biofilm drug candidates against *Pseudomonas aeruginosa*.

1.1 Problem Statement

Pseudomonas aeruginosa is an opportunistic pathogen which is the main cause of morbidity and mortality in cystic fibrosis and immunocompromised patients. They use quorum sensing mechanism to regulate virulence factors in bacterial biofilm which help them to resist multiple antibiotics. So, its eradication has become more challenging. Increasing extent of pathogenic resistance to drugs has encouraged the search for new anti-biofilm drugs.

1.2 Aim of Study

The aim of current research was to better understand the quorum sensing mechanism of *Pseudomonas aeruginosa* as well as identification of new, natural and non-toxic quorum sensing inhibitors from *Reynoutria japonica* which may have anti-biofilm properties for respective bacteria and can combat its associated infections.

1.3 Research Objectives

To achieve the desired aim following objectives were designed:

1. To identify various bioactive compounds of *Reynoutria japonica* as potential inhibitors of target proteins.

2. To analyze the binding conformation between targeted proteins and other inhibitors as standard anti-quorum sensing potential.

3. To identify the lead compound as anti-biofilm drug candidate.

1.4 Scope

People prefer traditional medicines over synthetic ones, because of their lower cost and lesser side effects. The present research was an attempt to determine new and effective anti-quorum sensing agents from *Reynoutria japonica* which can reduce the virulence factors in *Pseudomonas aeruginosa* biofilm without causing drug resistance to the pathogens. If successful on clinical trials, selected quorum sensing inhibitors will be instrumental in future to fight emerging bacterial infections and may have potential to prove itself as anti-biofilm drug candidate.

Chapter 2

Review of Literature

2.1 Quorum Sensing

For hundreds of years, bacteria were thought to be alone and silent. But recently, it has been discovered that they can perform their actions at the population level by creating, perceiving, and responding to small signaling molecules known as auto-inducers [20]. The mechanism of communication of bacteria through signaling molecules, when specific threshold concentration is reached then they start living in the form of colonies. This process is known as quorum sensing [21]. Auto-Inducers can coordinate interactions between microorganisms such as intra-species or inter-species interactions as well as between the host by inducing the expression of genes that encode virulence factors such as those involved in biofilm development and strengthen motility [22]. Biofilm is the origin of 80% of human bacterial infections.

Based on quorum sensing control in microbial pathogens, innovative diagnostic approaches and antibiotic drugs have been developed [23]. However, the fundamental significance of quorum sensing is in the biofilm development process [24]. Antibiotic resistance is caused primarily by biofilms, which are ordered structures and are responsible for number of important biological problems especially related to human health such as the formation of dental plaque is due to complex biofilm [25]. *Pseudomonas aeruginosa* is an opportunistic pathogen which attaches itself

to medical equipment's or medical devices and other hospital surfaces as a result healthcare-associated infections develop such as nosocomial infections, extensive inflammation and sepsis. This type of infections is most common in immune-compromised patients similarly burn victims and those who suffer from malignant tumor or HIV. It rarely affects healthy individuals. It may also most frequent cause of persistent infection of the lungs in cystic fibrosis patients. It may also develop microbial keratitis in those individuals who wear their lenses for a long time [6].

2.1.1 The Association Between Quorum-Sensing and Biofilms

Through quorum sensing, microorganisms have an ability to communicate with each other as well as to form biofilm during the process. Quorum sensing controls the metabolic activity of planktonic cells and can lead to the production of microbial biofilms and enhanced pathogenicity [26]. A biofilm is an organized bacterial community. Exopolysaccharides, proteins, and extracellular DNA are all found in bacteria which are enclosed in a biopolymer matrix. Biofilms produced by gram-negative bacteria like *Pseudomonas aeruginosa* are extremely difficult to disseminate. It has a potential to produce biofilms that are resistant to antibiotics. Biofilm is formed when biofilm-producing bacteria attach to solid surfaces in an aqueous environment and develop a network of extracellular polymeric substances, adopting a multicellular lifestyle. Biofilms pose serious problems in terms of infection prevention and treatment [27].

To disrupt biofilms, drug designing and in-silico approaches to measure antimicrobial medicine effectiveness can be developed. Anti-biofilm molecules, such as herbal active compounds, chelating agents, peptide antibiotics have been discovered or tested, along with their structures, mechanisms of action, and applications [28]. Bacteria within a biofilm are protected from the host's defensive mechanisms, harsh surroundings, and antibiotics. The biofilm assembly process is completed in four phases.

- Adhesion of bacteria to a substrate
- Development of micro-colonies
- Maturation of biofilm
- Detachment of bacteria from a surface to colonize new area.
- Bacteria in biofilms are extremely resistant to environmental stresses, particularly antibiotics. Biofilms are a major public health issue because bacteria growing as a biofilm cause 60-80% of human microbial infection [29].

2.1.2 Types of Auto-Inducers

Alteration in gene expression through quorum sensing depends upon signal molecules termed as auto-inducers which are of different types. Most common auto-inducers are acyl-homoserine lactones also known as AHLs which are synthesized by most of gram-negative bacteria and autoinducer peptides also called AIP which are observed in quorum sensing mechanism of gram-positive bacteria like *Staphylococcus aureus* [30].

2.1.2.1 AHL Based Quorum Sensing System

In gram-negative bacteria, this form of quorum sensing mechanism exists. Gene expression in AHL dependent systems is regulated by single synthase-regulator complex. The signal molecules are located in and around the cells and are produced constitutively at smaller amounts by the synthase gene [31]. Signal molecules adheres to its receptor at high cell concentration and triggers the transcriptional activator, which then binds to the DNA, thus stimulating the expression of virulent genes regulated by the quorum sensing system. More than one auto-inducer can be found in a single species, for example, there are two AHLs in *Pseudomonas aeruginosa* [32]. An autoinducer synthase and a transcriptional activator are components of each quorum-sensing system. The following quorum sensing systems

are required to produce and sense these signals. In *Pseudomonas aeruginosa*, the quorum-sensing systems i-e Las and Rhl both are complete and semi-independent. LasR or RhlR is a transcriptional activator, while LasI or RhlI is an autoinducer synthase of quorum sensing system. The production of AHLs requires quorum sensing systems like LasI/R and RhlI/RhlR. LasI produces N-3-oxo-dodecanoyl-L-homoserine lactone/OdHL and its bind with LasR protein, while RhlI produces N-butanoyl-L-homoserine lactone /BHL and binds to regulatory protein Rh1R and cause some virulence genes like exotoxins and proteases to be expressed [33].

2.1.2.2 Quinolone Based Quorum Sensing System

The quinolone-based is a third quorum-sensing system which is present in *Pseudomonas aeruginosa*. It is required for *Pseudomonas* quinolone signal synthesis usually 4-hydroxy-2-alkylquinolines or HAQs. *Pseudomonas* quinolone signal binds to the MvfR/PqsR. This in turn causes the PqsABCDE operon to be expressed and stimulating the production of biofilms and the expression of different virulence factors such as exotoxins and proteases [33].

2.1.3 *Pseudomonas aeruginosa* Quorum Sensing Mechanism

Quorum sensing causes bacteria to release chemical signals or auto-inducers, which accumulate in the bacterial environment. Bacteria exchange information by detecting changes in signal concentrations. A number of signaling molecules related to quorum sensing in bacteria have been discovered. Bacterial quorum sensing can be classified depending on the type of signaling and sensor systems [34]. LasI/R and RhlI/R quorum sensing systems, as well as the PqsABCDE/PqsR system, have been identified in *Pseudomonas aeruginosa* as shown in figure 2.1. The quorum sensing signals such as OdHL, BHL, PQS are produced and binds with their respective receptors [1]. Quorum sensing signals are therefore essential for the formation of bacterial biofilms. The biofilm matrix acts as a physical barrier to

host antimicrobials. It has a high tolerance mechanism in biofilms for heavy metals such as zinc, copper, and lead. Metal-based drugs are unable to clear chronic infections due to this tolerance [35]. Chronic and acute infections in cystic fibrosis patients or ventilator associated infections in immune-compromised patients both are possible with *Pseudomonas aeruginosa*. Chronic infections have been linked to biofilms, whereas acute infections have been linked to a mechanism involving type III secretion system also known as TTSS [36]. TTSS-releasing effectors such as ExoS are also expressed in biofilm which causes ocular damage in microbial keratitis patients [37].

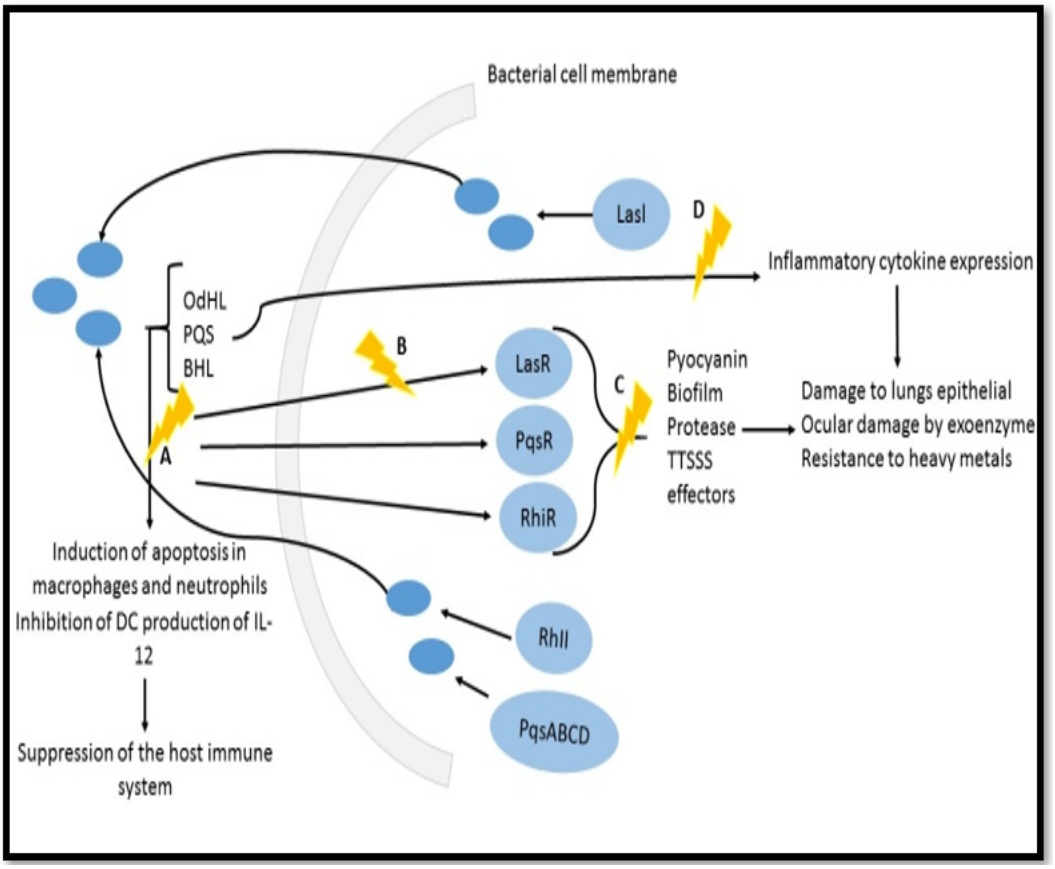


FIGURE 2.1: Mechanism of quorum sensing and virulence in *Pseudomonas aeruginosa [38]*.

2.1.3.1 Relationship Between Quorum Sensing and Secretion System

Quorum sensing is the communication mechanism that allows bacteria to communicate with each other, form biofilms, express virulence genes, produce secondary

metabolites and adaptation of stress mechanism or secretion system etc [39]. Different species have their own QS systems as shown in Table 2.1 for regulation of gene expression.

TABLE 2.1: The following table represented quorum-sensing systems in different bacteria

Signal Molecule	**Pathways**	**Key Points**	**Bacteria**	**References**
AHL	LuxlR	LuxI/LuxR	*V. scheri*	[40]
	LasI/R-	Lasl/LasR	*P.aeruginosa*	[33]
	RhlI/R	and RhlI/RhlR		
AIP	Agr	Agr A,B,C	*S. areus*	[31]
	Fsr	Fsr A,B,C	*E. faecalis*	[40]
AI-2	Lux S	Lux S	*V. harveyi*	[41]
			S. agalactiae	[42]
Others	PQS	Pqs protein	*P. aeruginosa*	[21]
	IQS	AmbBCDE	*P. aeruginosa*	[43]

2.1.4 Target Proteins of Quorum Sensing Systems

AHL based and quinolone-based quorum sensing systems consist of proteins which are responsible for establishment of infections in *Pseudomonas aeruginosa*. These proteins are described as under.

2.1.4.1 LasI Protein

Pseudomonas aeruginosa produces several virulence factors in response to cell density, which is controlled by quorum-sensing mechanism such as las system. las system consists of two proteins LasI and LasR. LasI protein involves the synthesis of autoinducer such as N-3-oxododecanoyl homoserine lactone which binds to LasR [44].

2.1.4.2 Transcriptional Activator Protein LasR

LasR is a transcriptional activator protein in *Pseudomonas aeruginosa*. LasI protein involves the synthesis of autoinducer such as N-3-oxododecanoyl homoserine lactone which binds to LasR. Induction of several virulence genes which encodes elastase etc. requires LasR [44].

2.1.4.3 RhlI Protein

Pseudomonas aeruginosa produces several virulence factors in response to cell density, which is controlled by another quorum-sensing mechanism such as rhl system. This system consists of two proteins such as RhlI and RhlR [45]. RhlI protein involves the synthesis of autoinducer such as N-butyryl homoserine which binds with RhlR [44].

2.1.4.4 Transcriptional Activator Protein RhlR

RhlR is a transcriptional activator protein in *Pseudomonas aeruginosa*. RhlI protein involves the synthesis of autoinducer such as N-butyryl homoserine which binds with RhlR [33], [46].

2.1.4.5 MvfR Protein

MvfR is multiple virulence factors regulating protein. MvfR regulates the expression of several quorum sensing regulated virulence factors such as pyocyanin, proteases etc. As well as the expression of the PqsA-E genes, these genes encode functions that require for the biosynthesis of 4-hydroxy-2-alkylquinolines HAQs .

2.1.5 Quorum Sensing Induced Worries

Quorum sensing based biofilms formed by infectious bacteria are big cause of spreading human infections. Quorum sensing mechanism involves upregulation

of motility, virulence and pathogenic factors which step towards the formation of biofilms which further cause disease. Different bacterial species are major source of different infections e.g., Pseudomonas species decline health of cystic fibrosis patients. Fishery department is under-threat due to some disease-causing bacteria such as *Aeromonas hydrophila, Aeromonas salmonicida, Vibrio anguillarum* and *Vibrio harveyietc* which express their pathogenicity through quorum sensing systems. Agriculture, aquaculture, drinking water processing and waste water treatment plants are some of the other areas where biofilms have been found to affect process efficiencies [47]. There are certain other areas like drinking water sources, treatment plants, buildings, agriculture which are affected by biofilms produced by *Salmonella enterica* and *Escherichia coli* [48] [49].

2.2 Antibiotic Usage and Resistance

The 20th century was good with relation to health aspects as it was the era of antibiotic discovery. Enormous medication was done by using antibiotics. But as the excess of everything is bad and due to evolutionary nature of bacteria they got resistant to those antibiotics in the 21st century [50]. *P. aeruginosa* causes multidrug-resistant bacterial infections, especially nosocomial infections like urinary tract infections, lungs infection, and surgical site wound infections and so on. Infections like these are growing more widespread all across the world. Gram-negative pathogens become resistant to various antibiotics like fluoroquinolones, aminoglycosides and Beta-lactams including Carbapenems, Monobactam, Cephalosporins, and wide range of Penicillin's [51]. Antibiotic resistance in *P. aeruginosa* was shown in Table 2.2 respectively.

TABLE 2.2: Resistance of *Pseudomonas aeruginosa* to a variety of antibiotics

Bacteria	**Antibiotic family**	**Examples**	**References**
P.aeruginosa	Aminoglycosides	Tobramycin Gentamicin Amikacin	[52]

TABLE 2.2: Resistance of *Pseudomonas aeruginosa* to a variety of antibiotics

Bacteria	**Antibiotic family**	**Examples**	**References**
P.aeruginosa	Quinolone	Ciprofloxacin Levofloxacin	[53]
P.aeruginosa	Beta-lactam	Penicillin Cephalosporin Carbapenem Monobactam	[54]

Antimicrobial resistance or AMR is a major public health concern around the world. It leads to an increase in mortality as well as excessive health-care costs. MDR bacterial infections, often known as multidrug-resistant bacteria have serious clinical and economic consequences [55]. *Pseudomonas aeruginosa* acquiring antibiotic resistance mechanism was shown in Table 2.3. Alternative techniques to fight against multidrug-resistant bacteria are required.

TABLE 2.3: The acquisition of antibiotic resistance mechanism in *Pseudomonas aeruginosa*

S/No	**Mechanism**	**Mechanism of Action**	**References**
1	Intrinsic resistance	This type of antibiotic resistance can be achieved by expression of efflux pumps and synthesis of antibiotic-inactivating enzymes which remove antibiotics from the cell mutational changes.	[56]

TABLE 2.3: The acquisition of antibiotic resistance mechanism in *Pseudomonas aeruginosa*

S/No	Mechanism	Mechanism of Action	References
2	Acquired resistance	It could attain through horizontal resistance gene transfer (conjugation, transformation, and transduction) or mutational changes.	[56]
3	Adaptive resistance	It involves the formation of biofilm in infected lungs patients or cystic fibrosis patients which act as a diffusion barrier, limiting antibiotic access access to the bacterial cell.	[27]

It is the need of the hour because approximately 33000 people die each year as a result of bacterial infections. It is therefore mandatory to search for effective medication development proposals.It is therefore mandatory to search for effective medication development proposals. Inhibition of quorum sensing is yet to be best proposed suggestion in this regard. This will protect us from repeated antibiotic usage and their harmful side effects [57].

2.3 Biofilm-Mediated Resistance

Formation of biofilm by *Pseudomonas aeruginosa* in cystic fibrosis patients which acts as a diffusion barrier, limiting antibiotic access to the bacterial cells [27]. Multidrug-tolerant persister cells can also form in biofilms. These cells are the

source of long-term and recurrent infection [58]. Several new treatment techniques have been discovered recently to disrupt the quorum sensing mechanism in *Pseudomonas aeruginosa* [5].

2.4 Quorum Quenching

While overuse of antibiotics has resulted in a significant resistance problem and inefficiency in *Pseudomonas aeruginosa* infection treatments, biofilms are already distinctly resistant to antibiotic therapy. Quorum sensing inhibition is proposed as an alternative approach to control bacterial infections by increasing biofilm sensitivity to drugs and reducing virulence factors. It can be blocked at the signal, signal receptor, or signal transduction levels. Bacteria within biofilms multiply and cause most of the infectious diseases. These biofilms could be destroyed by using bioactive compounds. These compounds can be obtained from different natural sources such as plants. Those bioactive compounds that disturb the quorum sensing mechanism are called inhibitors. This phenomenon of interruption of bacterial communication through quorum sensing inhibitors is termed as quorum quenching [59].

2.4.1 General Strategies to Block Quorum Sensing

Quorum quenching interrupts bacterial communication and prevents group actions such as the expression of disease-causing factors. Quorum quenching can be done by many ways and it is now considered to be one of best strategies in signal inhibition and destruction [59].

2.4.1.1 Inhibition of Signaling Molecules

Signals are main components of quorum sensing. By controlling enzymes which take part in signal production, the signal synthesis can be blocked and in this

way quorum sensing can be controlled e.g., enoyl-ACP reductase is involved in formation of AHL signals. Triclosan is seen effective to reduce action of this enzyme [60].

2.4.1.2 Competitive Inhibition

In this strategy some structures similar to signals are selected. These similar structures can bind with receptors by process of competition and thus can block the binding of signals with receptor proteins e.g. halogenated furanones have structure similarity with AHLs and can be used for this purpose [61].

2.4.1.3 Degradation of Signal Molecules

For quorum sensing a large number of signals is required. This number can be reduced by using special enzymes that can degrade signals and resulting in their lesser production. MacQ is used for this purpose in gram-negative bacteria [62].

2.4.2 Mechanism of Quorum Quenching

As various types of signals exist in different bacterial species so the mechanism of quorum quenching is also different according to type of bacteria.

2.4.2.1 AHL Based Quorum Quenching

The quorum quenching of AHLs has received the greatest attention because AHLs are involved in regulating disease causing factors. Either of these methods can be used to suppress or block AHL- mediated quorum sensing system [63].

1. Disturbing the production of AHL signals
2. Interrupting with the signal transmission
3. Antagonizing the AHL receptors

2.4.2.2 AIP Based Quorum Quenching

Due to cause of widespread diseases and resistant nature, *Staphylococcus aureus* has always remained a reference organism with respect to AIP based quorum sensing. A lot of research has done on this system to disturb it signaling system. Many ways to quench quorum sensing in *Staphylococcus aureus* are discussed under [64].

2.4.2.3 AHL Based Quorum Quenching Mechanism

There are three strategies to block AHL-mediated quorum sensing systems in *Pseudomonas aeruginosa*:

a. **Interfering with the production of AHL**: Lowering the concentration of active AHL in the media has the potential to disturb the bacterial communication. Lactone hydrolysis happens naturally in AHLs because of high pH levels. As a result, enzymatic reactions can potentially initiate it [65] [66]. In terms of enzymatic degradation, the AHL structure can be disrupt by four different reactions. Lactonase or decarboxylase enzymes catalyze two chemical reactions. They break homoserine lactone or HSL ring. While amidases and deaminases catalyze the other two chemical reactions, they split the HSL ring from the acyl chain [67].

b. **Interfering with signal transmission:** By lowering the concentration of active AHL in the media has the potential to disrupt bacterial communication. When the pH level rises, lactone hydrolysis starts naturally in AHLs but it can also be triggered by using enzymes [66]. In terms of enzymatic degradation, the AHL structure can be disrupt by four different reactions. Lactonase or decarboxylase enzymes catalyze two chemical reactions. They break homoserine lactone or HSL ring [67].

c. **Antagonizing AHL receptors:** Signal transduction in quorum sensing mechanism can be blocked by using antagonists that restrict signal and receptor union and prevent quorum sensing signals from being transmitted.

So, non-competitive antagonists bind to different regions of the receptor and have little or no structural similarity to AHLs. LuxN-type receptors are vigorous antagonist of the LuxR family [68]. Some natural compounds that target quorum sensing circuitry in *Pseudomonas aeruginosa* were shown in Table 2.4.

TABLE 2.4: Natural compounds targeting quorum sensing circuitry in *Pseudomonas aeruginosa*

Inhibitors	**Target**	**Mechanism of Action**	**References**
AHL lactonases	AHLs	They breakdown AHLs of different acyl chain lengths, especially the 3-oxo-Cl2-HSL, to prevent the production of *Pseudomonas aeruginosa* biofilm.	[69]
Decarboxylases	AHLs	Attack the lactone ring.	[69]
2-alkyl-3- hydroxy -4(1H)-quinolone- 2,4-dioxygenase	PQS	It cleaves PQS.	[69]
Tetrazole (PD12)	LasR	Elastase and pyocyanin, two virulence factors that are dependent on quorum sensing, were inhibited.	[70]
V-06-018	LasR	Elastase and pyocyanin, two virulence factors that are dependent on quorum sensing, were inhibited.	[70]

TABLE 2.4: Natural compounds targeting quorum sensing circuitry in *Pseudomonas aeruginosa*

Inhibitors	Target	Mechanism of Action	References
PPAR8 agonists	PPAR8, OdHL	Bind to peroxisome proliferator-activated receptors to PPARS and NF-KB-dependent pro-inflammatoory genes should not be activated.	[71]
Ionic Silver	Biofilms	Ionic silver in high quantities disperses biofilm	[72]

2.4.3 Quorum Sensing Inhibitors and Their Sources

Quorum sensing inhibitors mainly contribute in disruption of biofilms. They work primarily by quenching the quorum sensing. Different molecules are used as quorum sensing inhibitors. They may be from different sources like plants etc.

2.4.3.1 Synthetic Quorum Sensing Inhibitors

Various synthesized chemicals were tested as quorum sensing inhibitors and shown to be helpful in combating infections e.g., Penicillic acid have been shown to be advantage against *Pseudomonas aeruginosa*. Baicalin hydrate was found to be beneficial in treating *Burkholderia* infections. Vancomycin is also helpful against *Candida elegans*. The XYD-11G2 antibody was found to be efficient against *Pseudomonas aeruginosa* [73].

2.4.3.2 Natural Quorum Sensing Inhibitors

Natural sources of quorum sensing inhibitors have been found to be effective in reducing the quorum sensing activity of several harmful bacteria. Such as methanol

extract of Psoralea corylifolia L., have been found to be effective in reducing the quorum sensing activity of *Pseudomonas aeruginosa, Serratia marcescens,* and *Aeromonas hydrophila* [74]. An Indian medicinal plant such as Cuminum cyminum is effective in inhibiting biofilm formation in harmful bacteria like *Pseudomonas aeruginosa, Proteus mirabilis, and Serratitia marcescens* [75]. Development of biofilms in *Pseudomonas aeruginosa* is inhibited by a halogenated furanone. Its chemical formula is $C_5H_3BrO_3$. It is isolated from the Australian edible macroalga Delisea pulchra [76].

2.5 *Reynoutria japonica*

The medicinal plant *Reynoutria japonica* is native to Japan although it can also be found in South Korea, China, Europe and North America. It is also known as *Polygonum cuspidatum*. It is the member of the plant family Polygonaceae.

2.5.1 Botany

It is herbaceous plant and its length is estimated around 1-2 meters. Its stem and root are used as medicinal purpose against various diseases. This species develops huge, straight, hollow stems with distinct nodes that resemble bamboo but are softer and have large-leaved foliage. as shown in figure 2.2 [77]. The stems are also distinguished by prominent purple speckles. The leaves are placed oppositely, are around 10-15cm long, and form on a zig-zagging stem. The leaves are heart to shield-shaped, with flat bases and pointy tips. At the plant's base, a crown of rhizomes may be visible, producing white shoots. It can grow to be over 4m tall in a single season. Flowers are small and cream-white in color and range in length from 6 to 15cm. They bloom from late summer to early fall. It has invasive rhizomes that are difficult to eradicate [78]. This plant is used for many purposes like medicines, fodder and decoration etc. Young shoots of this plant are edible and rich source of Vitamin A [13]. Its hierarchical classification was shown in Table 2.5.

FIGURE 2.2: *Reynoutria japonica* (A) Whole plant, and the arrow represented the stems of Polygonum cuspidatum (B) flowers and leaves (C) dried roots of *Reynoutria japonica* [77] .

TABLE 2.5: Hierarchical classification of *Reynoutria japonica* [79].

Serial No	**Domain**	**Eukarya**
1	Kingdom	Plantae
2	Phylum	Tracheophytes
3	Class	Angiosperms
4	Order	Caryophyllales
5	Family	Polygonaceae
6	Genus	*Reynoutria*
7	Species	*R. japonica*

2.5.2 Phytochemistry

Since the early 1950s, many chemical compounds have been discovered in *Reynoutria japonica* or *Polygonum cuspidatum* in China, Japan, Korea, and other countries. Quinones, stilbenes, flavonoids, coumarins and other polyphenolic compounds have all been isolated from this plant and they have anti-inflammatory, anticancer, antiviral and antibacterial properties [80].

2.5.3 Traditional Usages

A number of useful metabolites are found in roots and stems of *Reynoutria japonica*. It has rich source of secondary metabolites such as quinones and flavonoids etc. which are present in the roots of this plant. The root of this plant has also been used in herbal medicines. It may also effective for the treatment of inflammatory and infectious diseases, jaundice, skin burns and chronic bronchitis. The stem of this plant is useful in everyday meals, and the roots have been used as a coloring agent of rice. Its pharmacological effects were shown in figure 2.3 [13].

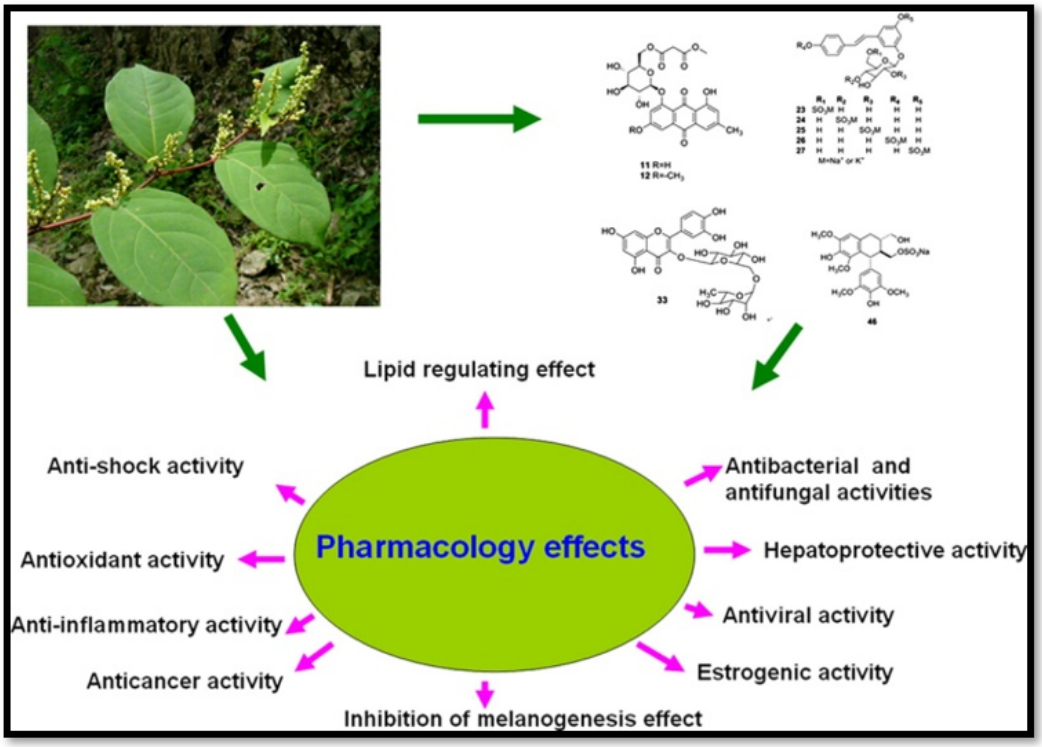


FIGURE 2.3: Indicated multiple pharmacological effects of *Reynoutria japonica* [13] .

2.5.4 Naphthoquinone as a Natural Inhibitor

Naphthoquinones are one of these several secondary metabolites of *Reynoutria japonica*, with a variety of biologically significant features. Its chemical structure was shown in figure 2.4. Recent researches have explored nine juglone derivatives in the treatment of *Helicobacter pylori* infections. Among those, first four

showed good results. Their name and chemical structure were shown in Table 2.6 [19]. Many 1, 4-naphthoquinones, particularly 5-hydroxy-1, 4-naphthoquinones (juglone) and their derivatives have been discovered. They have antibacterial, antifungal, antiviral, anticancer, and cytotoxic properties [81]. Five anthraquinones, two stilbenes and a 1,4-naphthoquinone derivative from the root portion of *Reynoutria japonica* have previously been reported for anti- *Helicobacter pylori* activity. As a natural chemical, the 2-methoxy-6-acetyl-7- methyl-juglone showed the most antibacterial activity among the isolates [19]. Due to the better efficacy of these bioactive compounds having anti-bacterial properties, the present study is focused on their positive contribution in controlling resistant strains of *Pseudomonas aeruginosa*.

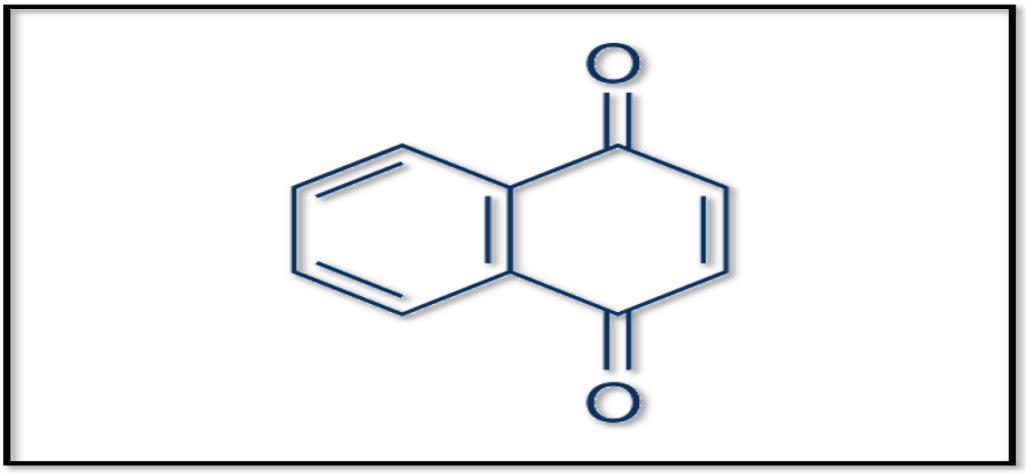
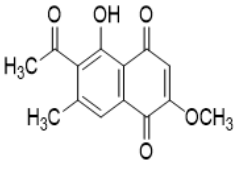
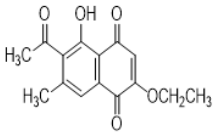
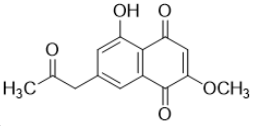
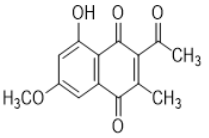


FIGURE 2.4: Chemical structure of 1, 4-naphthoquinones [19] .

TABLE 2.6: Structural analogs of 1-4 Naphthoquinone [19].

Secondary metabolite of *Reynoutria Japonica*	**Chemical Component**	**Chemical Structure**
1,4 Napthoquinone	2-Methoxy-6-acetyl-7-methyjuglone	

2-Ethoxy-6-acetyl-7-methyjuglone	
2-methyl-2-metyljuglone	
3-Acetyl-7-methoxy-2-metyljuglone	

2.6 Bioactive Compounds of -*Reynoutria japonica* which act as Inhibitors

Bioactive compounds have been described as compounds that cause a specific biological reaction in animals and humans. Bioactive compounds of *Reynoutria japonica* which act as inhibitors described below in Table 2.7 and their chemical structure has been shown in Figure 2.5 respectively.

TABLE 2.7: Chemical compounds isolated from *Reynoutria Japonica*

Sr.No	**Classification**	**Chemical component**	**Part of plant**	**Reference**
1.	Quinones	Juglone(5-hydroxy-1, 4,-naptho-quinone)	Root	[82]
2.	Quinones	Anthraqui--none or Emodin-8-o-b-glucos-ide	Root	[82]

TABLE 2.7: Chemical compounds isolated from *Reynoutria Japonica*

Sr.No	Classification	Chemical component	Part of plant	Reference
3.	Quinones	Emodin	Root	[83]
4.	Stilbenes	Polydatin	Root	[84]
5.	Stilbenes	Resveratrol	Root	[84]
6.	Quinones	2-Methoxy -6-acetyl-7 methyljug- lone (MAM)	Root	[85]
7.	Quinones	2-Ethoxy-6- acetyl-7-meth -yljuglone	Root	[19]
8.	Quinones	2-methyl-7- acetyl quinoline	Root	[86]
9.	Quinones	Physcion	Root	[84]
10.	Quinones	Chrysophanol	Root	[84]

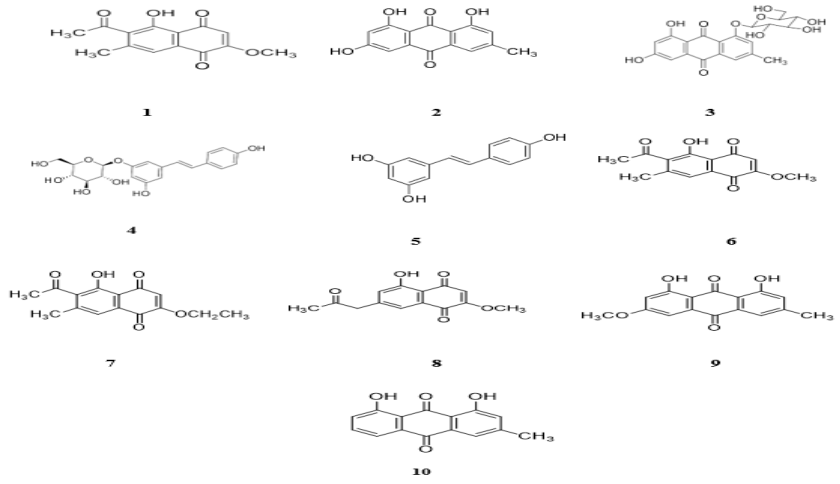


FIGURE 2.5: Chemical structure of bioactive compounds of *Reynoutria japonica* [84] .

2.6.1 5-Hydroxy-1, 4-Naphthoquinone

5-hydroxy-1,4- naphthoquinone, often known as juglone. Juglone is a plant metabolite produced by many plant species but most commonly *Juglans nigra*. It is a naphthoquinone's compound also isolated from Chinese plants such as *Polygonum cuspidatum* or *Reynoutria japonica*. Juglone has traditionally been used as a natural dye for clothes, especially wool. But it is often used as herbicide. Juglone is currently being studied because it has anticancer properties. Juglone has been shown to have antimicrobial, antifungal, oxidizing, antihypertensive, and mainly anti-proliferative properties. So, 1,4-naphthoquinone is a valuable for medicinal chemistry and used to treat many diseases such as cancer, allergies and bacterial infections etc [87].

2.6.2 Emodin

Emodin is an anthraquinone derivative found naturally in Chinese plants like *Rheum palmatum*. Many cultures particularly in Eastern Asia have long employed these herbs as traditional treatments [88]. Scientists are now studying the pharmacological effects of this substance. Previous studies confirm its anticancer and anti-inflammatory activities. Emodin has also been shown to have a wide range of pharmacological actions including antiviral, antibacterial, antiallergic, antidiabetic, immunosuppressive, neuroprotective and hepatoprotective propertie [89].

2.6.3 Emodin 8-o-b-Glucoside

Emodin-8-o-b-glucoside is a dihydroxyanthraquinone. It is glycosylated derivative of Emodin. It is involved in phosphorylation of mitogen-activated protein kinase pathway and phagocytosis. It also stimulates the secretion of proinflammatory cytokines. It is an anthraquinone that can be found in the roots and barks of variety of plants. It has antiproliferative effect in cancer cells that are mediated

by many signaling pathways. It has anti-cancer, anti-depressant and antimicrobial properties [83]. It is also taken as an effective anti-aging, anti-hyperlipidemia and anti-inflammatory agent in pre-clinical and clinical therapy to improve immunomodulation, neuroprotection and the recovery of other diseases [90]

2.6.4 Polydatin

Polydatin is a stilbenoid monocrystalline compound isolated from *Polygonum cuspidatum*. It is also detected in grapes, peanut, hop cones, red wines, hop pellets, cocoa-containing products, chocolate products and many daily diets. Anti-platelet aggregation, low-density lipoprotein's anti-oxidative action, cardioprotective activity, anti-inflammatory, and immune control functions are among few biomedical properties of Polydatin [91]. It also exhibited neuroprotective and lung protective effect [92]. Anti-tumor and anti-oxidative effects are also shown by Polydatin. Effective anti-bacterial activity is also exhibited by Polydatin. It inhibits *Streptococcus* mutans and greatly lowers glycolytic acid generation at a low level. Despite the fact that Polydatin's bioactivities have been demonstrated in laboratory animals, organs, and cells. A few molecular mechanisms of action are understood and the definitive target proteins bound by Polydatin are unknown, which require further clinical applications of polydatin [93] [94] [95].

2.6.5 Resveratrol

Resveratrol is a polyphenolic antioxidant found in nature that belongs to the stilbene family [96]. Resveratrol can be found in a variety of plants, including peanuts, blueberries, and cranberries. *Polygonum cuspidatum*, also known as Japanese knotweed is a traditional Asian herbal remedy as well as a natural source for human consumption in grapevines. Numerous studies have shown that Resveratrol has antibacterial, antifungal, antioxidant properties as well as it also exhibit anti-tumor activity [97]. Piceid, piceatannol glucoside, and resve ratroloside these are three glycosylated resveratrol analogues derived from the invasive

plant *Polygonum cuspidatum* [98]. And they possess antibacterial compounds [99]. Past researches showed that it reduced the growth of *Bacillus cereus, Helicobacter pylori, Vibrio cholerae, E.coli and Neisseria gonorrhoeae* [100]. The capacity of resveratrol to inhibit biofilm formation in diverse bacterial pathogens has been investigated. Resveratrol has also been shown to reduce the ability of *Vibrio vulnificus* to swarm [101] [102].

2.6.6 2-Methoxy-6-acetyl-7-methyljuglone

It is a naphthoquinone or natural bioactive juglone derivative which is isolated from *Reynoutria japonica*. It is also called 2-Methoxystypandrone or MAM. It is a quinoid compound that has been isolated from several Juglandaceae members. Furthermore it is a bioactive element found in specific plant parts and has been used in ethnomedicine to treat a variety of diseases including allergies and bacterial infections [103]. Naphthoquinones are one of several secondary metabolites found in Reynoutria japonica and they have biologically important applications. It has anti-inflammatory properties. It has anticancer, antioxidant, antimicrobial, and anti-HIV activities as well. MAM also improved the brain function through protecting the integrity of the blood-brain barrier and aid neurogenesis [85].

2.6.7 2-Ethoxy-6-acetyl-7-methyljuglone

It is a naphthoquinone or natural bioactive juglone derivative which is isolated from *Reynoutria japonica*. Naphthoquinones are one of several secondary metabolites and they have biologically important applications. It showed remarkable therapeutic activity against bacteria, fungi and algae. It is seen effective in treatment of tumors and diabetes. It is also useful in human intestinal infections [104] [105]. Many juglone derivatives have been found to be effective in the treatment of *Helicobacter pylori* infections in recent studies. These derivates can be synthesized from *Reynoutria japonica* by using the acetate-polymalonate pathway.

Their positive results against *Helicobacter pylori* led to the hypothesis that naphthoquinones could be employed against other bacterial strains to restrict growth and treat infections [19].

2.6.8 2-Methyl-7-acetyl- quinoline

It is juglone derivatives which are derived from *Reynoutria japonica*. They show remarkable antimicrobial and anticancer properties. 2-methyl-7- acetyl- quinoline is a novel natural juglone derived from this plant showed strong potent anti-*Helicobacter pylori* activity [81]. Further studies investigated that in HeLa cells, it promotes the expression of NFR2 target genes. NFR2 or nuclear factor erythroid 2-related factor 2 is a transcription factor that regulates the expression of genes involved in drug detoxification and antioxidative stress. Inflammatory reactions, cardiovascular diseases, and cancers have all been associated to NRF2 [86].

2.6.9 Physcion

Physcion is a major bioactive ingredient isolated from *Reynoutria japonica* and used in the traditional Chinese medicine. It is naturally occurring anthraquinone derivative and has wide range of pharmacological actions, including laxative, hepatoprotective, anti-inflammatory, anti-microbial, and anti-proliferative properties [106].

2.6.10 Chrysophanol

Chrysophanol is a natural anthraquinone and used in the traditional Chinese medicine. Some of the pharmacological effects of Chrysophanol include neuroprotection, anticancer, antibacterial, antiviral, antioxidation, and blood lipid regulation, and it can be used to treat disorders such as cancer, atherosclerosis, asthma, diabetes and diabetic complications, and Alzheimer's disease [107].

2.7 Molecular Docking in Drug Discovery

Molecular Docking is technique used to estimate the strength of a bond between a ligand and a target protein through a special scoring function and to determine the correct structure of the ligand within the target binding site. The 3D structure of the target proteins and the ligands is taken as the input for docking. It represents a frequently used approach in structure-based drug design since it requires a 3D structure of a target protein. It can be used to determine the correct structure of the ligand within the target binding site, and to estimate the strength of the binding between the ligand and the target proteins through a specific scoring function [108]. It also helps in the recognition of new small molecular compounds, revealing the essential properties, such as high interaction between binding with target protein having reasonable absorption, distribution, metabolism and excretion, which help in the selection of lead compound for the target [109]. So, the docking process includes following compounds which are discussed below:

1. The docking process requires a 3D structure of protein which is downloaded from protein data bank (PDB).
2. Minimum size of molecules or compounds or virtual compounds that contain a database is required.
3. A computational framework is also needed to perform the docking and find the scoring process.

Protein and ligand docking is one of the key areas of molecular docking, which is obtain high popularity and appreciation due to its role in structure-based drug designing [110]. Molecular dynamics, distance geometry method and genetics algorithm are most widely used algorithm in molecular docking and the most frequent software used for molecular docking are Auto Dock vina, Auto Dock, CB Dock and ICM etc [111].

The target proteins of *Pseudomonas aeruginosa* and ligands from *Reynoutria japonica* for docking purpose were shown in Table 2.8. A significant number of

information is present on the effectiveness of quorum quenching techniques in the battle against infectious diseases till date. No doubt researchers have focused their attention on disruption of quorum sensing in this regard but the majority of their work is related to signal antagonism and very little research was done on signal enzymatic destruction, signal inhibition, or signal sequestration [112]. *Pseudomonas aeruginosa* has adverse history related to clinical aspects and undoubtedly many antibiotics had been tried for treatment but there is very little search to control it signaling mechanism which could be more effective than antibiotics usage. Therefore, it is need to search innovate strategies to suppress signaling and non-toxic compounds preferentially from herbal sources for drug designing purpose [113].

TABLE 2.8: Bioactive compounds with target proteins for docking purpose

Sr.No	**Selected Ligands**	**Target Proteins**
1.	Juglone	LasI/LasR
2.	Emodin	
3.	Emodin-8-o-b-glucoside	Rh1I/Rh1R
4.	Polydatin	
5.	Resveratrol	MvfR (PqsR)
6.	Physcion	
7.	Chrysophanol	
8.	2-Methoxystypandrone	
9.	2-Ethoxy-6-acetyl -7-methyljuglone	
10.	2-Methyl-7-acetyl -quinoline	

In the light of above overall discussions, it can be concluded that past studies suggested variety of efforts which have been used to fight against emerging bacterial infections. Due to massive use of antibiotics in clinical practices cause bacteria

to become resistant against them. With the evolution of virulent pathogens into multi-resistant bugs have compelled the researchers to come with some new research ideas. As bacteria's have become smart with changing environment so we need to become even smarter to make ourselves more sustainable in the world of infections. The options which are using in present research are to quench the signaling pathways of bacteria at any possible level of their production and dissemination. There are strong chances that through multiple *Insilico* approaches, the use of this knotweed against the infectious proteins (which are used in quorum sensing of bacteria) would make it quite easy to locate the binding sites of proteins available for metabolites to interact with and will assist in choosing the best one and further investigating the pathways towards the field of anti-microbial drug candidate.

Chapter 3

Research Methodology

3.1 Methodology Flowchart

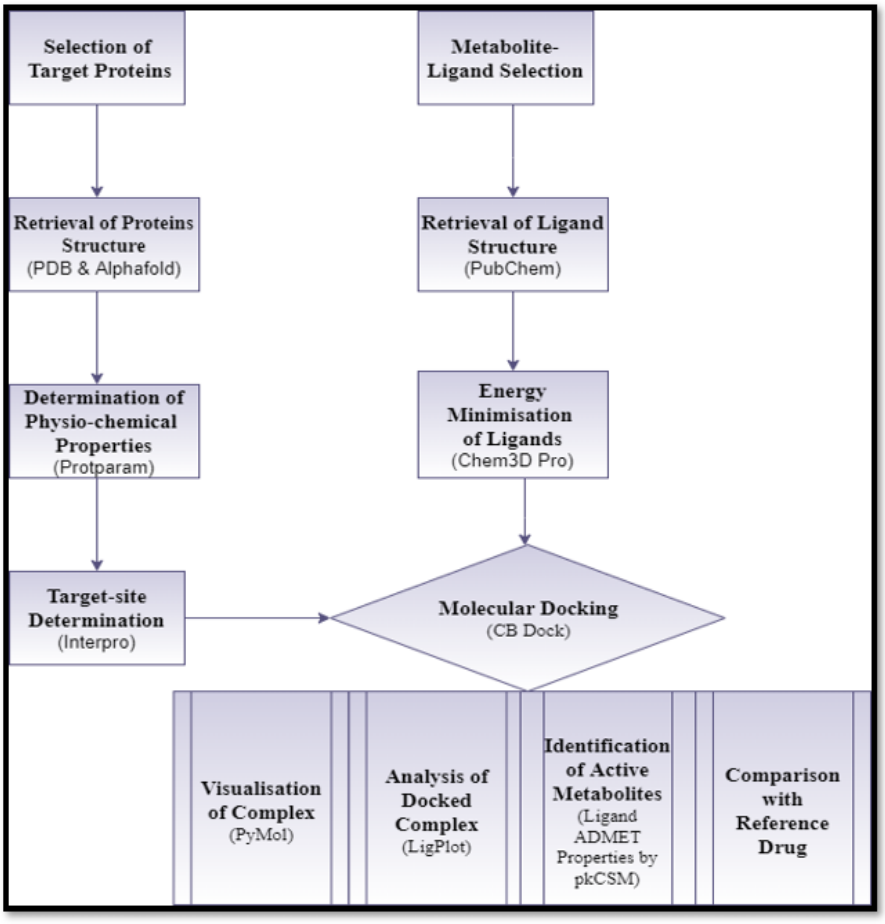


FIGURE 3.1: The flowchart of research methodology.

Present research was based on several computational approaches which are described below under headings sequentially.

3.2 Selection of Problem

In case of bacterial infections antibiotic treatment is recommended but it have seen now that certain bacteria become resistant to those antibiotics because of their massive use [114]. Patients with cancer, cystic fibrosis, and burns who are hospitalized frequently develop numerous *Pseudomonas aeruginosa* infections such as nosocomial infections etc. because it has an ability to resist multiple antibiotics and situation has become more challenging. In case of *Pseudomonas aeruginosa*, the main contributors of pathogenicity and virulence are AHL and PQS based quorum sensing systems. It has been seen the proteins of quorum sensing systems play a key role in establishment of bacterial infections [115]. By targeting those proteins through natural metabolic compounds severe bacterial infections could be control.

3.3 Target Identification

Certain proteins in the bacterial quorum sensing pathways were responsible for the development of life-threatening illnesses. Target proteins which were selected from *Pseudomonas aeruginosa* on the basis of their virulence and pathogenicity factors were LasI protein, LasR protein, RhlI protein, RhlR protein and transcriptional regulator protein MvfR [116].

3.4 Primary Sequence Retrieval

Primary sequence of target proteins (LasI, LasR, RhlI, RhlR and MvfR) was taken in FASTA format from protein sequence database UniProt `http://www.uniprot.`

org/ under accession number A0A0M4BU37, A9JPX1, A9JPX5, A0A411F6T9 and AOA1C6ZJJ6 with residues length of 401, 236,196, 238 and 292 respectively [117].

3.5 Analysis of Physicochemical Properties

Physicochemical properties play an important role in determining the function of proteins. ProtParam https://web.expasy.org/protparam/ was used to predict these properties of LasI, LasR, Rh1I, Rh1R and MvfR [118]. The number of positively charged residues (Arg+ Lys) and negative charged residues (Asp+ Glu), theoretical pI, molecular weight, Ext. Coefficient (Cyst included), Ext. Coefficient (Cyst not included), instability index, aliphatic index and grand average of hydrophobicity were computed through ProtParam [118] [119].

3.6 Protein Structure Retrieval

3D Structures of *Pseudomonas aeruginosa* proteins such as LasI, LasR, RhlI, RhlR and MvfR were downloaded directly from RCSB Protein Data Bank or PDB https://www.rcsb.org/. Alternatively, Alphafold could be used if some structures were missing on PDB. Alphafold https://alphafold.com/ another authentic protein structure database used for 3D structure prediction of proteins [120].

3.7 Structural Analysis by Use of PyMOL

PyMOL https://pymol.org/ a cross platform molecular graphics tool that has been used world widely for the three-dimensional analysis and visualization of many proteins, small molecules including nucleic acids, densities of different electrons and varying surfaces and also the trajectories. It could be used for editing the molecules and to make animations and movies. The software based on python

and also contain many plugin tools in order to enhance its use and also facilitate the drug targeting and designing. After downloading the protein structure, its 3D conformation was analyzed and extra constituents attached to the protein was eliminated by using an open-source system PyMOL [121].

3.8 Functional Domain Identification

InterPro http://www.interpro.com/ is an online database which was used to identify the functional domains of targeted proteins such as LasI, LasR, RhlI, RhlR and MvfR [122]. Conserved domains involved in sequence/structure/relationship of proteins.

3.9 Identification of Binding sites and Favorable Ligand Binding Moieties

The ligand showed maximum or highest interactions with the protein where the target protein has its active site. In the complex of ligand to protein synthesis, amino acids played a significant role. Proteins binding pockets were identified by using CASTp software http://sts.bioe.uic.edu/castp/ [128].

3.10 Retrieval of Chemical Structure of Ligands

PubChem https://pubchem.ncbi.nlm.nih.gov/ is the world's largest repository of easily accessible chemical information database. So, the chemical compounds that could be used as ligands were selected directly from PubChem database. The selected ligands were refined through Chem Draw Ultra version 12.0.2 software. Those ligands were selected that had previously shown some antibacterial properties. The selected ligands were Juglone, Emodin, Emodin 8-o-b glucoside,

Polydatin, Resveratrol, Physcion, Chrysophanol, 2-Methyl-7-acetonyl-juglone, 2-Methoxy-6-acetyl-7-methyl-juglone and 2-Ethoxy-6-acetyl-7-methyl-juglone respectively. If in case the selected ligand's structure was not available on PubChem, then our next attempt would be to download the canonical similes from PubChem and then insert them in the software ChemDraw and after obtaining their 3D structure then repeat the energy minimization step by using Chem pro software. This is a mandatory step in the preparation of ligands for docking because unstable ligands will show unreliable vina scores in docking results. In the end the SDF format was selected to save energy minimized structure of the ligands [123].

3.11 Virtual Screening and Molecular Docking

The purpose of molecular docking was to find the best conformational interaction between target proteins and bioactive compounds. The two essential requirements for docking were the target protein and the candidate ligand. Active metabolites of *Reynoutria japonica* found after the review of literature could be used as the ligands and target proteins were LasI protein, LasR protein, RhlI protein, RhlR protein and transcriptional regulator protein MvfR from *P. aeruginosa*. Virtual screening of targeted proteins against natural compounds was done by using CB dock. CB dock `http://clab.labshare.cn/cbdock/php/blinddock.php` is an online docking server which automatically predict the binding sites and used to perform docking. It can simplify docking procedures and improve accuracy of ligands by predicting target protein binding sites [125].

3.11.1 Process of Molecular Docking

The first step in performing the docking process was to create ligand and target protein files. Firstly, the target protein file was compiled following a few steps. PDB file of target proteins was given to CB dock as input file one by one. After these amendments target protein file was saved in pdbqt format. After compilation

of protein files, the ligands files were prepared by following the same procedure and saved in PDB format in same directory. Then setting up Grid box around protein ligand structure was performed. For this purpose, macromolecules option was selected from Grid and pdbqt target protein file was opened then from set map type option ligand structure was opened [126].

After performing these steps from grid, grid box option was selected to design grid around protein- ligand complex. Grid box with all parameters appeared and file was saved as Grid Parameters File in same directory after selecting parameters. Utilizing docking related commands docking was performed. These commands help to get the directory path where the docking readable prepared protein ligand files have been saved along grid parameters file. Docking files were created for chosen data set after completion of this step and results were saved in pdbqt format [127].

3.12 Analysis and Output Visualization of Drug Target and Protein

The docked poses were listed based on the corresponding docking scores after molecular docking was complete. CB dock `http://clab.labshare.cn/cbdock/php/blinddock.php` was used to predict free energy for ligands binding to the receptor [125]. PyMOL was used to examine the 3D conformation ligand- receptor [121].

3.13 Ligand-Protein Interaction Studies

The interaction between the active pockets of the proteins and the ligands (docked complex) were calculated for the interpretation of their interactions. Two types of interactions were studied, hydrogen bonding and hydrophobic bonding by using LigPlot plus (version v.1.4.5). The PDB file of protein-ligand complex was given

to this software. This software automatically generated the schematic diagrams of the protein-ligand interaction and results could be visualized [142].

3.14 Absorption, Distribution, Metabolism and Excretion (ADME) Screening and Toxicity Analysis

Chemical compounds that could be used as ligands were selected from PubChem database. Selected compounds follow the Lipinski rule of five and could be used as active drug in humans. The potential success of a compound depends on its ADME screening and toxicity analysis. pkCSM https://omictools.com/pkcsm-tool is an online tool which was used to predict pharmacokinetic properties and percent human oral absorption values of the compounds. The server predicts different properties including log P, % human oral absorption in intestine and many other properties. For Lipinski score calculations, the ligand in SMILE format was uploaded to pkCSM. The physicochemical properties and Lipinski Rule of Five were also analyzed by pkCSM. The acceptability of the compounds was assessed using the Lipinski's rule of five, a crucial standard for oral absorption. The guidelines listed as follow:

1. The log P value of most "drug-like" molecules should be limited to 5 and molecular weight should be under 500.
2. Maximum number of H-bond acceptor should be 10 and maximum number of H-bond donor should be 5 [124].

3.15 Lead Compound Identification

After a detailed analysis of protein and ligand interactions, ADMET properties and docking score studies, the most active inhibitor was identified. The selected

compound was potential lead compound.

3.16 Reference Anti-bacterial Drug Identification and Selection

This step was performed for identification of drugs that are already being used for bacterial diseases treatment purpose. Drug Bank https://go.drugbank.com/database was used for drug identification because it helps to analyze the disease in detail along with its pathways [129].

3.17 Prediction of Different Parameters of Selected Drug

The identified drugs must be filtered in order to select the most effective drug. This was done through a detailed study of identified drugs and most effective drug was identified by setting parameters i.e., physicochemical properties, effective ADMET analysis, effective mechanism of action and minimal side effects using PubChem, Drug Bank and pkCSM databases respectively. The selected reference drug was then docked with target proteins to identify the inhibition efficiency. CB dock (Cavity-detection guided Blind Docking) an online docking server which was used to perform docking. It could simplify docking procedure and improve accuracy by predicting target protein binding sites [130].

3.18 Reference Drug and Lead Compound Comparison

The comparison between reference anti-bacterial drug and the proposed lead compound was done through comparing docking values, physicochemical properties

and ADME screening and toxicity analysis.

Hence it is concluded here, that virtual screening of the selected natural compounds of *Reynoutria japonica* and five targeted proteins of *Pseudomonas aeruginosa* was performed by using multiple computational approaches. Therefore, 3D structures of the bacterial proteins and selected natural compounds were downloaded from their respective databases. After downloading their structures, they were refined by removing extra constitutes attached with them. Molecular docking was performed to identify the potential inhibitors for *Pseudomonas aeruginosa*. For the purpose of further prioritization of the natural compounds absorption, distribution, metabolism, excretion (ADME) and toxicity parameters for the potential inhibitors were predicted and selected lead compound was then compared with reference anti-bacterial drug.

Chapter 4

Results and Discussions

Bacterial infections especially with multi-drug resistant *Pseudomonas aeruginosa* are hard to treat because of their high resistant profile and potential to form biofilm through quorum sensing. A growing list of infections such as pneumonia, nosocomial infections, inflammation and respiratory infections are becoming harder and impossible to treat while antibiotics become less effective. Currently, there are no effective drugs against these bacteria. The present study was undertaken to evaluate protein-ligand interactions of respective bacteria (*P. aeruginosa*) targeted proteins with natural compounds from a *Reynoutria japonica* in order to identify potential inhibitors of related bacteria by using multiple in -silico approaches. For the purpose of further prioritization of the natural compounds absorption, distribution, metabolism, excretion (ADME), and toxicity parameters for the potential inhibitors were predicted and selected lead compound was then compared with reference anti-bacterial drug. All of these steps are described below under headings sequentially.

4.1 *In silico* Protein Preparation

For *in silico* protein preparation following properties were required to identify such as primary sequence retrieval, physicochemical properties prediction, 3D structures

identification, functional domains identification and active sites identification of targeted proteins. .

4.1.1 Primary Sequence Retrieval

FASTA sequence of selected target proteins was retrieved through UniProt http://www.uniprot.org/. These proteins were selected on basis of their pathogenicity and virulence causing factors. The FASTA sequence of LasI, LasR, Rh1I, Rh1R and transcriptional regulator protein MvfR were downloaded from uniport under accession number A0A0M4BU37, A9JPX1, A9JPX5, A0A411F6T9 and A0A1C6ZJJ6 with 401,236,196, 238 and 292 residues length respectively. The primary sequence of the targeted proteins was shown in Figure 4.1 respectively.

>tr|A0A0M4BU37|A0A0M4BU37_PSEAI Acyl-homoserine-lactone synthase (Fragment) OS=Pseudomonas aeruginosa OX=287 GN=lasI PE=3 SV=1
KLLGEMHKLRAQVFKERKGWDVSVIDEMEIDGYDALSPYYMLIQEDTPEAQVFGCWRILD
TTGPYMLKNTFPELLHGKEAPCSPHIWELSRFAINSGQKGSLGFSDCTLEAMRALARYSL
QNDIQTLVTVTTVGVEKMMIRAGLDVSRFGPHLKIGIERAVALRIELNAKTQIALYGGVL
VEQRLAVS

>tr|A9JPX1|A9JPX1_PSEAI LasR protein OS=Pseudomonas aeruginosa OX=287 GN=lasR PE=3 SV=1
MALVDGFLELERSSGKLEWSAILQKMASDLGFSKILFGLLHKDSQDYENAFIVGNYPAAW
HEHYDRTGYARVDPTVSQCTQNVLPIFWEASIYQTRKQHEFFEEASAAGLVYGLTMPLHG
ARGELGALSLSVEAENRAEANRFMESVLPTLWMLKDYALQSGAGLAFEHPVSKPVVLTSR
EKEVLQWCAIGKTSWEISVICNCSEANVNFHMGNIRRKFGVTSRRVAAIMAVNLGLITL

>tr|A9JPX5|A9JPX5_PSEAI Acyl-homoserine-lactone synthase OS=Pseudomonas aeruginosa OX=287 GN=rhlI PE=3 SV=1
MIAELGRYRHQVFIEKLGWDVVSTSRVRDQEFDQFDHPQTRYIVAMGRQGICGCARLLPT
TDAYLLKEVFAYLCSETPPSDPSVWELSRYAASAADDPQLAMKIFWSSLQCAWYLGASSV
VAVTTTAMERYFVRNGVILQRLGPPQKVKGETLVAISFPAYQERGLEMLLPYHPEWLQGV
PLSMAV

>tr|A0A411F6T9|A0A411F6T9_PSEAI Regulatory protein RhlR (Fragment) OS=Pseudomonas aeruginosa OX=287 GN=rhlR PE=3 SV=1
RNDGGFLLWWDGLRSEMQPIHDSQGVFAVLEKEVRRLGFDYYAYGVRHTIPFTRPKTEVH
GTYPKAWLERYQMQNYGAVDPAILNGLRSSEMVVWSDSLFDQSRMLWNEARDWGLCVGAT
LPIRAPNNLLSVLSVARDQQNISSFEREEIRLRLRCMIELLTQKLTDLEHPMLMSNPVCL
SHREREILQWTADGKSSGEIAIILSISESTVNFHHKNIQKKFDAPNKTLAAAYAAALGLI

>tr|A0A1C6ZJJ6|A0A1C6ZJJ6_PSEAI LysR family transcriptional regulator OS=Pseudomonas aeruginosa OX=287 GN=mvfR PE=1 SV=1
MPIHNLNHVNMFLQVIASGSISSAARILRKSHTAVSSAVSNLEIDLCVELVRRDGYKVEP
TEQALRLIPYMRSLLNYQQLIGDIAFNLNKGPRNLRVLLDTAIPPSFCDTVSSVLLDDFN
MVSLIRTSPADSLATIKQDNAEIDIAITIDEELKISRFNQCVLGYTKAFVVAHPQHPLCN
ASLHSIASLANYRQISLGSRSGQHSNLLRPVSDKVLFVENFDDMLRLVEAGVGWGIAPHY
FVEERLRNGTLAVLSELYEPGGIDTKVYCYYNTALESERSFLRFLESARQRLRELGRQRF
DDAPAWQPSIVETVQRRSGPKALAYRQRAAPE

FIGURE 4.1: Sequence Retrieval

4.1.2 Physicochemical Characterization of Target Proteins

ProtParam is a tool of Expasy which is used online for the prediction of different parameters including both physical and chemical properties of selected proteins. These several parameters calculate and estimate the following through ProtParam: molecular weight, composition of amino acid, theoretical value of protein index, atomic composition of protein, extinction coefficient, estimated half-life of protein instability, aliphatic index and grand average of hydropathicity which was abbreviated as GRAVY. The physicochemical properties of the LasI, LasR, RhlI, RhlR and MvfR were shown in Table 4.1 respectively.

TABLE 4.1: Physicochemical Properties of Target Proteins.

Physico-chemical Properties	**Target proteins**				
	Las I	Las R	Rh1I	Rh1R	MvfR
MW	21088.45	26661.53	21010.22	27466.40	37241.51
PI	6.12	5.99	5.92	6.73	7.09
NR	23	26	19	28	36
PR	21	22	17	27	36
Ext.Co1	25565	43680	41160	49055	27390
Ext.Co2	25440	43430	41160	49055	27390
Instability Index	40.94	43.75	35.92	57.04	46.82
Aliphatic Index	98.03	91.05	88.60	91.46	101.05
GRAVY	-0.061	-0.030	-0.030	-0.287	-0.131

MW stands for molecular weight, pl for theoretical isoelectric point at which protein is neutral, without any charge), NR for total number of negatively charged residues (Asp + Glu), PR for total number of positively charged residues (Arg

+Lys), Ext.Co1 for extinction coefficients when assuming all pairs of Cyst residues form cystines, Ext. Co2 for extinction coefficients when assuming all Cyst residues are reduced and GRAVY for grand average of hydropathicity. All these parameters which were selected for this research work were taken according to previous research work [131].

The calculated PI greater than 7 represents the basic nature of the protein while less than 7 shows acidic nature of protein. PI value of MvfR was 7.09 so this protein is basic in nature while other targeted proteins (LasI, LasR, RhlI, RhlR) have shown less PI value so they are acidic basic in nature. Extinction coefficient represents light absorption. Instability index if less than 40 show stability of the protein while greater than 40 indicates the instability of protein [132]. Instability index of RhlI was less than 40. The aliphatic index represents the aliphatic content of a protein. The high value of the aliphatic index indicates the thermostability of the protein. MvfR has highest aliphatic index value among others. Molecular weight contains both positive and negative charged residues of protein. Low GRAVY shows better interaction with water molecules. LasR, RhlI showed lower GRAVY values among others.

4.1.3 Protein Structure Retrieval

3D Structures of targeted proteins LasI, LasR, RhlI, RhlR and MvfR were downloaded from RCSB PDB in PDB format. Protein Data Bank is a three-dimensional database of complex molecules of living organisms like proteins and nucleic acids [133]. Alphafold could be used if some structures were missing on PDB database. Alphafold `https://alphafold.com/` is also a protein structure database used for 3D structure prediction of proteins [120]. The 3D structures of LasI and MvfR proteins were taken from RCSB PDB in PDB format under 1RO5 and 6B8A IDs respectively. The 3D structures of LasR, Rh1I and Rh1R proteins were taken from Alphafold in PDB format under P25084, P54291 and P54292 IDs respectively. The protein structures were prepared in PyMOL by removing water molecules and extra ligands if existed. After the removal of ligands and other atoms the missing

polar hydrogens were added. This step was performed to get the stable conformation by preventing overlaps and saved the modified file in PDB format.

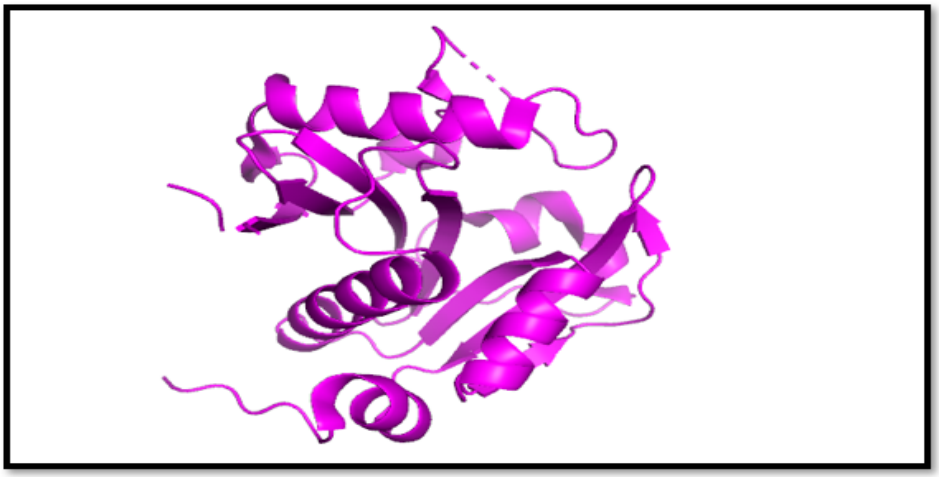


FIGURE 4.2: 3D Structure of LasI Protein

Above figure 4.2 represented three-dimensional structure of LasI protein which is part of AHL based quorum sensing system of *Pseudomonas aeruginosa*. It is an autoinducer molecule that is required to bind with transcriptional activator LasR and leads to the upregulation of some pathogenic and virulent factors.

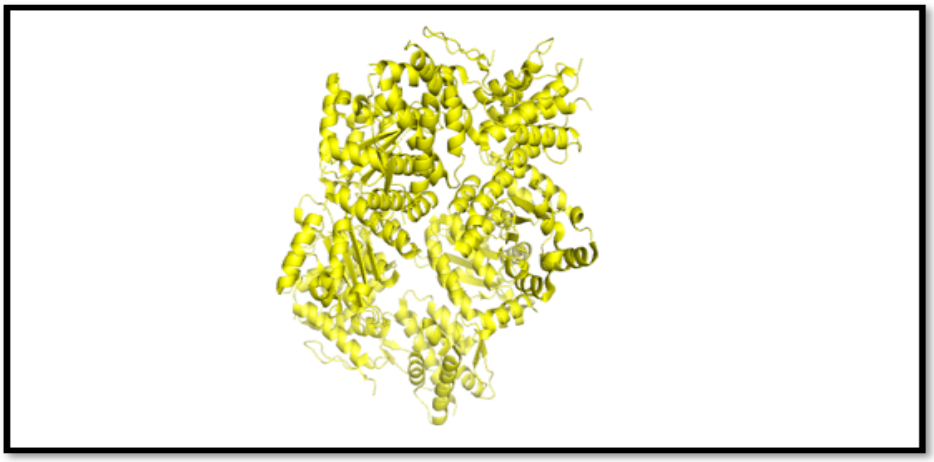


FIGURE 4.3: 3D Structure of LasR Protein

Above figure 4.3 represented three-dimensional structure of transcriptional activator protein LasR which is also part of AHL based quorum sensing system of *Pseudomonas aeruginosa*. The regulation of virulence factor gene expression is controlled by this protein.

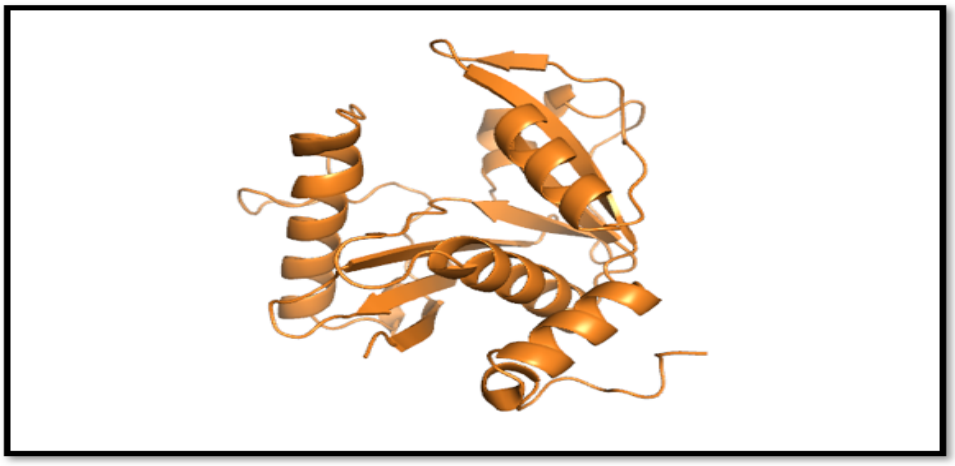


FIGURE 4.4: 3D Structure of RhlI Protein

Above figure 4.4 represented the three-dimensional structure of RhlI Protein, which is also a part of AHL based quorum sensing system of *Pseudomonas aeruginosa*. It is an autoinducer molecule that is required to bind with transcriptional activator RhlR and leads to upregulation of some pathogenic and virulent factors.

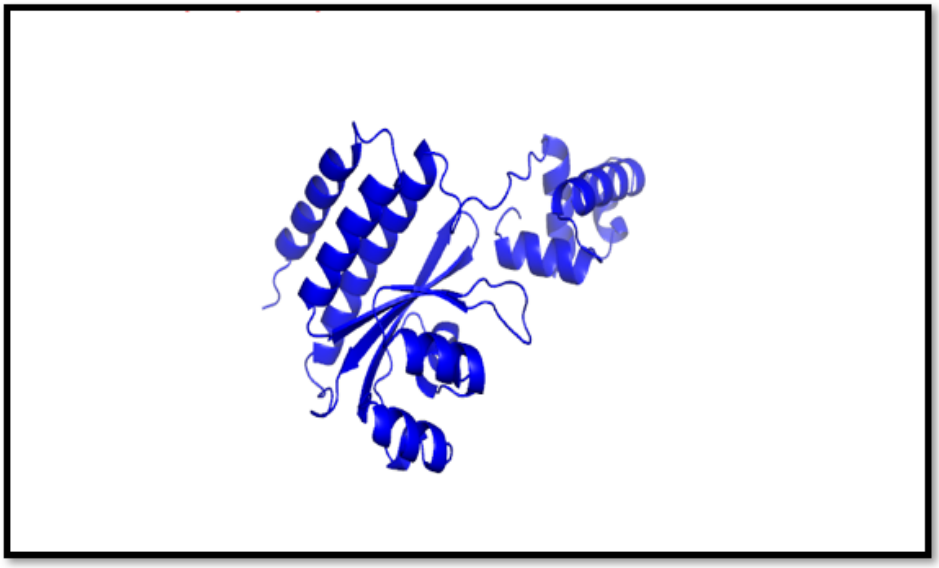


FIGURE 4.5: 3D Structure of RhlR Protein

Above figure 4.5 represented the three-dimensional structure of transcriptional activator protein RhlR which is also a part of AHL based quorum sensing system of *Pseudomonas aeruginosa*. The regulation of virulence factor gene expression is controlled by this protein.

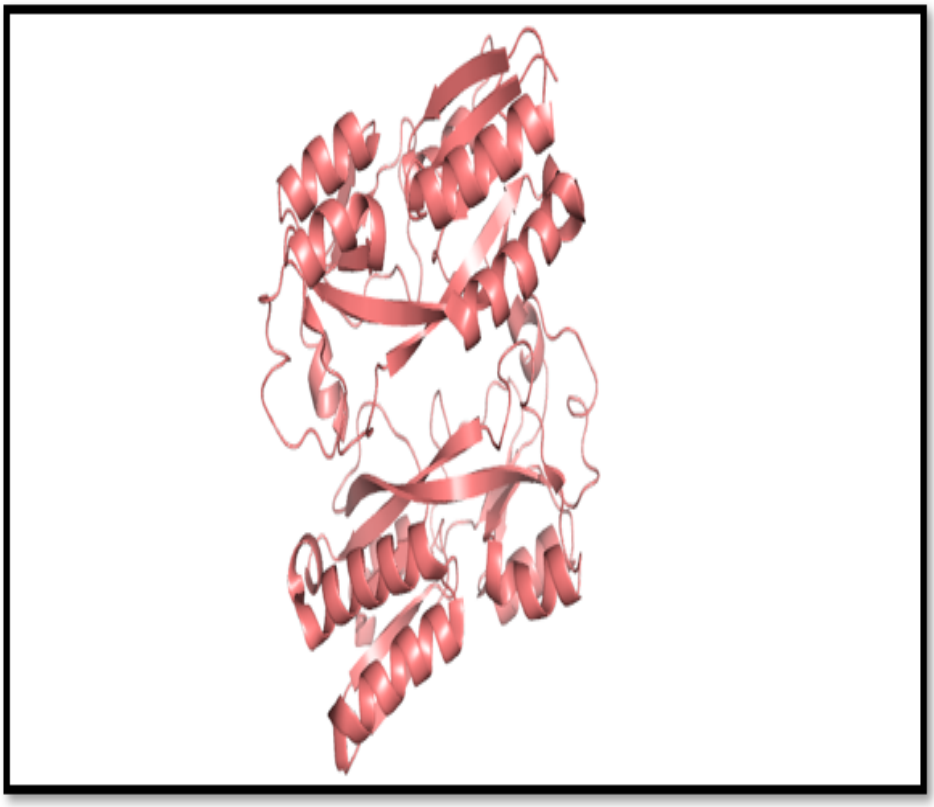


FIGURE 4.6: 3D Structure of MvfR Protein

Above figure 4.6 represented the three-dimensional structure of transcriptional regulator MvfR which is part of PQS based quorum sensing system of *Pseudomonas aeruginosa* and required for its *Pseudomonas quinolone* signal production and its virulence factors expression.

4.1.4 Protein Functional Domains Identification

Database InterPro was used to identify the domains and functional sites of selected proteins. InterPro is a resource for functional analysis of protein sequences. Conserved domains are involved in sequence/structure/relationship of proteins. Proteins can have more than one functional domain that perform different functions. Functional domain is the active part of a protein that is involved in interactions of proteins with other substances. All these parameters which were selected for this research work were taken according to previous research work [134]. Functional domains of the target proteins were shown in figure 4.7, 4.8, 4.9, 4.10 and 4.11 .

4.1.4.1 Functional Domain Identification of LasI

LasI protein is 401aa long consisting of single domain which is Acyl-CoA-acyltransferase domain starting from residue 3 and ending at 179 and its function as sugar and fat metabolism.

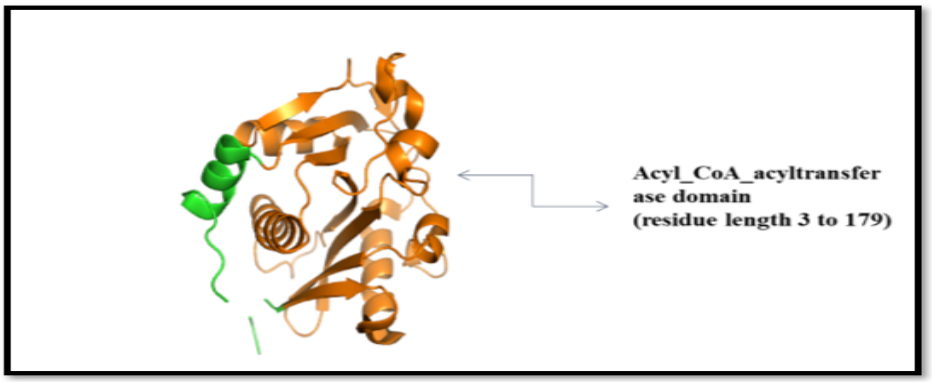


FIGURE 4.7: Functional domain of LasI protein with residues length

Above figure 4.7 represented the domain of LasI protein. This is 401aa long protein consists of single domain. Orange color showing Acyl-CoA-acyltransferase domain starting from residue 3 and ending at 179.

4.1.4.2 Functional Domains Identification of LasR

LasR protein is 236aa long consists of two domains called Tscrpt-reg-LuxR-C domain which is DNA binding domain, starting at residue 170 and ending at 235 and another domain is TF-LuxR-autoind-bd-dom which is signals binding domain starting at residue 18 and ending at 160.

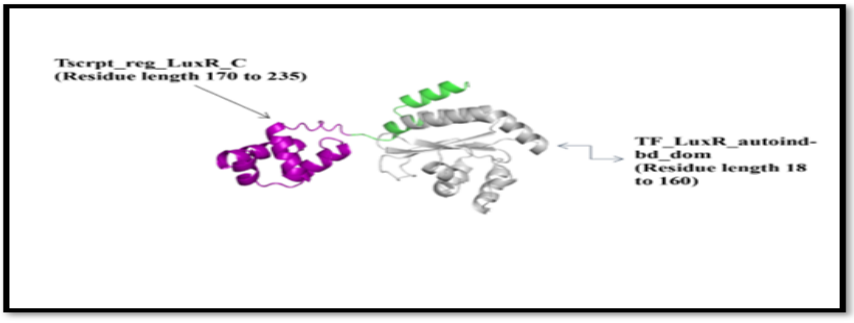


FIGURE 4.8: Functional domains of LasR protein with residues length

Above figure 4.8 represented the domains of LasR protein. This is 236aa long protein consisting of two domains. Gray color showing TF-LuxR-autoind-bd-dom starting from residue 18 and ending at 160 while the purple color showing Tscrpt-reg-LuxR-C, domain starting at residue 170 and ending at 235.

4.1.4.3 Functional Domain Identification of RhlI

RhlI protein with sequence of 196aa long consists of single domain called Acyl-CoA-acyltransferase domain starting at residue 2 and ending at 162.

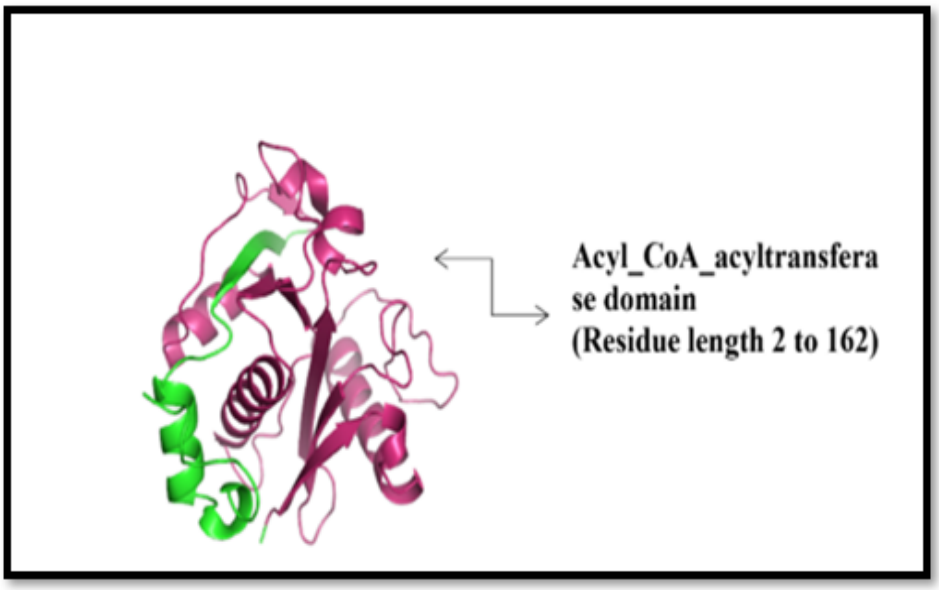


FIGURE 4.9: Functional domain of RhlI protein with residues length

Above figure 4.9 represented the domain of RhlI protein. This is 196aa long protein consists of single domain. Pink color showing Acyl-CoA-acyltransferase domain starting from residue 2 and ending at 162.

4.1.4.4 Functional Domains Identification of RhlR

RhlR protein with sequence of 238aa long consists of two domains one is TF-LuxR-autoind-bd-om starting at residue 25 and ending at 163 and other is Tscrpt-reg-LuxR-C starting at residue 173 and ending at 238.

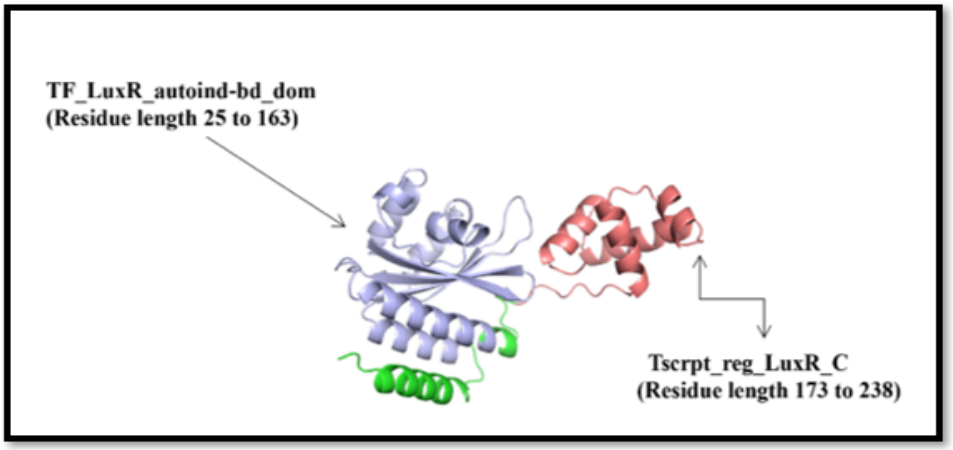


FIGURE 4.10: Functional domains of RhlR protein with residues length

Above figure 4.10 represented the domains of RhlR protein. This is 238aa long protein consisting of two domains. Red color showing Tscrpt-reg-LuxR-C domain starting from residue 173 and ending at 238 while the blue-magenta color showing domain called TF-LuxR-autoind-bd-dom starting at residue 25 and ending at 163.

4.1.4.5 Functional Domains Identification of MvfR

Transcriptional regulator protein MvfR with sequence of 292aa consists of single domain which is LysR-subst-bd starting from residue 139 and ending at 292

FIGURE 4.11: Functional domain of MvfR protein with residues length

Above figure 4.11 represented that transcriptional regulator protein MvfR with sequence of 292aa consists of single domain which is LysR-subst-bd showing in color blue, starting from residue 139 and ending at 292.

4.1.5 Identification of Binding sites and Favorable Ligand Binding Moieties

The active sites present on a protein's surface that carry out a its functions are known as protein-ligand binding sites. Determining these binding sites is often frequently important in researching protein functions and structure-based drug discovery. There are many computational tools developed in recent decades, So CASTp an online tool proved that it combines the results of many methods and improve its prediction results [135]. To identify active sites of proteins LasI, LasR, RhlI, RhlR and MvfR, CASTp software was used which predicted available pockets for ligand binding and also give information about surface area and volume of pockets [136]. Tables 4.2, 4.3, 4.4,4.5 and 4.6 illustrated the surface area and volume of LasI, LasR, Rh1I, Rh1R and MvfR binding pockets respectively.

4.1.5.1 Identification of Druggable cavities on LasI Protein Surface

The identified area and volume of binding pockets of LasI was shown in Table 4.2 and protein surface structure of available pockets for ligand binding was shown in Figure 4.12 respectively.

TABLE 4.2: Area and volume of binding pockets of LasI obtained by CASTp.

Pocket ID	Area (SA)	Volume (SA)
1	712.477	532.707
2	56.618	39.791
3	54.192	26.239
4	35.487	13.910
5	15.007	3.003

TABLE 4.2: Area and volume of binding pockets of LasI obtained by CASTp.

Pocket ID	**Area (SA)**	**Volume (SA)**
6	10.642	1.195
7	6.233	0.853
8	2.974	0.373
9	4.030	0.316
10	3.612	0.241
11	2.974	0.165
12	3.205	0.146
13	1.622	0.142
14	1.543	0.116
15	0.725	0.095
16	0.880	0.069
17	0.900	0.057
18	0.936	0.042
19	0.817	0.034
20	0.811	0.019
21	0.522	0.018
22	0.301	0.008
23	0.071	0.001
24	0.060	0.000

Above table 4.2 represented binding pocket IDs with area and volume of transcription regulator protein LasI along with surface area and volume. It showed that there were twenty-four pockets available for protein LasI. The largest binding pocket has surface area 712.477 whereas its volume was 532.707 while the smallest binding pocket has surface area 0.060 and its volume was 0.000. Below figure 4.12 represented transcription regulator protein LasI structure. Red color showed the available ligand-binding pockets on protein surface. Binding pocket is the region where ligand can bind. The number of pockets with size and volume was already shown in above Table 4.2.

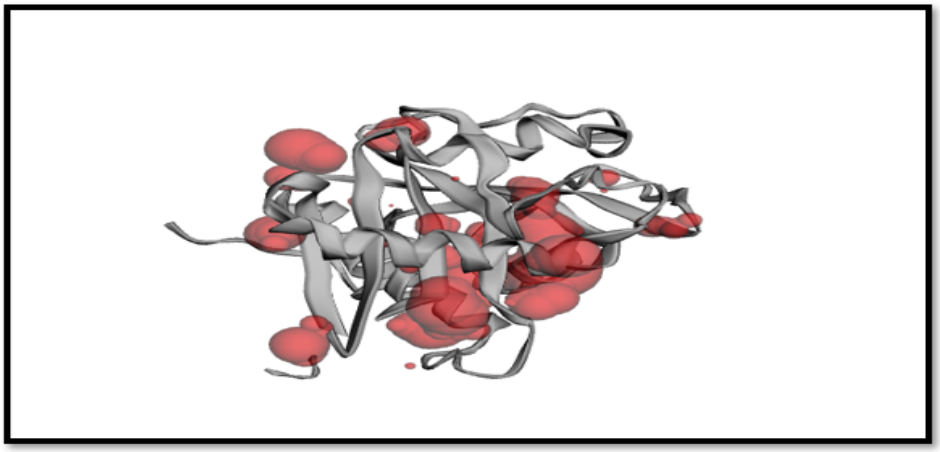


FIGURE 4.12: Structure of LasI protein showing available pockets for ligands

4.1.5.2 Identification of Druggable cavities on LasR Protein Surface

The identified area and volume of binding pockets of LasR was shown in Table 4.3 and protein surface structure of available pockets for ligand binding was shown in Figure 4.13 respectively.

TABLE 4.3: Area and volume of binding pockets of LasR obtained by CASTp

Pocket ID	Area (SA)	Volume (SA)
1	2523.01	3330.72
2	2447.49	3218.23
3	1742.68	2236.07
4	480.833	498.316
5	460.043	462.459
6	387.515	333.671
7	558.739	279.687
8	277.033	194.574
9	191.541	155.615
10	281.661	146.919
11	267.007	135.924
12	145.738	126.151
13	270.061	121.247

TABLE 4.3: Area and volume of binding pockets of LasR obtained by CASTp

Pocket ID	**Area (SA)**	**Volume (SA)**
14	244.725	117.305
15	216.194	115.799
16	204.738	93.343
17	150.997	87.99
18	173.349	75.895
19	64.593	62.497
20	53.221	54.242
21	91.639	48.566
22	60.637	35.473
23	31.997	30.685
24	94.625	24.86
25	20.219	3.273
26	2.145	0.131
27	0.015	0

Above table 4.3 represented binding pocket IDs with area and volume of transcription regulator protein LasR along with surface area and volume. It showed that there were twenty-seven pockets available for protein LasR. The largest binding pocket has surface area 2523.009 whereas its volume was 3330.724 while the smallest binding pocket has surface area 0.015 and its volume was 0.000.

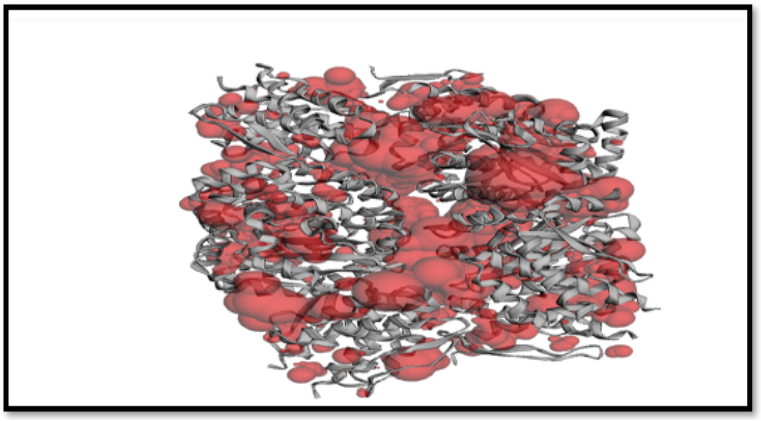


FIGURE 4.13: Structure of LasR protein showing available pockets for ligands

Above figure 4.13 represented the transcription regulator protein LasR structure. Red color showed the available ligand-binding pockets on protein surface. Binding pocket is the region where ligand can bind. The number of pockets with size and volume was already shown in above Table 4.3.

4.1.5.3 Identification of Druggable cavities on RhlI Protein Surface

The identified area and volume of binding pockets of RhlI was shown in Table 4.4 and protein surface structure of available pockets for ligand binding was shown in Figure 4.14 respectively.

TABLE 4.4: Area and volume of binding pockets of RhlI obtained by CASTp

Pocket ID	**Area (SA)**	**Volume (SA)**
1	11857.717	7222.781
2	5.838	8.116
3	6.924	3.160
4	1.016	0.360
5	0.513	0.260
6	1.201	0.234
7	0.311	0.020
8	0.077	0.001
9	0.016	0.000

Above table 4.4 represented binding pocket IDs with area and volume of transcription regulator protein RhlI along with surface area and volume. It showed that there were nine pockets available for protein RhlI. The largest binding pocket has surface area 11857.717 whereas its volume was 7222.781 while the smallest binding pocket has surface area 0.016 and its volume was 0.000. Below figure 4.14 represented transcription regulator protein RhlI structure. Red color showed the available ligand-binding pockets on protein surface. Binding pocket is the region where ligand can bind. The number of pockets with size and volume was already shown in above Table 4.4.

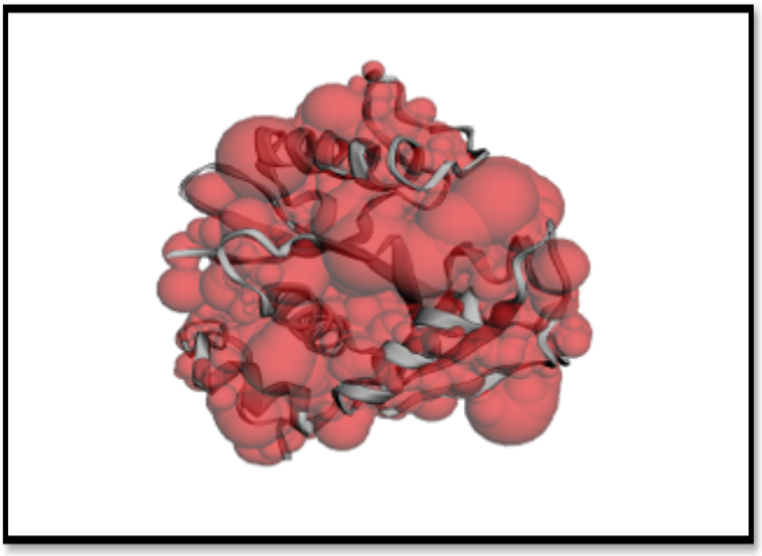


FIGURE 4.14: Structure of RhlI protein showing available pockets for ligands

4.1.5.4 Identification of Druggable cavities on RhlR Protein Surface

The identified area and volume of binding pockets of RhlR was shown in Table 4.5 and protein surface structure of available pockets for ligand binding was shown in Figure 4.15 respectively.

TABLE 4.5: Area and volume of binding pockets of RhlR obtained by CASTp

Pocket ID	**Area (SA)**	**Volume (SA)**
1	13778.524	7762.963
2	3.311	2.178
3	2.300	0.867
4	1.269	0.555
5	0.723	0.230
6	1.015	0.166
7	0.928	0.109
8	0.621	0.055
9	0.397	0.046
10	0.341	0.009
11	0.142	0.003

TABLE 4.5: Area and volume of binding pockets of RhlR obtained by CASTp

Pocket ID	**Area (SA)**	**Volume (SA)**
12	0.051	0.000
13	0.003	0.000

Above table 4.5 showed binding pocket IDs with area and volume of transcription regulator protein RhlR along with surface area and volume. It showed that there were thirteen pockets available for protein RhlR. The largest binding pocket has surface area 13778.524 whereas its volume was 7762.963 while the smallest binding pocket has surface area 0.003 and its volume was 0.000.

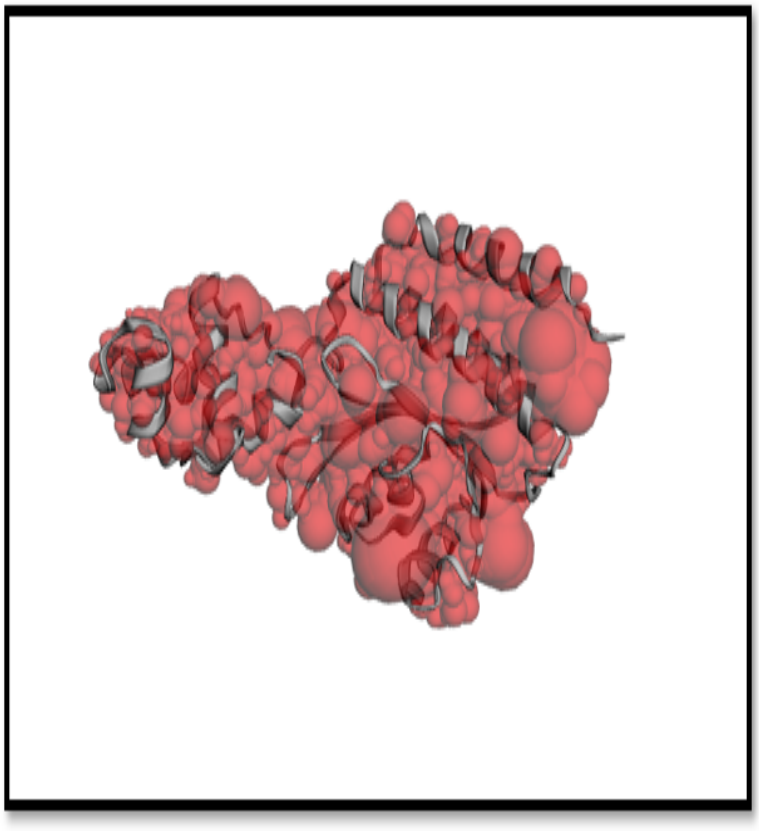


FIGURE 4.15: Structure of RhlR protein showing available pockets for ligands

Above figure 4.15 represented transcription regulator protein RhlR structure. Red color showed the available ligand-binding pockets on protein surface. Binding pocket is the region where ligand can bind. The number of pockets with size and volume is already shown in above Table 4.5.

4.1.5.5 Identification of Druggable cavities on MvfR Protein Surface

The identified area and volume of binding pockets of MvfR was shown in Table 4.6 and protein surface structure of available pockets for ligand binding was shown in Figure 4.16 respectively.

TABLE 4.6: Area and volume of binding pockets of MvfR obtained by CASTp

Pocket ID	Area (SA)	Volume (SA)
1	26406.279	18508.494
2	1.387	6.860
3	13.697	4.679
4	5.235	3.439
5	1.290	0.816
6	0.225	0.020
7	0.138	0.004
8	0.000	0.000

Above table 4.6 represented binding pocket IDs with area and volume of transcription regulator protein MvfR along with surface area and volume. It showed that there were eight pockets available for protein MvfR. The largest binding pocket has surface area 26406.279 whereas its volume was 18508.494 while the smallest binding pocket has surface area 0.000 and its volume was 0.000.

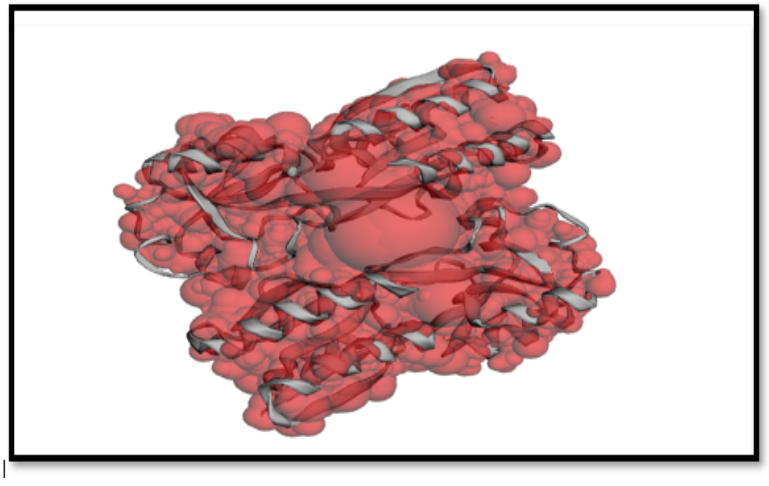


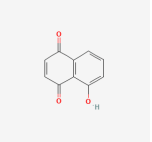
FIGURE 4.16: Structure of MvfR protein showing available pockets for ligands

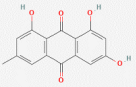
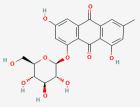
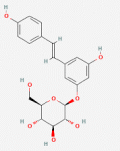
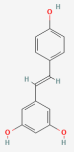
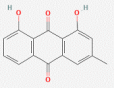
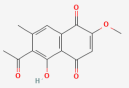
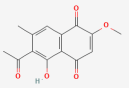
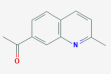
Above figure 4.16 represented transcription regulator protein MvfR structure. Red color showed the available ligand-binding pockets on protein surface. Binding pocket is the region where ligand can bind. The number of pockets with size and volume was already shown in above Table 4.6.

4.2 Ligands Selection

Protein data bank contains a large amount of protein ligand complex, especially for the protein target. Therefore, the selection of ligands is based on the best resolution of the structure, the chemical class of the co-crystal ligand bound to the protein structure and gives the best binding affinity. Conformational selection is a process in which ligand selectively binds to one of these conformers, strengthening it and increasing its population with respect to the total population of the protein ultimately resulting in the observed complex [137]. Ligands were searched out from the chemical information database PubChem https://pubchem.ncbi.nlm.nih.gov. From this database, the information can be accessed freely around the globe. Their 3D structures were downloaded from PubChem in SDF format. After selection of ligands, energy minimization was carried out by ChemDraw Pro software (chem 3D v 12.0.2) [138]. This was a mandatory step in the preparation of ligands for docking because unstable ligands will show unreliable vina scores in docking results. Bioactive anti-virulence compounds of *Reynoutria japonica* were selected as ligands for the present study. Selected ligands with molecular formula, molecular weight and chemical structure were represented in Table 4.7.

TABLE 4.7: Ligands and their related properties

S. No	Ligands Name	Molecular formula	Molecular weight	Structure
1	Juglone	$C_{10}H_6O_3$	174.155 g/mol	

2	Emodin	$C_{15}H_{10}O_5$	270.24 g/mol	
3	Emodin-8-o-b-glucoside	$C_{21}H_{20}O_{10}$	432.4 g/mol	
4	Polydatin	$C_{20}H_{22}O_8$	390.4 g/mol	
5	Resveratrol	$C_{14}H_{12}O_3$	228.24 g/mol	
6	Physcion	$C_{16}H_{12}O_5$	284.267 g/mol	
7	Chrysophanol	$C_{15}H_{10}O_4$	254.241 g/mol	
8	2-Methoxy-stypandrone	$C_{14}H_{12}O_5$	260.245 g/mol	
9	2-ethoxy-6-acetyl-7-methyl-juglone	$C_{14}H_{12}O_5$	260.245 g/mol	
10	2-methyl-7-acetyl-quinoline	$C_{12}H_{11}NO$	185.226 g/mol	

The selected ligands were Juglone, Emodin, Emodin 8-o-b glucoside, Polydatin, Resveratrol, Physcion, Chrysophanol, 2-Methoxy-6-acetyl-7-methyl-juglone, 2 -Methyl-7-acetyl- quinoline, 2- Ethoxy -6-acetyl-7-methyl-juglone. The ligand selection is based upon Lipinski rule of five. The Lipinski rule deals with certain

parameters like Molecular weight which should be less than or equal to 500, log P less than or equal to 5, H-bond donors less than or equal to 5, H-bond acceptors less than or equal to 10. These rules are to be followed by orally active compounds. The drug-like is dependent on the mode of administration. A compound is considered a drug when it follows 3 or more rules and if a compound violates two or more rules it is considered poorly absorbed [139].

4.3 Virtual Screening and Molecular Docking

Molecular Docking is technique used to estimate the strength of a bond between a ligand and a target protein through a special scoring function and to determine the correct structure of the ligand within the target binding site. The 3D structure of the target proteins and ligands is taken as the input for docking. It represents a frequently used approach in structure-based drug designing [140]. It can also be used to determine the correct structure of the ligand within the target binding site and to estimate the strength of the binding between the ligand and the target proteins through a specific scoring function. It also helps in the recognition of new small molecular compounds, revealing the essential properties such as high interaction between binding with target protein having reasonable absorption, distribution, metabolism and excretion which help in the selection of lead compound for the target [141].

The docking was performed using transcription regulator proteins such as LasI/R, Rh1I/R and MvfR and ligands were Juglone, Emodin, Emodin 8-o-b glucoside, Polydatin, Resveratrol, Physcion, Chrysophanol, 2-Methoxy-6-acetyl-7- methyl-juglone, 2-Ethoxy-6-acetyl-7-methyl-juglone and 2-Methyl-7-acetyl- quinoline. Virtual screening of targeted proteins against ligands was done by using CB Dock. It is user-friendly blind docking web server, was used to automatically anticipate binding modes in the absence of binding site information's which predicts and estimates a binding site for a given protein and calculate centers and sizes with a novel rotation cavity detection method and perform docking with the popular

docking program named Auto dock Vina [142]. CB dock generates five best interacting confirmations for each ligand molecule. These confirmations are arranged based on binding affinity and then finest confirmation selection is done on the basis of highest affinity score of protein-ligand interaction. Ligands with best binding score values with target proteins were represented in Table 4.8 and 4.9.

TABLE 4.8: Results of CB dock

Properties	**Juglone**	**2-Methoxy stypan drone**	**Resveratrol**	**Polydatin**	**Physcion**
Binding score	-9.0	-9.5	-8.7	-9.5	-9.9
Cavity size	1598	1598	1598	1598	1598
Grid Map	21	19	21	24	20
Minimum Energy (Kcal/mol)	0.00	0.00	0.00	0.00	0.00
Maximum Energy (Kcal/mol)	1.6E+00	1.6E+00	1.6E+00	1.6E+00	1.6E+00

TABLE 4.9: Results of CB dock

Properties	**Chryso -phanol**	**Emodin**	**Emodin-8-o-b-glucoside**	**2-ethoxy-6-acetyl-7-methyl-juglone**	**2-methyl-7-acetyl -quinoline**
Binding score	-10.1	-9.6	-10.1	-9.5	-8.9

TABLE 4.9: Results of CB dock

Properties	**Chryso -phanol**	**Emodin**	**Emodin-8-o-b-glucoside**	**2-ethoxy-6-acetyl-7-methyl-juglone**	**2-methyl-7-acetyl -quinoline**
Cavity size	1598	1598	1598	1598	1598
Grid Map	19	19	22	19	18
Minimum Energy (Kcal/mol)	0.00	0.00	0.00	0.00	0.00
Maximum Energy (Kcal/mol)	1.6E+00	1.6E+00	1.6E+00	1.6E+00	1.6E+00

After docking process, the docked poses were listed based on the corresponding docking scores the dock structures were then selected for further analysis. The 3D conformation ligand receptor was analyzed using PyMOL. On the basis of docking score, cavity size, Grid map, binding energy one can select best docked structure. After detailed analysis on docking scores three best scoring phytocompounds namely as 2-Methoxy-6-acetyl-7-methyljuglone (2-Methoxystypandrone), Chrysophanol and Physcion were identified as hit compounds and their binding score with respective bacterial proteins was following as -9.5, -10.1 and -9.9. Chrysophanol gave the highest binding score among them which was -10.1.

4.4 Ligand Protein Interaction

A ligand protein interaction study was done for validated protein structures. The interaction of the active pockets of the ligand and the protein are calculated for

the interpretation of docking results. Two types of interactions were studied, hydrogen bonding and hydrophobic bonding interaction. Using LigPlot plus (version v.1.4.5) the protein ligand interactions were studied [142]. By using LigPlot plus the interaction of active confirmation of ligands and the target protein has been identified. The saved conformations for ligand receptor complex of each molecule were analyzed in detail. This software automatically generates schematic diagrams of the protein-ligand interactions of the given docked complex in the PDB file. The docked files were uploaded in PDB format to get hydrogen and hydrophobic interactions. A significant number of hydrophobic and hydrogen bond interactions were observed between the ten ligands and the five target proteins. Ligand-receptor complex shows strong hydrogen bonding and hydrophobic interactions [143].Following diagrams showed the ligand-receptor interactions while Table **??** summarized the results of all these interactions.

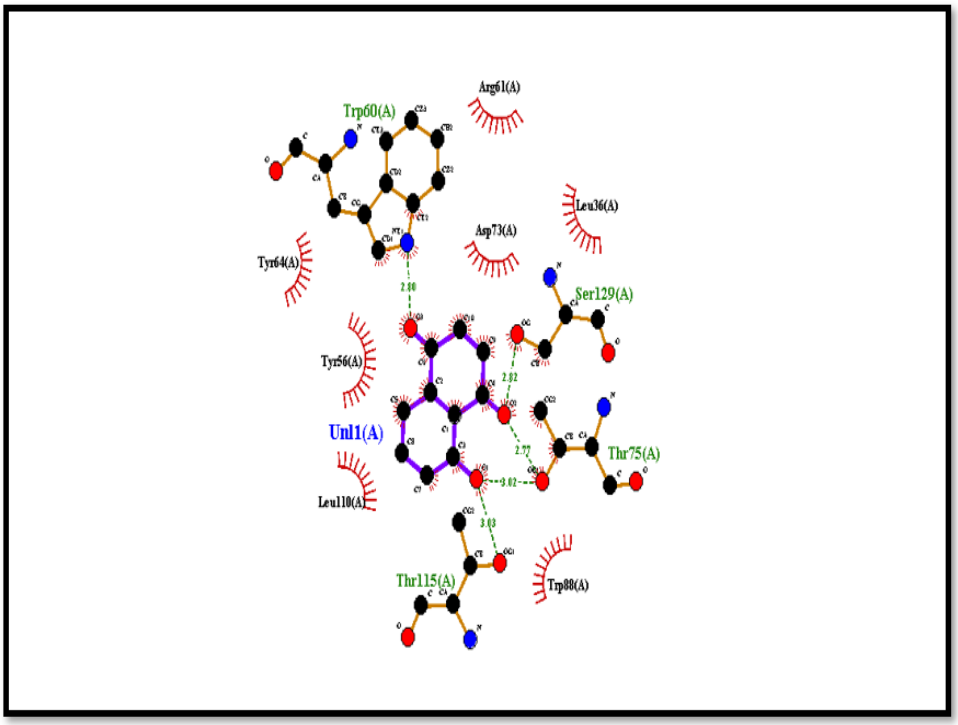


FIGURE 4.17: Interactions of 5-Hydroxy-1,4-naphthoquinone or Juglone by LigPlot

Figure 4.17 represented the interactions of juglone with transcriptional regulator protein. Seven hydrophobic residues and four intermolecular hydrogen bonds showed close contact with them.

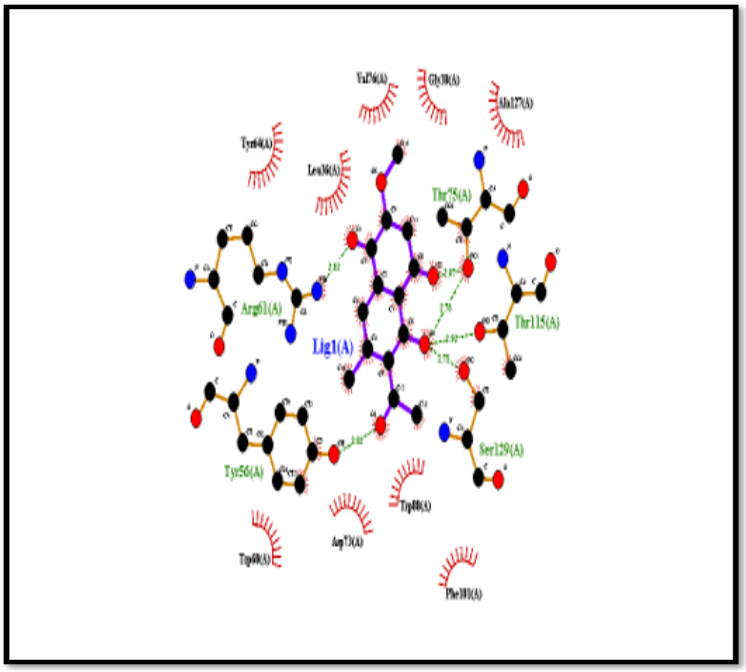


FIGURE 4.18: Interactions of 2-Methoxy-6-acetyl-7-methyljuglone by LigPlot

Figure 4.18 represented the interactions of 2-Methoxy-6-acetyl-7-methyljuglone with receptor protein. It showed that 2-Methoxy-6-acetyl-7-methyljuglone has formed nine hydrophobic interactions and five hydrogen bonds with it.

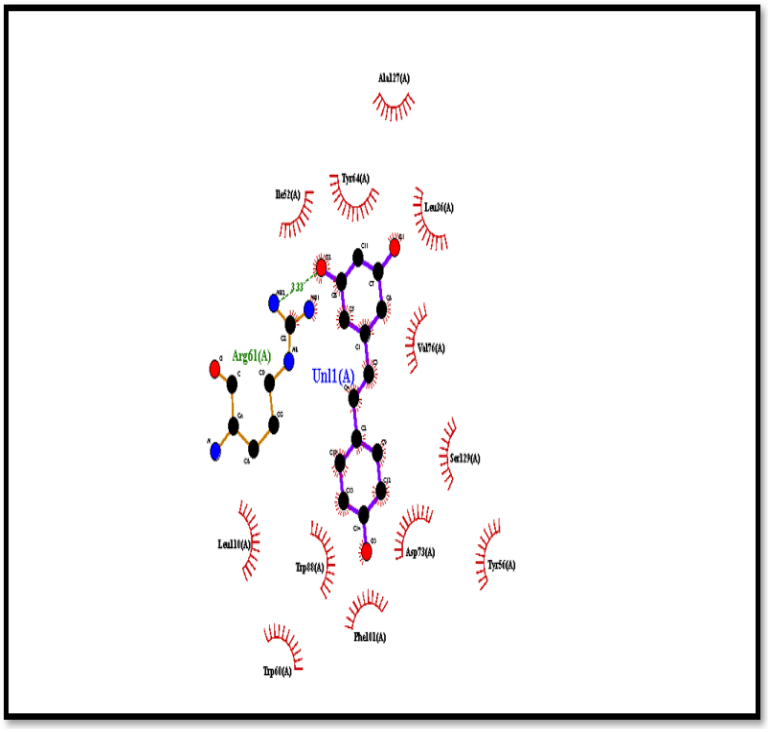


FIGURE 4.19: Interactions of Resveratrol by LigPlot

Figure 4.19 represented the interactions of Resveratrol with transcriptional regulator protein. Twelve hydrophobic residues and one intermolecular hydrogen bond showed close contact with them.

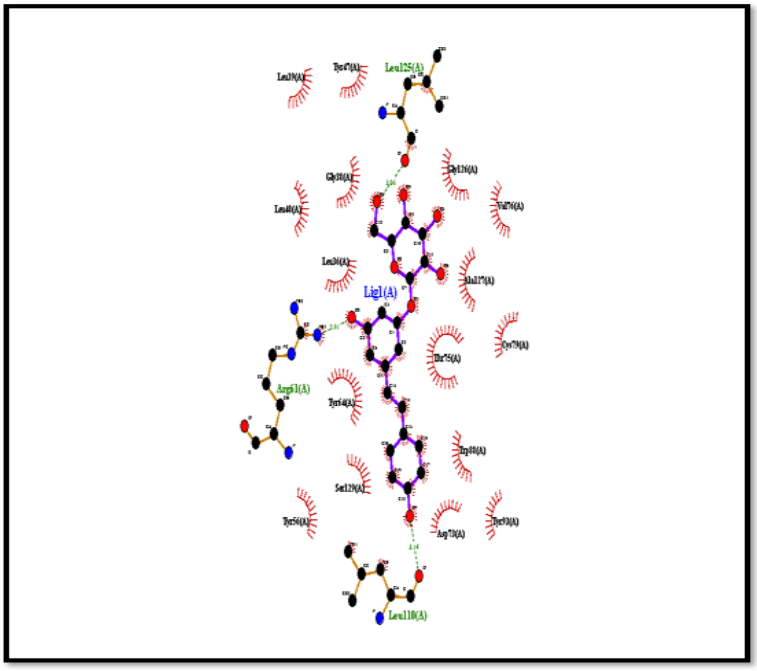


FIGURE 4.20: Interactions of Polydatin by LigPlot

Figure 4.20 represented the interactions of Polydatin with receptor protein. It showed that Polydatin has formed sixteen hydrophobic interactions and three hydrogen bonds with it.

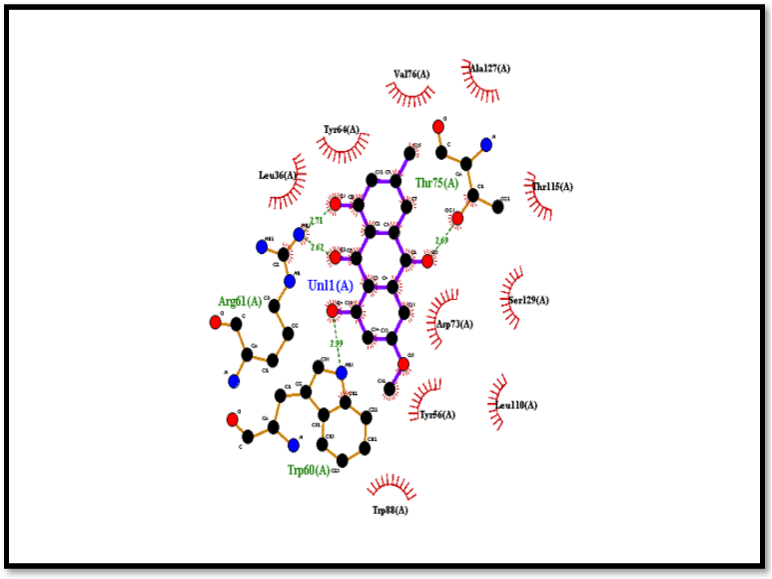


FIGURE 4.21: Interactions of Physcion by LigPlot

Figure 4.21 represented the interactions of Physcion with transcriptional regulator protein. Ten hydrophobic residues and three intermolecular hydrogen bonds showed close contact with them.

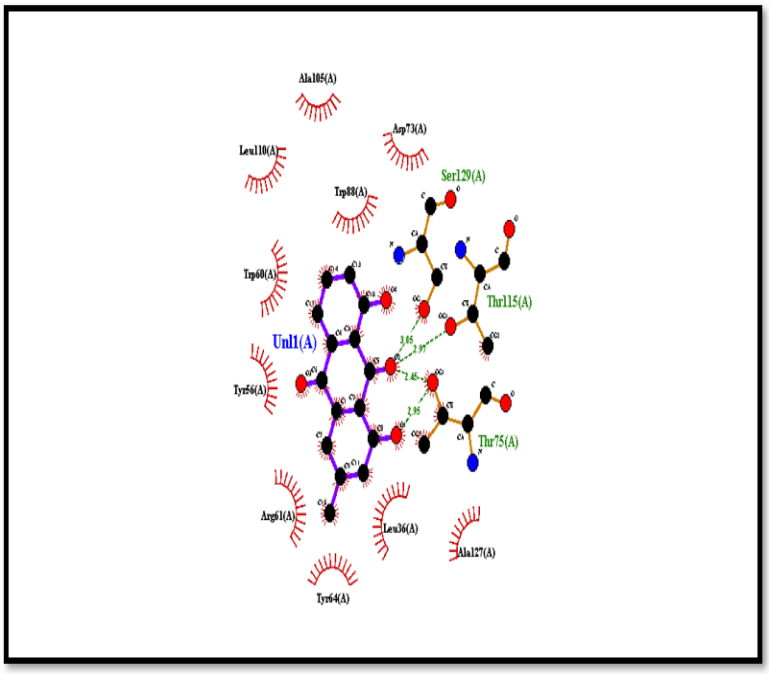


FIGURE 4.22: Interactions of Chrysophanol by LigPlot

Figure 4.22 represented the interactions of Chrysophanol with transcriptional regulator protein. Ten hydrophobic residues and three intermolecular hydrogen bonds showed close contact with them.

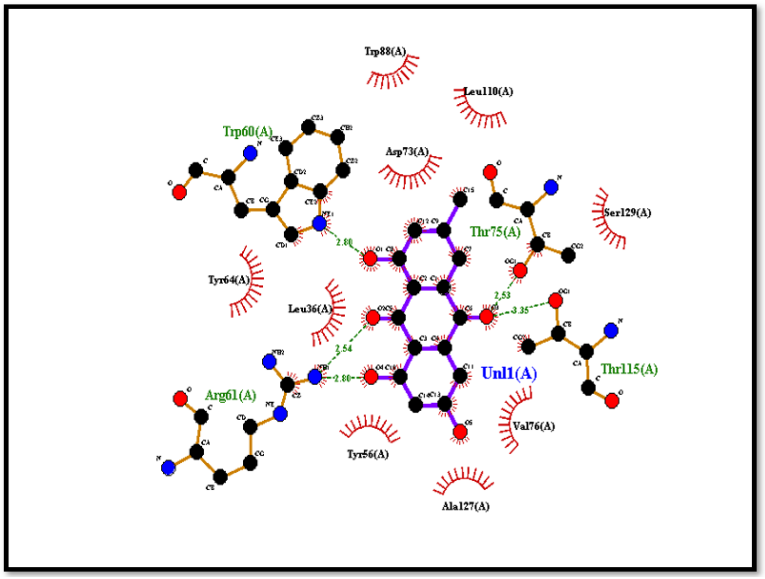


FIGURE 4.23: Interactions of Emodin by LigPlot

Figure 4.23 represented the interactions of Emodin with transcriptional regulator protein. Nine hydrophobic residues and four intermolecular hydrogen bonds showed close contact with them.

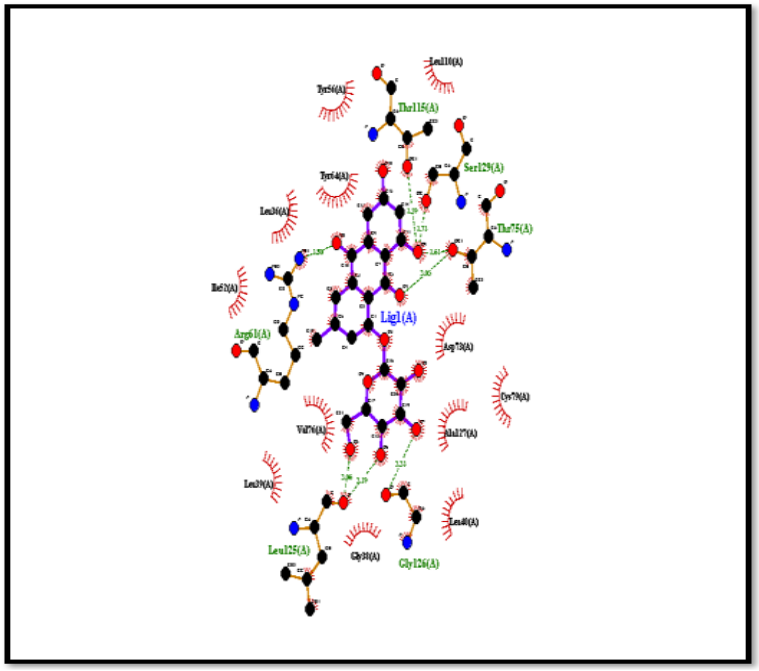


FIGURE 4.24: Interactions of Emodin-8-o-b-glucoside by LigPlot

Figure 4.24 represented the interactions of Emodin-8-o-b-glucoside with receptor protein. It showed that Emodin-8-o-b-glucoside has formed nine hydrophobic interactions and six hydrogen bonds with it.

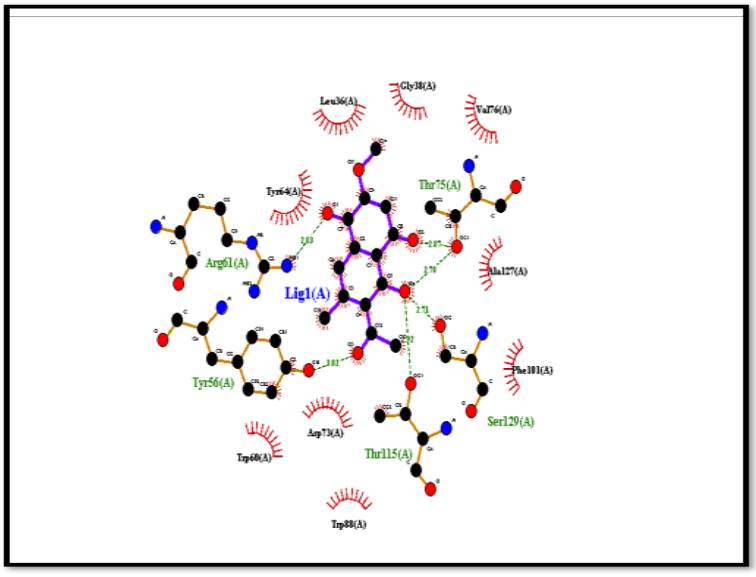


FIGURE 4.25: Interactions of 2-Ethoxy-6-acetyl-7-methyl-juglone by LigPlot

Figure 4.25 represented the interactions of 2-Ethoxy-6-acetyl-7-methyl-juglone with receptor protein. It showed that 2-Ethoxy-6-acetyl-7-methyl-juglone has formed nine hydrophobic interactions and five hydrogen bonds with it.

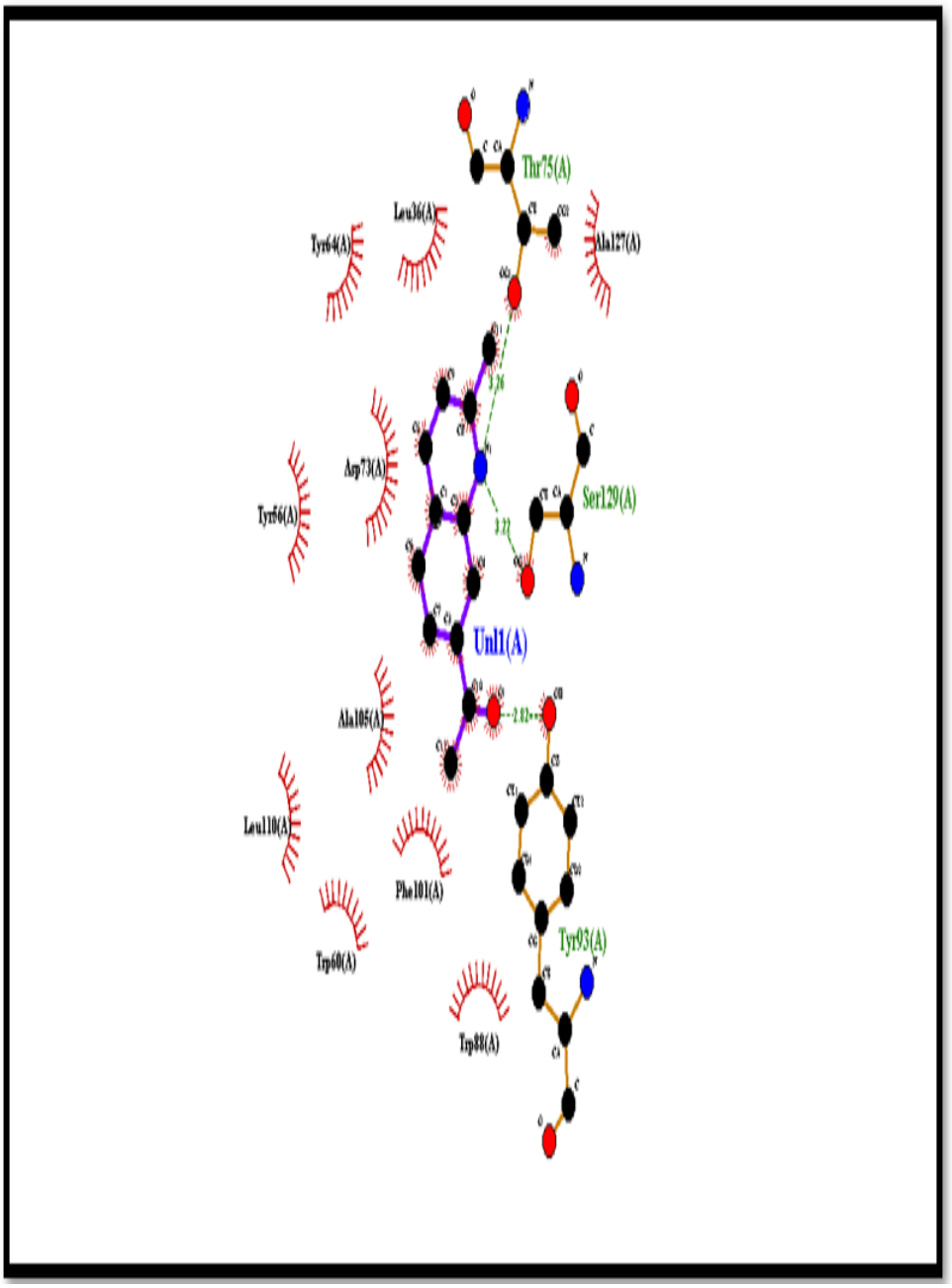


FIGURE 4.26: Interactions of 2-Methyl-7-acetylquinoline by LigPlot

Figure 4.26 represented the interactions of 2-Methyl-7-acetylquinoline with transcriptional regulator protein. Ten hydrophobic residues and three intermolecular hydrogen bonds showed close contact with them.

TABLE 4.10: Combined results of compounds obtained by LigPlot

Compound Name	**Docking Score**	**No. of H-bond Interaction**	**Residues in Molecular Interaction**	**Hydrophobic Residues**	**H-bond Distance**
Juglone	-9.0	4	Trp60, Ser129, Thr75, Thr115	Arg61, Asp73, Leu36, Tyr64, Tyr56, Leu110, Trp88	3.02
2-Methoxy stypandrone	-9.5	5	Arg61 Tyr56 Ser129 Thr115 Thr75	Tyr64, Leu36, Val76, Gly38, Ala127, Trp60	3.02
Resveratrol	-8.7	1	Arg61	Ala127, Leu36, IIe52, Tyr64, Val176, Ser129, Asp73, Tyr56, Phe101, Trp88, Trp60, Leu110	3.33

TABLE 4.10: Combined results of compounds obtained by LigPlot

Compound Name	**Docking Score**	**No. of H-bond Interaction**	**Residues in Molecular Interaction**	**Hydrophobic Residues**	**H-bond Distance**
Polydatin	-9.5	3	Leu125 Arg61 Leu110 Thr75	Leu39, Tyr47, Leu40, Gly38, Leu36, Tyr64, Ser129, Tyr56, Gly126, Val76, Ala127, Tyr64, Leu36, Thr115, Ser129, Asp73, Ala105, Leu110, Asp73, Trp88, Trp60, Tyr56, Arg61, Tyr64, Leu36, Ala127	3.14
Physcion	-9.9	3	Arg61 Trp60 Ser129 Thr115 Thr75		2.99
Chrysophanol	-10.1	3			3.05

TABLE 4.10: Combined results of compounds obtained by LigPlot

Compound Name	**Docking Score**	**No. of H-bond Interaction**	**Residues in Molecular Interaction**	**Hydrophobic Residues**	**H-bond Distance**
Emodin	-9.6	4	Trp60 Thr75 Thr115 Arg61	Trp88, Leu110, Ser129, Asp73, Val76, Ala127, Tyr56, Leu36,	3.35
Emodin-8 -o-b- glucoside	-9.1	6	Thr115 Ser129 Thr75 Gly126 Leu125 Arg61	Tyr64 Leu110, Tyr56, Tyr64, Leu36, IIe52, Val76, Leu39, Gly38, Leu40	

TABLE 4.10: Combined results of compounds obtained by LigPlot

Compound Name	**Docking Score**	**No. of H-bond Interaction**	**Residues in Molecular Interaction**	**Hydrophobic Residues**	**H-bond Distance**
2-Ethoxy-6-acetyl-7-methyl-juglone	-9.5	5	Arg61 Tyr56 Ser129 Thr115 Thr75	Tyr64, Leu36 Gly38, Val76, Ala127, Phe101 Trp88, Trp60 Asp73 Leu36, Tyr64, Asp73, Tyr56, Ala105, Leu110, Trp60, Phe101, Trp88, Ala127	
2-Methyl-7-acetyl quinoline	-8.9	3	Thr75 Ser129 Tyr93		3.26

Above results suggested that 2-Methoxy-6-acetyl-7-methyljuglone (2-Methoxy-stypandrone), Chrysophanol and Physcion were identified as hit compounds on the basis of their binding score with LasR was following as -9.5, -10.1 and -9.9. The presence of intermolecular hydrogen bonds and hydrophobic regions indicated tight interactions between the ligands and the receptor proteins. The interactions outcome demonstrated that the majority of hydrogen bond donors were from the

proteins that bind to the acceptors on the corresponding ligands. The compound 2-Methoxystypandrone binds to Arg61, Tyr56, Ser129, Thr115, Thr75 residues (H-bond distance of 3.02 Å) of the LasR protein and form hydrophobic interactions with Tyr64, Leu36, Val76, Gly38, Ala127, Trp60, Asp73, Trp88, Phe101 residues.

The compound Chrysophanol binds to Ser129, Thr115, Thr75 residues (H-bond distance of 3.05 Å) of the LasR protein and form hydrophobic interactions with Ala105, Leu110, Asp73, Trp88, Trp60, Tyr56, Arg61, Tyr64, Leu36, Ala127 residues. The compound Physcion binds to Thr75, Arg61, Trp60 residues (H-bond distance of 2.99 Å) of the LasR protein and form hydrophobic interactions with Val76, Ala127, Tyr64, Leu36, Thr115, Ser129, Asp73, Leu110, Tyr56, Trp88 residues.

4.5 ADME Analysis of all Potential Ligands

ADME screening was performed for all potential ligands. Lipinski's five-drug law used as a first step in assessing verbal bioavailability and artificial availability. Along with ADME analysis, oral absorption was also assessed using Lipinski's rule of five. pkCSM is an online tool which was used to predict ADMET properties [144]. The physicochemical properties and Lipinski Rule of Five were also analyzed by pkCSM. For Lipinski score calculations, the ligand in SMILE format was uploaded to pkCSM.

4.5.1 Pharmacodynamics

Pharmacodynamics branch of pharmacology in which we study the effect of drugs on the body.

4.5.2 Pharmacokinetics

In pharmacokinetics we study the absorption of drugs, distribution of drugs, metabolism of the drug and excretion of the drugs.

4.5.3 Absorption Analysis of Potential Ligands

In pharmacology specifically pharmacokinetics, the transfer of a drug from the bloodstream into the tissues is called absorption. So, the chemical composition of a drug, as well as the environment into which a drug is placed, work together to determine the rate and extent of drug absorption. A medicine must pass through cellular barrier such as epithelial or endothelial cells in order to be absorbed. Only a few medications pass cellular barriers in an active manner that demands the use of energy and transports the drug from a low concentration to a higher concentration. Most medications on the other hand pass past cellular barriers by passive diffusion in which they travel from a high-concentration area to a low-concentration area by diffusing through cell membranes. This sort of drug movement does not involve any energy expenditure but it is controlled by the drug size and solubility [145]. The absorption level of the substances was predicted using Caco-2 permeability, intestinal absorption (human), skin permeability, and P-glycoprotein substrate or inhibitor. Absorption properties of ligands were shown in Table 4.11 and 4.12 respectively.

TABLE 4.11: Absorption properties of ligands

Properties	**Juglone**	**2-Methoxy stypandrone**	**Resveratrol**	**Polydatin**	**Physcion**
Water solubility (mol/L)	-0.835	-2.777	-3.235	-3.113	-3.156
CaCO2 permeability (cm/S)	1.232	1.374	1.196	0.167	1.26
Intestinal absorption (Human) %	94.085	94.947	87.933	42.758	95.924

TABLE 4.11: Absorption properties of ligands

Properties	**Juglone**	**2-Methoxy stypandrone**	**Resveratrol**	**Polydatin**	**Physcion**
Skin permeability (Log/Kp)	-2.77	-3.277	-2.748	-2.735	-2.8
P-glyco -protein Substrate	No	No	Yes	Yes	Yes
P-glyco -protein I inhibitor	No	No	No	No	No
P-glyco -protein II inhibitor	No	No	No	No	No

TABLE 4.12: Absorption properties of ligands

Properties	**Chryso-phanol**	**Emodin**	**Emodin-8-o-b-glucoside**	**2-ethoxy-6-acetyl-7-methyl -juglone**	**2-methyl-7-acetyl -quinoline**
Water solubility (mol/L)	-3.238	-3.271	-2.856	-2.777	-2.612
CaCO2 permeability (cm/S)	1.235	0.259	-0.651	1.374	1.186
Intestinal absorption (Human) %	96.007	71.316	39.796	94.947	98.077

TABLE 4.12: Absorption properties of ligands

Properties	**Chryso-phanol**	**Emodin**	**Emodin-8-o-b-glucoside**	**2-ethoxy-6-acetyl-7-methyl-juglone**	**2-methyl-7-acetyl-quinoline**
Skin perme--ability (Log/Kp)	-2.811	-2.741	-2.735	-3.277	-2.247
P-glyco-protein Substrate	Yes	Yes	Yes	No	No
P-glyco-protein I inhibitor	No	No	No	No	No
P-glyco-protein II inhibitor	No	No	No	No	No

When the predicted value is less than 0.90; thus, the compound has high CaCO2 permeability and is easy to absorb. Absorption of less than 30% is regarded as being poorly absorbed in terms of intestinal absorption (in humans). The chemical is thought to have a relatively poor skin permeability because the log Kp greater than -2.5 [154]. CaCO2 permeability of all the given ligands was considered normal. Skin permeability for all ligands except 2-methyl-7-acetyl-quinoline was considered as greater than -2.5 (log Kp). While Intestinal absorption of Juglone, Physcion, 2-Methoxystypandrone, Chrysophanol, 2-Ethoxy-6-acetyl-7-methyl-juglone and 2-Methyl-7-acetyl-quinoline was considered more than 90% while it was average for Emodin, Resveratrol and remaining ligands. The findings revealed that Resveratrol, Polydatin, Physcion, Chrysophanol Emodin, and Emodin-8-o-b-glucoside all

were P-glycoprotein substrates, suggesting that P-glycoprotein may actively expel them from cells.

4.5.4 Distribution Analysis of Potential Ligands

Distribution in pharmacology is a branch of pharmacokinetics which deals with the movement of drug within the body from one location to another location. To describe the distribution of substances, the distribution volume (VDss), fraction unbound (human), blood-brain barrier membrane permeability (logBB), and CNS permeability those all parameters are used. VDss is considered low if it is less than 0.71 L/kg and when VDss is higher than 2.81L/kg it is thought that the distribution volume is quite high. If VDss is high it means that more of the drug is still distributed to the tissues than to plasma [154] . The compounds are believed to easily penetrate the blood-brain barrier when logBB greater than 0.3, which indicates membrane permeability for the blood-brain barrier. A logBB -1 indicated that the compounds had difficulty crossing the blood-brain barrier [146]. The distribution properties of ligands were shown in Table 4.13 and 4.14.

TABLE 4.13: Distribution properties of ligands

Properties	**Juglone**	**2-Methoxy stypandrone**	**Resveratrol**	**Polydatin**	**Physcion**
VDss (human) (L/kg)	-0.13	-0.03	0.022	0.103	0.206
Fraction unbound (human) (Fu)	0.418	0.458	0.089	0.177	0.133

TABLE 4.13: Distribution properties of ligands

Properties	**Juglone**	**2-Methoxy stypandrone**	**Resveratrol**	**Polydatin**	**Physcion**
BBB Perme -ability (Human) (Log BB)	-0.209	-0.26	-0.152	-0.994	-0.035
CNS perme -ability (Log PS)	-2.203	-2.905	-2.113	-3.862	-2.278

TABLE 4.14: Distribution properties of ligands

Properties	**Chryso-phanol**	**Emodin**	**Emodin-8-o-b-glucoside**	**2-ethoxy-6-acetyl-7-methyl-juglone**	**2-methyl-7-acetyl-quinoline**
VDss (human) (L/kg)	0.258	0.313	0.368	-0.03	0.01
Fraction unbound (human) (Fu)	0.129	0.159	0.226	0.458	0.332
BBB Perme -ability (Human) (Log BB)	0.18	-0.861	-1.553	-0.26	0.021

TABLE 4.14: Distribution properties of ligands

Properties	**Chryso-phanol**	**Emodin**	**Emodin-8-o-b-glucoside**	**2-ethoxy-6-acetyl-7-methyl-juglone**	**2-methyl-7-acetyl-quinoline**
CNS perme -ability (Log PS)	-2.087	-2.304	-4.228	-2.905	-2.253

The above results showed that the VDSS of all ligands was low. BBB permeability of all ligands was in range so they couldn't easily cross the blood brain except Emodin-8-o-b-glucoside and Polydatin. Juglone, 2-Methoxystypandrone, Resveratrol, Physcion Chrysophanol, and Emodin were expected to have low CNS permeability (logPS is less than -3) and so be unable to penetrate the CNS.

4.5.5 Metabolic Analysis of Potential Ligands

CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 models of the various isoforms of Cytochrome P450 which is an important cleansing enzyme found in the liver. This enzyme reacts to xenobiotics to facilitate their release. Some drugs are triggered by this enzyme while most drugs are neutralized by it [147]. Cytochrome P450 has two primary subtypes: CYP2D6 and CYP3A4. Metabolic properties of ligands were shown in Table 4.15 and 4.16 respectively.

TABLE 4.15: Metabolic properties of ligands

Properties	**Juglone**	**2-Methoxy stypandrone**	**Resveratrol**	**Polydatin**	**Physcion**
CYP-2D6 substrate	No	No	No	No	No
CYP-3A4 substrate	No	No	No	No	No

Table 4.15: Metabolic properties of ligands

Properties	**Juglone**	**2-Methoxy stypandrone**	**Resveratrol**	**Polydatin**	**Physcion**
CYP-1A2 inhibitor	No	No	Yes	No	No
CYP-2C19 inhibitor	No	No	No	No	Yes
CYP-2C9 inhibitor	No	No	No	No	No
CYP-2D6 inhibitor	No	No	No	No	No
CYP-3A4 inhibitor	No	No	No	No	No

Table 4.16: Metabolic properties of ligands

Properties	**Chryso -phanol**	**Emodin**	**Emodin-8-o-b-glucoside**	**2-ethoxy-6-acetyl-7-methyl-juglone**	**2-methyl-7-acetyl-quinoline**
CYP-2D6 substrate	No	No	No	No	No
CYP-3A4 substrate	Yes	No	No	No	No
CYP-1A2 inhibitor	Yes	Yes	No	No	Yes
CYP-2C19 inhibitor	No	No	No	No	No
CYP-2C9 inhibitor	No	No	No	No	No
CYP-2D6 inhibitor	No	No	No	No	No

TABLE 4.16: Metabolic properties of ligands

Properties	**Chryso -phanol**	**Emodin**	**Emodin-8-o-b-glucoside**	**2-ethoxy-6-acetyl-7-methyl-juglone**	**2-methyl-7-acetyl-quinoline**
CYP-3A4 inhibitor	No	No	No	No	No

The above results showed that all ligands were not substrates for the two subtypes of Cytochrome P450; CYP2D6 and CYP3A4 but Chrysophanol was a substrate for CYP-3A4. A strong inhibitor CYP-1A2, which was present only in Resveratrol, Chrysophanol, Emodin and 2-methyl-7-acetyl-quinoline that may be metabolized in liver.

4.5.6 Excretory Analysis of Potential Ligands

The organs involved in drug excretion are the kidneys (renal excretion) and the liver (biliary excretion). Other organs may also be involved in drug excretion are lungs (for volatile or gaseous agents). Drugs can also be excreted in sweat, saliva and tears. The molecular weight and hydrophilicity of substances affect drug elimination. Models of Excretion property are Total Clearance expressed as log (CL tot) in ml/min/kg and second one is Renal OCT2 substrate which predicts results as Yes /No. OCT2 (organic cation transporter 2) is a renal uptake transporter that plays role in disposition and renal clearance of drugs [148]. Excretory properties of all ligands were listed below in Table 4.17.

TABLE 4.17: Excretion properties of ligands

Sr No.	**Ligands**	**Total Clearance (ml/kg)**	**Renal OCT2 substrate**
1	Juglone	0.022	No

TABLE 4.17: Excretion properties of ligands

Sr No.	Ligands	Total Clearance (ml/kg)	Renal OCT2 substrate
2	2-Methoxystypandrone	0.5	No
3	Resveratrol	0.094	No
4	Polydatin	0.144	No
5	Physcion	0.431	No
6	Chrysophanol	0.004	No
7	Emodin	0.352	Yes
8	Emodin-8-o-b-glucoside	0.403	No
9	2-ethoxy-5-acetyl-7-methyl juglone	0.5	No
10	2-methyl-7-acetyl quinoline	0.288	No

Above prediction suggested that all compounds exhibited fair total clearance value while all the compounds showed negative result for model Renal OCT2 substrate except Emodin.

4.5.7 Virtual Screening and Toxicity Prediction of Ligands

PkCSM is an online tool used to find the ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of bioactive compounds and drugs. The maximum tolerated dose (MTD) provides a measure of toxic chemical on individuals. This will help in directing the first recommended dose of the treatment regimen in phase 1 clinical trials. MTD is expressed in the form of logarithms (log mg / kg / day). In a given compound MTD less than or equal to 0.477 log (mg / kg /day) is considered to be lower and higher if it is more than 0.477 log (mg / kg / day) [149]. Hepatotoxicity reveals drug-induced liver damage and is a major safety concern for drug development. Skin sensitivity is a potential adverse effect of skin care and applied products. In Minnow toxicity LC50 values below 0.5

mM (log LC 50 less than-0.3) are regarded as high acute toxicity [150]. Toxicity prediction of ligands were shown in Table 4.18 and 4.19 respectively.

TABLE 4.18: Toxicity prediction of ligands

Properties	**Juglone**	**2-Methoxy stypandrone**	**Resveratrol**	**Physcion**	**Polydatin**
Max. tolerated dose	0.658	0.3726	0.561	0.293	0.374
hERG I inhibitor	No	No	No	No	No
hERG II inhibitor	No	No	No	No	No
Oral Rat Acute Toxicity	1.845	1.747	2.216	2.152	2.397
Oral Rat Chronic Toxicity	2.673	1.491	1.761	1.694	3.817
Hepato -toxicity (log ug/L)	Yes	No	No	No	No
Skin Sensitization	No	No	No	No	No
T.Pyriformis toxicity	0.509	0.471	0.982	0.629	0.285
Minnow toxicity	2.048	2.209	1.367	1.191	0.864

TABLE 4.19: Toxicity prediction of ligands

Properties	**Chryso -phanol**	**Emodin**	**Emodin 8-o-b-glucoside**	**2-ethoxy-5-acetyl-7-methyl juglone**	**2-methyl-7-acetyl quinoline**
Max.tol erated dose	-0.331	0.166	0.539	0.362	0.563
hERG I inhibitor	No	No	No	No	No
hERG II inhibitor	No	No	No	No	No
Oral Rat Acute Toxicity	2.219	2.281	2.789	1.747	2.06
Oral Rat Chronic Toxicity	2.054	1.78	4.112	1.491	2.136
Hepato -toxicity (log ug/L)	No	No	No	No	No
Skin Sensitization	No	No	No	No	No
T. Pyriformis toxicity	0.898	0.62	0.285	0.471	0.563
Minnow toxicity	1.572	1.428	4.942	2.209	0.876

Above results predicted that only juglone may be hepatotoxic whereas all of the ligands may inhibit the hERG channel and not cause cardiotoxicity or skin sensitization.

4.5.8 Lipinski Rule of Five

For Lipinski score calculations, the ligand in SMILE format was uploaded to pkCSM. The physicochemical properties and Lipinski Rule of Five were also analyzed by pkCSM. Further analysis was not performed on compounds that violate more than two of Lipinski's rule of five. So, following rules (mentioned below) was applied on natural compounds and hence analysis of different ligands of *Reynoutria japonica* was checked and results were shown in Table 4.20 respectively.

Lipinski's rule of five are as follow:

1. The log P value of most "drug-like" molecules should be limited to 5.
2. Molecular weight should be under 500.
3. Maximum number of H-bond acceptor should be 10.
4. Maximum number of H-bond donor should be 5 [151].

TABLE 4.20: Applicability of Lipinski rule on ligands

Ligands	**Log P**	**Molecular weight**	**H-bond acceptor**	**H-bond donor**
Juglone	1.3274	174.155	3	1
2-Methoxy stypandrone	1.81252	260.245	5	1
Resveratrol	2.9738	228.247	3	3
Polydatin	0.4469	390.388	8	6
Physcion	2.19022	284.267	5	2
Chrysophanol	2.18162	254.241	4	2
Emodin	1.88722	270.24	5	3
Emodin-8-o-b-glucoside	-0.63968	432.381	10	6

TABLE 4.20: Applicability of Lipinski rule on ligands

Ligands	**Log P**	**Molecular weight**	**H-bond acceptor**	**H-bond donor**
2-ethoxy-5-acetyl-7-methyl juglone	1.81252	260.245	5	1
2-methyl-7-acetyl quinoline	2.74582	185.226	2	0

The Table 4.20 showed molecular weight in g/mol, , log P values, hydrogen bond acceptor and donor values of ligands of *Reynoutria japonica*. These rules were to be followed by orally active compounds. The drug-like is dependent on the mode of administration. A compound is considered a drug when it follows 3 or more rules and if a compound violates two or more rules it is considered poorly absorbed [151]. Except Emodin-8-O-b-glucoside, 2-Methyl-7-acetyl quinoline and Polydatin nearly all the ligands followed Lipinski rule of five. Emodin 8-o-b glucoside and Polydatin has one extra hydrogen bond donor and their log P value was nearly equal to 0. The compound 2-Methyl-7-acetyl quinoline has zero H-bond donor and also have low H-bond acceptors. Hence, Emodin 8-o-b glucoside, Polydatin and 2-Methyl-7-acetyl quinoline did not obey Lipinski rule of five so they could knock out in primary screening.

4.6 Binding Interaction of Potential Lead Compound

Physicochemical and pharmacokinetics properties determined the final destiny of compounds as drug or non-drug compounds. Physicochemical properties or Lipinski rule of five works as primary filter and pharmacokinetics studies as secondary

filter in screening of potential compounds. Emodin 8-o-b glucoside, Polydatin and 2-Methyl-7-acetyl quinoline do not obey Lipinski rule of five so they knock out in primary screening. On the basis of binding score, ADMET analysis, physico-chemical properties and Lipinski rule of five, Chrysophanol was selected as lead compound among others which could inhibit target proteins in quorum sensing process of respective bacteria. Chrysophanol showed highest binding score among other ligands such as -10.1 with target protein and selected as lead compound. It also obeyed Lipinski rule of five as its log P value was 2.18162 and its MW was 254.241. It has four H-bond acceptors and two H-bond donors and its ADME analysis was also good among others. So, all these properties determined the final destiny of Chrysophanol as drug.

4.7 Identification of Reference Anti-bacterial Drug

The selection of most efficient anti-bacterial drug was based on the physiochemical, ADMET analysis along their mechanism of action with side effects. For physico-chemical properties, PubChem online database was used and for ADMET analysis of drugs pkCSM online tool was used. Mechanism of action was identified through Drug Bank database. Selected anti-bacterial drug and its related properties were shown in Table 4.21 respectively.

TABLE 4.21: Reference anti-bacterial drug and its related properties

S. No	Properties	Meropenem (Carbapenem)
1	Chemical Formula	$C_{17}H_{25}N_3O_5S$
2	Absorption	Not absorbed after oral administration.
3	Water Solubility (mg/l)	-2.591
4	Log P	-0.308
5	H-bond Donor	3
6	H-bond Acceptor	6

TABLE 4.21: Reference anti-bacterial drug and its related properties

S. No	Properties	Meropenem (Carbapenem)
7	Side Effects	Ataxia,

4.7.1 Meropenem

Meropenem is a broad-spectrum carbapenem antibiotic. It is active against Gram-positive and Gram-negative bacteria. *Pseudomonas* infection is caused by bacterial strains present in the environment. Carbapenems are a type of antibiotic that was commonly used to treat bacteria that were resistant to other antibiotics. Because of the excessive use of these antibiotics, certain *P. aeruginosa* strains have developed carbapenem resistance, and these bacteria are known as carbapenem-resistant *P. aeruginosa* (CRPA) [152], [153].

4.7.2 Meropenem Mechanism of Action

The bactericidal activity of Meropenem results from the inhibition of cell wall synthesis. Meropenem readily penetrates the cell wall of most Gram-positive and Gram-negative bacteria to reach PBP targets. Its strongest affinities are toward PBPs 2, 3 and 4 of *E. coli and P. aeruginosa*; and PBPs 1, 2 and 4 of *S. aureus* .

4.7.3 Drug ADMET Analysis

ADMET analysis of FDA approved antibacterial drug were explored by pkCSM prediction tool.

4.7.3.1 Absorption Analysis of Drug

The absorption properties of selected drug Meropenem were shown Table 4.22,4.23.

TABLE 4.22: Absorption properties of drug[1a]

Drug	Water solubility (mol/L)	CaCO2 Permeability (cm/S)	Intestinal absorption (human)	Skin Permeability (Log/Kp)
Meropenem	-2.591	0.095	37.317	-2.735

TABLE 4.23: Absorption properties of drug[1b]

Drug	P-glycoprotein substrate	P-glycoprotein I inhibitor	P-glycoprotein II inhibitor
Meropenem	Yes	No	No

The drug has lower CaCO2 permeability and is not easy to absorb. Absorption of less than 30% is regarded as being poorly absorbed in terms of intestinal absorption (in humans) [154]. The selected drug has 37.317% for intestinal absorption. The chemical is thought to have a relatively poor skin permeability because the log Kp greater than -2.5. The selected drug has -2.735 log Kp value for skin permeability.

4.7.3.2 Distribution Analysis of Drug

Distribution properties of selected drug Meropenem were shown in Table 4.24.

TABLE 4.24: Distribution properties of drug

Drug Name	VDss (Human) (L/kg)	Fraction unbound (Human) (Fu)	BBB Permeability (Human) (Log BB)	CNS Permeability (Log PS)
Meropenem	-0.922	0.682	-0.867	-3.673

The above results showed that the VDSS of drug was high. Meropenem was expected to have high CNS permeability (logPS is less than -3) and so be able to

penetrate the CNS.

4.7.3.3 Metabolic Analysis of Drug

Metabolic properties of selected drug Meropenem were shown in Table 4.25 and 4.26.

TABLE 4.25: Metabolic properties of drug [1a]

Drug Name	**CYP-2D6 Substrate**	**CYP-3A4 Substrate**	**CYP-2D6 Inhibitor**	**CYP-2619 Inhibitor**
Meropenem	Yes	No	No	No

The above and below results tables showed that Meropenem was only substrate for CYP2D6 from one of the two subtypes of Cytochrome P450.

TABLE 4.26: Metabolic properties of drug [1b]

Drug Name	**CYP-269 Inhibitor**	**CYP-2D6 Inhibitor**	**CYP-3A4 Inhibitor**
Meropenem	No	No	No

4.7.3.4 Excretion Analysis of drug

Excretion properties of selected drug Meropenem were shown in Table 4.27.

TABLE 4.27: Excretion properties of drug

S. No	**Drug Name**	**Total Clearance (ml/Kg)**	**Renal OCT2 Substrate**
1	Meropenem	0.449	No

Above prediction results suggested that drug exhibited fair total clearance value while it showed negative result for model Renal OCT2 substrate.

4.7.3.5 Toxicity Analysis

Toxicity properties of selected drug Meropenem were shown in Table 4.28.

TABLE 4.28: Toxicity properties of drug

S. No	Toxicity Properties	Meropenem
1	Max tolerated dose (Human) (mg/kg)	1.692
2	hERG1 Inhibitor	No
3	hERG II inhibitor	No
4	Oral rat acute toxicity (mol/kg)	2
5	Oral rat chronic toxicity (mg/kg)	2.567
6	Hepatoxicity (Log ug/L)	Yes
7	Skin Sensitization	No
8	*T. pyriformis* activity (Log ug/L)	0.285
9	Minnow toxicity (Log mM)	3.397

Above mentioned results predicted that the drug may be hepatotoxic whereas it may not cause skin sensitization.

4.7.3.6 Lipinski Rule of Five

Table 4.29 showed the properties of selected drug Meropenem according to Lipinski rule of five.

TABLE 4.29: Applicability of Lipinski rule on drug

S. No	Ligands	Log P value	Molecular Weight	H-bond acceptor	H-bond donor
1	Meropenem	-0.308	383.47 g/mol	6	3

Above mentioned results show that Meropenem has lower log P value.

4.7.3.7 Meropenem Docking

For the docking purpose, CB Dock online docking tool is used. It gives 5 best confirmation results and finest one was selected. Meropenem was used as ligand and LasI protein, LasR protein, RhlI protein, RhlR protein and transcriptional regulator protein MvfR were selected as receptors. As the mechanism of action showed that meropenem inhibits those proteins which involve in infection. So, docking helped us to find out the inhibition value, the inhibitory value was shown in Table 4.30.

TABLE 4.30: Results of CB dock of Meropenem

Drug Name	Binding Score	Cavity Size	Grid Map	Minimum energy (Kcal/mol)	Maximum energy (Kcal/mol)
Meropenem	-8.0	8355	34	0.00	1.6E+00

The docking score of Meropenem was -8.0 whereas its cavity size was 8355.

4.8 Meropenem and Lead Compound Comparison

The comparison between Meropenem and Chrysophanol helped us to identify the better treatment for infectious diseases. Comparison was being performed through

parameters like ADME screening, toxicity analysis and physiochemical properties of both compounds.

4.8.1 ADMET Analysis Comparison

ADMET analysis between reference drug and lead compound was given below

4.8.1.1 Absorption Analysis Comparison

Meropenem and Chrysophanol absorption properties were given in Table 4.31.

TABLE 4.31: Absorption analysis comparison of natural compound and reference drug

S.No	Properties	Chrysophanol	Meropenem
1	Water solubility (mol/L)	-3.238	-2.591
2	CaCO2 permeability (cm/S)	1.235	0.095
3	Intestinal absorption (Human) \%	96.007	37.317
4	Skin permeability (Log/Kp)	-2.811	-2.735
5	P-glycoprotein Substrate	Yes	Yes
6	P-glycoprotein I inhibitor	No	No
7	P-glycoprotein II inhibitor	No	No

When the predicted value is greater than 0.90; thus, the compound has high CaCO2 permeability and is easy to absorb. Absorption of less than 30% is regarded

as being poorly absorbed in terms of intestinal absorption (in humans) [154]. Above results predicted that Chrysophanol has high intestinal absorption (%) and higher CaCO2 permeability as compared to Meropenem.

4.8.1.2 Distribution Analysis Comparison

The distribution properties of Meropenem and Chrysophanol were shown in Table 4.32.

TABLE 4.32: Distribution analysis comparison of natural compound and reference drug

Sr. No	Properties	Chrysophanol	Meropenem
1	VDss (human) (L/kg)	0.258	-0.922
2	Fraction unbound (human) (Fu)	0.129	0.682
3	BBB Permeability (Human) (Log BB)	0.18	-0.867
4	CNS permeability (Log PS)	-2.087	-3.673

The above results showed that the VDSS of both compounds was low. BBB permeability of Chrysophanol was in range so it couldn't easily cross the blood brain

as compared to drug. Chrysophanol was expected to have low CNS permeability (log PS is less than -3) as compared to Meropenem so it may be unable for Chrysophanol to penetrate the CNS.

4.8.1.3 Metabolic Analysis Comparison

The metabolic properties of Meropenem and Chrysophanol were given in Table 4.33.

TABLE 4.33: Metabolic analysis comparison of natural compound and reference drug

Sr. No	Properties	Chrysophanol	Meropenem
1	CYP-2D6 substrate	No	Yes
2	CYP-3A4 substrate	Yes	No
3	CYP-1A2 inhibitor	Yes	No
4	CYP-2C19 inhibitor	No	No
5	CYP-2C9 inhibitor	No	No
6	CYP-2D6 inhibitor	No	No
7	CYP-3A4 inhibitor	No	No

The above results showed that Chrysophanol and Meropenem were not substrates for both subtypes of Cytochrome P450; CYP2D6 and CYP3A4 but Chrysophanol was a substrate for CYP-3A4 and Meropenem was a substrate for CYP2D6. A strong inhibitor CYP-1A2, which was present only in Chrysophanol as compared to drug that may be metabolized in liver.

4.8.1.4 Excretory Analysis Comparison

The excretion properties of Meropenem drug and Chrysophanol were shown in Table 4.34.

TABLE 4.34: Excretory analysis comparison of natural compound and reference drug

Sr No.	**Ligands**	**Total Clearance (ml/kg)**	**Renal OCT2 substrate**
1	Chrysophanol	0.004	No
2	Meropenem	0.449	No

4.8.1.5 Toxicity Analysis

The toxicity ranges of Meropenem drug and Chrysophanol was given in Table 4.35.

TABLE 4.35: Toxicity analysis comparison of natural compound and reference drug

Sr No.	**Properties**	**Chrysophanol**	**Meropenem**
1	Max. tolerated dose	-0.331	1.692
2	hERG I inhibitor	No	No
3	hERG II inhibitor	No	No
4	Oral Rat Acute Toxicity	2.219	2
5	Oral Rat Chronic Toxicity	2.054	2.567
6	Hepatotoxicity (log ug/L)	No	Yes
7	Skin Sensitization	No	No
8	*T.Pyriformis* toxicity	0.898	0.285
9	Minnow toxicity	1.572	3.397

Above results predicted that only Meropenem may be hepatotoxic as compared to Chrysophanol. whereas both compounds may inhibit the hERG channel and not cause cardiotoxicity or skin sensitization [155]. The max tolerated dose for

Meropenem was 1.692 and for Chrysophanol was -0.331 and oral rat acute toxicity of Chrysophanol was greater and chronic toxicity value of Meropenem was higher.

4.8.1.6 Lipinski Rule of Five

Meropenem and Chrysophanol Lipinski rule of five was given in Table 4.36.

TABLE 4.36: Meropenem and Chrysophanol Lipinski rule of five

S. No	Ligands	Log P value	Molecular Weight	H-bond acceptor	H-bond donor
1	Meropenem	-0.308	383.47 g/mol	6	3
2	Chrysophanol	2.18162	254.241	4	2

Above data showed that Chrysophanol bioactive compound showed better result over Meropenem with respect to log P value.

4.8.2 Docking Score Comparison

Both the standard drug and the lead compound were docked against the target proteins and the docking result provided the best binding score. Table 4.37 showed that the lead compound Chrysophanol has higher vina score than that of the standard drug which was Meropenem.

TABLE 4.37: Docking results comparison

S. No	Properties	Chrysophanol	Meropenem
1	Binding score	-10.1	-8.00
2	Cavity size	1598	8355
3	Grid Map	19	34
4	Minimum Energy (Kcal/mol)	0.00	0.00

TABLE 4.37: Docking results comparison

S. No	Properties	Chrysophanol	Meropenem
5	Maximum Energy (Kcal/mol)	1.6E+00	1.6E+00

Previous studies have suggested that several antibiotic candidates have been explored for their anti-virulence effectiveness against bacteria. The research showed that these compounds inhibited the early stages of bacterial communication. Currently, there are hundreds of thousands of natural compounds that can be used for screening to find new therapeutic targets. Three compounds namely 2-Methoxy-6-acetyl-7-methyljuglone, Chrysophanol, and Physcion, were identified as hit compounds in the current study using virtual screening of ten natural compounds from *Reynoutria japonica*. The binding affinity of these compounds with *Pseudomonas aeruginosa* proteins was following as -9.5, -10.1 and -9.9 and their log P value was 1.81252, 2.18162 and 2.19022 respectively. Intestinal absorption of Chrysophanol was 96.007% while it was low for 2-Methoxy-6-acetyl-7-methyljuglone and Physcion. Our analysis predicted that Chrysophanol showed highest binding affinity with LasR protein among them and its activity was also better in comparison to synthetic drug Meropenem. Chrysophanol has higher vina score such as -10.1 than that of the standard drug which was Meropenem with -8.00 vina score. Chrysophanol has formed 5 hydrophobic interactions and single hydrogen bond with LasR protein. And the log P value of Chrysophanol was 2.18162 while log P value of Meropenem was -0.308. Chrysophanol showed tight interactions with transcriptional regulator protein LasR. And the log P value of Chrysophanol was 2.18162 while log P value of Meropenem was -0.308. However, these findings revealed that Chrysophanol had demonstrated itself as a promising potential anti-biofilm agent against *Pseudomonas aeruginosa* proteins. Consequently, it might be an excellent candidate for drugs to treat bacterial infections.

Chapter 5

Conclusions and Recommendations

Bacterial infections especially with multidrug resistant *Pseudomonas aeruginosa* are hard to treat due to their high resistance profile and potential to produce biofilm. Due to massive use of antibiotics in clinical practices caused respective bacteria to become resistant against multiple antibiotics. Antibiotic failure forced researchers to look for alternatives. There was an urgent need of development of innovative therapeutics and new strategies to fight emerging infections. One of these strategies was to disturb quorum sensing system (which is cell density dependent communication mechanism and responsible for virulence or biofilm formation in multidrug resistant strains of respective bacteria) by natural means. This strategy is called quorum quenching. The aim of present research was to identify bioactive compounds from *Reynoutria japonica* through multiple computational methods which could suppress the regulation of virulence factors in *Pseudomonas aeruginosa* biofilm. After performing data mining studies on literature databases ten ligands from *Reynoutria Japonica* were selected for the current research work. The proteins used for virtual screening were LasI, LasR, RhlI, RhlR and transcriptional regulator protein MvfR from respective bacteria. CB Dock automated version of Auto Dock vina was used for the docking studies. Protein-ligand interactions were analyzed using LigPlot plus version v.1.4.5. All the software's and

tools used in the current research study were reliable and authentic. After the detailed analysis of binding scores, physicochemical properties and ADMET parameters of all inhibitory agents, three best scoring phytocompounds namely as, 2-Methoxy-6-acetyl-7-methyljuglone, Chrysophanol and Physcion were identified as hit compounds and their binding score with respective bacterial proteins was following as -9.5, -10.1 and -9.9 and their log P value was 1.81252, 2.18162 and 2.19022 respectively. Intestinal absorption of Chrysophanol was 96.007% while it was low for 2-Methoxy-6-acetyl-7-methyljuglone and Physcion. Our analysis predicted that Chrysophanol showed highest binding affinity with LasR protein among them and its overall activity was also better in comparison to synthetic drug Meropenem. Chrysophanol has higher vina score such as -10.1 than that of the standard drug which was Meropenem with -8.00 vina score. Chrysophanol has formed 5 hydrophobic interactions and single hydrogen bond with LasR protein. And the log P value of Chrysophanol was 2.18162 while log P value of Meropenem was -0.308. From the above-mentioned findings suggest that Chrysophanol, a bioactive compound found in the roots of *Reynoutria japonica* could be a best choice for medicinal purposes and it can also be used to inhibit the quorum sensing mechanism of *Pseudomonas aeruginosa*. It showed lesser side effects as compared to synthetic ones and could be used for the treatment of infectious diseases. And in the future, it will be considered as an effective anti-biofilm drug.

It is now clear that the relation between quorum sensing and bacterial virulence represents a promising area from which new, effective anti-biofilm drugs can emerge. The identified compound showed itself as potential anti-biofilm. Consequently, it might be an excellent candidate for drugs to treat bacterial infections. But further in vitro validations are required to test its efficacy against *Pseudomonas aeruginosa*. *Insilico* technology alone cannot guarantee the identification of new, safe and effective lead compound. Although, future success is more practically dependent on the proper integration of new promising technologies with the knowledge and approaches of classical medicinal chemistry. However, more research is needed to explore their mechanism of action as well as the optimum levels of anti-quorum sensing compounds that can be used safely. Furthermore,

Reynoutria japonica is commonly used in combination with other herbs in traditional Chinese medicines, but modern experiments show that this plant alone has significant pharmacological effects, making it interesting and important to investigate the medical effects and molecular mechanisms of this plant combined with other herbs using modern disease pathophysiology concepts. Quorum sensing and quorum quenching both are research hotspots right now. We should pay more attention to the use of quorum sensing inhibitors and agonists in the future. Anti-quorum sensing techniques are developed so far but they have not yet been tested on a large-scale clinical trial. So, it is difficult to evaluate their full potential and limitations at this stage. It is evident, however, that we need to expand our antimicrobial targets and techniques, and inter-cellular communication interruption appears to be a viable and promising alternative for anti-biofilm drug discovery.

Bibliography

[1] A. P. Magalhães, P. Jorge, and M. O. Pereira, "Pseudomonas aeruginosa and Staphylococcus aureus communication in biofilm infections: insights through network and database construction," *Crit. Rev. Microbiol.*, vol. 45, no. 5–6, pp. 712–728, 2019, doi: 10.1080/1040841X.2019.1700209.

[2] M. B. Miller, K. Skorupski, D. H. Lenz, R. K. Taylor, and B. L. Bassler, "Parallel quorum sensing systems converge to regulate virulence in Vibrio cholerae," Cell, vol. 110, no. 3, pp. 303–314, 2002, doi: 10.1016/S0092-8674(02)00829-2.

[3] J. M. Junecko, " Transcribing virulence in Staphylococcus aureus ," *World J. Clin.* Infect. Dis., vol. 2, no. 4, p. 63, 2012, doi: 10.5495/wjcid.v2.i4.63.

[4] K. G. Chan, Y. C. Liu, and C. Y. Chang, "Inhibiting N-acyl-homoserine lactone synthesis and quenching Pseudomonas quinolone quorum sensing to attenuate virulence," *Front. Microbiol.*, vol. 6, no. OCT, pp. 1–7, 2015, doi: 10.3389/fmicb.2015.01173.

[5] Z. Pang, R. Raudonis, B. R. Glick, T. J. Lin, and Z. Cheng, "Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies," *Biotechnol. Adv.*, vol. 37, no. 1, pp. 177–192, 2019, doi: 10.1016/j.biotechadv.2018.11.013.

[6] A. Siryaporn, S. L. Kuchma, G. A. O'Toole, Z. Gitai, and F. M. Ausubel, "Surface attachment induces Pseudomonas aeruginosa virulence," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 111, no. 47, pp. 16860–16865, 2014, doi: 10.1073/pnas.1415712111.

[7] G. C. Palmer, P. A. Jorth, and M. Whiteley, "The role of two Pseudomonas aeruginosa anthranilate synthases in tryptophan and quorum signal production," Microbiol. (United Kingdom), vol. 159, no. PART 5, pp. 959–969, 2013, doi: 10.1099/mic.0.063065-0.

[8] A. C. Cooke et al., " Pseudomonas Quinolone Signal-Induced Outer Membrane Vesicles Enhance Biofilm Dispersion in Pseudomonas aeruginosa ," *mSphere*, vol. 5, no. 6, pp. 1–15, 2020, doi: 10.1128/msphere.01109-20.

[9] M. Romero, L. Acuna, and A. Otero, "Patents on Quorum Quenching: Interfering with Bacterial Communication as a Strategy to Fight Infections," *Recent Pat. Biotechnol.,* vol. 6, no. 1, pp. 2–12, 2012, doi: 10.2174/187220812799789208.

[10] M. Yang et al., "Effects of Natural Products on Bacterial Communication and Network-Quorum Sensing," *Biomed Res. Int.*, vol. 2020, 2020, doi: 10.1155/2020/8638103.

[11] J. Park et al., "Infection Control by Antibody Disruption of Bacterial Quorum Sensing Signaling," *Chem. Biol.*, vol. 14, no. 10, pp. 1119–1127, 2007, doi: 10.1016/j.chembiol.2007.08.013.

[12] C. L. Koh et al., "Plant-derived natural products as sources of anti-quorum sensing compounds," Sensors (Switzerland), vol. 13, no. 5, pp. 6217–6228, 2013, doi: 10.3390/s130506217.

[13] W. Peng, R. Qin, X. Li, and H. Zhou, "Botany, phytochemistry, pharmacology, and potential application of Polygonum cuspidatum Sieb.et Zucc.: A review," *J. Ethnopharmacol.*, vol. 148, no. 3, pp. 729–745, 2013, doi: 10.1016/j.jep.2013.05.007.

[14] C. Yan, X. Zou, and M. Chemistry, "Protein-Ligand Docking In Silico Drug Discovery Tools," 2017.

[15] L. G. Ferreira, R. N. Dos Santos, G. Oliva, and A. D. Andricopulo, Molecular docking and structure-based drug design strategies, vol. 20, no. 7. 2015.

[16] S. Saha and J. Chowdhury, "Binding Interaction of Juglone with Lysozyme: Spectroscopic Studies Aided by In Silico Calculations," J. Photochem. Photobiol. B Biol., vol. 193, no. December 2018, pp. 89–99, 2019, doi: 10.1016/j.jphotobiol.2019.02.006.

[17] R. A. Friesner et al., "Glide: A New Approach for Rapid, Accurate Docking and Scoring. 1. Method and Assessment of Docking Accuracy," J. Med. Chem., vol. 47, no. 7, pp. 1739–1749, 2004, doi: 10.1021/jm0306430.

[18] S. Muhammad and N. Fatima, "In silico analysis and molecular docking studies of potential angiotensin-converting enzyme inhibitor using quercetin glycosides," Pharmacogn. Mag., vol. 11, no. 42, p. 123, 2015, doi: 10.4103/0973-1296.157712.

[19] A. A. K. Khalil et al., "A new anti-Helicobacter pylori juglone from Reynoutria japonica," Arch. Pharm. Res., vol. 42, no. 6, pp. 505–511, 2019, doi: 10.1007/s12272-019-01160-x.

[20] C. Fuqua and E. P. Greenberg, "Listening in on bacteria: Acyl-homoserine lactone signalling," Nat. Rev. Mol. Cell Biol., vol. 3, no. 9, pp. 685–695, 2002, doi: 10.1038/nrm907.

[21] K. H. Nealson, T. Platt, and J. W. Hastings, "Cellular control of the synthesis and activity of the bacterial luminescent system.," J. Bacteriol., vol. 104, no. 1, pp. 313–322, 1970, doi: 10.1128/jb.104.1.313-322.1970.

[22] K. Papenfort and B. L. Bassler, "Quorum sensing signal-response systems in Gram-negative bacteria," Nat. Rev. Microbiol., vol. 14, no. 9, pp. 576–588, 2016, doi: 10.1038/nrmicro.2016.89.

[23] P. Jorge, A. Lourenço, and M. O. Pereira, "New trends in peptide-based anti-biofilm strategies: a review of recent achievements and bioinformatic approaches," Biofouling, vol. 28, no. 10, pp. 1033–1061, 2012, doi: 10.1080/08927014.2012.728210.

[24] X. Zhao, Z. Yu, and T. Ding, "Quorum-sensing regulation of antimicrobial resistance in bacteria," Microorganisms, vol. 8, no. 3, 2020, doi: 10.3390/microorganisms8030425.

[25] R. Huang, M. Li, and R. L. Gregory, "Bacterial interactions in dental biofilm," Virulence, vol. 2, no. 5, pp. 435–444, 2011, doi: 10.4161/viru.2.5.16140.

[26] V. G. Preda and O. Săndulescu, "Communication is the key: biofilms, quorum sensing, formation and prevention," Discoveries, vol. 7, no. 3, p. e10, 2019, doi: 10.15190/d.2019.13.

[27] E. Drenkard, "Antimicrobial resistance of Pseudomonas aeruginosa biofilms," vol. 5, pp. 1213–1219, 2003, doi: 10.1016/j.micinf.2003.08.009.

[28] R. Roy, M. Tiwari, G. Donelli, and V. Tiwari, "Strategies for combating bacterial biofilms: A focus on anti-biofilm agents and their mechanisms of action," Virulence, vol. 9, no. 1, pp. 522–554, 2018, doi: 10.1080/21505594.2017.1313372.

[29] M. Crouzet et al., "Exploring early steps in biofilm formation: Set-up of an experimental system for molecular studies," BMC Microbiol., vol. 14, no. 1, pp. 1–12, 2014, doi: 10.1186/s12866-014-0253-z.

[30] F. Verbeke, "Peptides as quorum sensing molecules: Measurement techniques and obtained levels in vitro and in vivo," Front. Neurosci., vol. 11, no. APR, pp. 1–18, 2017, doi: 10.3389/fnins.2017.00183.

[31] S. T. Rutherford and B. L. Bassler, "Bacterial quorum sensing: Its role in virulence and possibilities for its control," Cold Spring Harb. Perspect. Med., vol. 2, no. 11, 2012, doi: 10.1101/cshperspect.a012427.

[32] J. M. Brint and D. E. Ohman, "Synthesis of multiple exoproducts in Pseudomonas aeruginosa is under the control of RhlR-RhlI, another set of regulators in strain PAO1 with homology to the autoinducer-responsive LuxR-LuxI family," J. Bacteriol., vol. 177, no. 24, pp. 7155–7163, 1995, doi: 10.1128/jb.177.24.7155-7163.1995.

[33] J. M. Farrow, Z. M. Sund, M. L. Ellison, D. S. Wade, J. P. Coleman, and E. C. Pesci, "PqsE functions independently of PqsR-Pseudomonas quinolone signal and enhances the rhl quorum-sensing system," J. Bacteriol., vol. 190, no. 21, pp. 7043–7051, 2008, doi: 10.1128/JB.00753-08.

[34] N. M. Mohamed, E. M. Cicirelli, J. Kan, F. Chen, C. Fuqua, and R. T. Hill, "Diversity and quorum-sensing signal production of Proteobacteria associated with marine sponges," Environ. Microbiol., vol. 10, no. 1, pp. 75–86, 2008, doi: 10.1111/j.1462-2920.2007.01431.x.

[35] I. Dawid ., "Gene expression in Pseudomonas aeruginosa biofilms," Nature, vol. 413, no. October, pp. 860–864, 2001.

[36] A. P. Tampakaki, V. E. Fadouloglou, A. D. Gazi, N. J. Panopoulos, and M. Kokkinidis, "Conserved features of type III secretion," Cell. Microbiol., vol. 6, no. 9, pp. 805–816, 2004, doi: 10.1111/j.1462-5822.2004.00432.x.

[37] H. Mikkelsen, N. J. Bond, M. E. Skindersoe, M. Givskov, K. S. Lilley, and M. Welch, "Biofilms and type III secretion are not mutually exclusive in Pseudomonas aeruginosa," Microbiology, vol. 155, no. 3, pp. 687–698, 2009, doi: 10.1099/mic.0.025551-0.

[38] J. Njoroge and V. Sperandio, "Jamming bacterial communication: New approaches for the treatment of infectious diseases," EMBO Mol. Med., vol. 1, no. 4, pp. 201–210, 2009, doi: 10.1002/emmm.200900032.

[39] R. T. Pena ., "Relationship between quorum sensing and secretion systems," Front. Microbiol., vol. 10, no. JUN, 2019, doi: 10.3389/fmicb.2019.01100.

[40] L. Ali, M. U. Goraya, Y. Arafat, M. Ajmal, J. L. Chen, and D. Yu, "Molecular mechanism of quorum-sensing in Enterococcus faecalis: Its role in virulence and therapeutic approaches," Int. J. Mol. Sci., vol. 18, no. 5, 2017, doi: 10.3390/ijms18050960.

[41] M. Guo, S. Gamby, Y. Zheng, and H. O. Sintim, "Small molecule inhibitors of AI-2 signaling in bacteria: State-of-the-art and future perspectives for anti-quorum sensing agents," Int. J. Mol. Sci., vol. 14, no. 9, pp. 17694–17728, 2013, doi: 10.3390/ijms140917694.

[42] B. Zhang., "The ai-2/luxsquorum sensing system affects the growth characteristics, biofilm formation, and virulence of haemophilus parasuis," Front. Cell. Infect. Microbiol., vol. 9, no. MAR, pp. 1–15, 2019, doi: 10.3389/fcimb.2019.00062.

[43] J. Lee , "A cell-cell communication signal integrates quorum sensing and stress response," Nat. Chem. Biol., vol. 9, no. 5, pp. 339–343, 2013, doi: 10.1038/nchembio.1225.

[44] A. Glessner, R. S. Smith, B. H. Iglewski, and J. B. Robinson, "Roles of Pseudomonas aeruginosa las and rhl quorum-sensing systems in control of twitching motility," J. Bacteriol., vol. 181, no. 5, pp. 1623–1629, 1999, doi: 10.1128/jb.181.5.1623-1629.1999.

[45] X. Cui, X. Ruan, J. Yin, M. Wang, N. Li, and D. Shen, "Regulation of las and rhl Quorum Sensing on Aerobic Denitrification in Pseudomonas aeruginosa PAO1," Curr. Microbiol., vol. 78, no. 2, pp. 659–667, 2021, doi: 10.1007/s00284-020-02338-z.

[46] G. Xiao et al., "MvfR, a key Pseudomonas aeruginosa pathogenicity LTTR-class regulatory protein, has dual ligands," Mol. Microbiol., vol. 62, no. 6, pp. 1689–1699, 2006, doi: 10.1111/j.1365-2958.2006.05462.x.

[47] V. C. Kalia, "Quorum sensing inhibitors: An overview," Biotechnol. Adv., vol. 31, no. 2, pp. 224–245, 2013, doi: 10.1016/j.biotechadv.2012.10.004.

[48] S. Yaron and U. Römling, "Biofilm formation by enteric pathogens and its role in plant colonization and persistence," Microb. Biotechnol., vol. 7, no. 6, pp. 496–516, 2014, doi: 10.1111/1751-7915.12186.

[49] T. C. Dakal and S. S. Cameotra, "Microbially induced deterioration of architectural heritages: Routes and mechanisms involved," Environ. Sci. Eur., vol. 24, no. 1, pp. 1–13, 2012, doi: 10.1186/2190-4715-24-36.

[50] R. J. Fair and Y. Tor, "Antibiotics and bacterial resistance in the 21st century," Perspect. Medicin. Chem., no. 6, pp. 25–64, 2014, doi: 10.4137/PMC.S14459.

[51] A. Z. Bialvaei and H. Samadi Kafil, "Colistin, mechanisms and prevalence of resistance," Curr. Med. Res. Opin., vol. 31, no. 4, pp. 707–721, 2015, doi: 10.1185/03007995.2015.1018989.

[52] O. Fleitas Martínez et al., "Interference With Quorum-Sensing Signal Biosynthesis as a Promising Therapeutic Strategy Against Multidrug-Resistant Pathogens," Front. Cell. Infect. Microbiol., vol. 8, no. February, 2019, doi: 10.3389/fcimb.2018.00444.

[53] K. J. Aldred and R. J. Kerns, "Mechanism of Quinolone Action and Resistance," 2014.

[54] K. Poole, "Cellular and Molecular Life Sciences Resistance to b -lactam antibiotics," vol. 61, pp. 2200–2223, 2004, doi: 10.1007/s00018-004-4060-9.

[55] M. Anwarul et al., "Antimicrobial Stewardship: Fighting Antimicrobial Resistance and Protecting Global Public Health," 2020, doi: 10.2147/IDR.S290835.

[56] D.Fuentenu and E.B.M. Breidenstein, Pseudomonas aerginos all road lead to resistance , vol.19,no.8, pp.419 to 426, 2011.

[57] M. Morgenstern, "Antibiotic resistance of commensal Staphylococcus aureus and coagulase-negative staphylococci in an international cohort of surgeons:

A prospective point-prevalence study," PLoS One, vol. 11, no. 2, pp. 1–16, 2016, doi: 10.1371/journal.pone.0148437.

[58] L. R. Mulcahy , "Emergence of Pseudomonas aeruginosa Strains Producing High Levels of Persister Cells in Patients with Cystic Fibrosis Emergence of Pseudomonas aeruginosa Strains Producing High Levels of Persister Cells in Patients with Cystic Fibrosis ," 2010, doi: 10.1128/JB.01651-09.

[59] O. Fleitas Martínez et al., "Interference With Quorum-Sensing Signal Biosynthesis as a Promising Therapeutic Strategy Against Multidrug-Resistant Pathogens," Front. Cell. Infect. Microbiol., vol. 8, no. February, 2019, doi: 10.3389/fcimb.2018.00444.

[60] T. L. Fischer, R. J. White, K. F. K. Mares, D. E. Molnau, and J. J. Donato, "UcFabV Requires Functional Reductase Activity to Confer Reduced Triclosan Susceptibility in Escherichia coli," J. Mol. Microbiol. Biotechnol., vol. 25, no. 6, pp. 394–402, 2015, doi: 10.1159/000441640.

[61] Y. Chang ., "Design, synthesis and evaluation of halogenated furanone derivatives as quorum sensing inhibitors in Pseudomonas aeruginosa," Eur. J. Pharm. Sci., vol. 140, p. 105058, 2019, doi: 10.1016/j.ejps.2019.105058.

[62] H. Kusada, H. Tamaki, Y. Kamagata, S. Hanada, and N. Kimura, "A novel quorumquenching N-acylhomoserine lactone acylase from Acidovorax sp. strain MR-S7 mediates antibiotic resistance," Appl. Environ. Microbiol., vol. 83, no. 13, 2017, doi: 10.1128/AEM.00080-17.

[63] Y. H. Dong, L. H. Wang, and L. H. Zhang, "Quorum-quenching microbial infections: Mechanisms and implications," Philos. Trans. R. Soc. B Biol. Sci., vol. 362, no. 1483, pp. 1201–1211, 2007, doi: 10.1098/rstb.2007.2045.

[64] P. C. Shih and C. T. Huang, "Effects of quorum-sensing deficiency on Pseudomonas aeruginosa biofilm formation and antibiotic resistance," J. Antimicrob. Chemother., vol. 49, no. 2, pp. 309–314, 2002, doi: 10.1093/jac/49.2.309.

[65] M. R. Parsek, D. L. Val, B. L. Hanzelka, J. E. Cronan, and E. P. Greenberg, "Acyl homoserine-lactone quorum-sensing signal generation," Proc. Natl. Acad. Sci. U. S. A., vol. 96, no. 8, pp. 4360–4365, 1999, doi: 10.1073/pnas.96.8.4360.

[66] E. A. Yates, "N-acylhomoserine lactones undergo lactonolysis in a pH-, temperature-, and acyl chain length-dependent manner during growth of Yersinia pseudotuberculosis and Pseudomonas aeruginosa," Infect. Immun., vol. 70, no. 10, pp. 5635–5646, 2002, doi: 10.1128/IAI.70.10.5635-5646.2002.

[67] H. B. Zhang, L. H. Wang, and L. H. Zhang, "Detection and analysis of quorum-quenching enzymes against acyl homoserine lactone quorum-sensing signals.," Curr. Protoc. Microbiol., vol. Chapter 1, pp. 1–15, 2007, doi: 10.1002/9780471729259.mc01c03s05.

[68] L. R. Swem et al., "A Quorum-Sensing Antagonist Targets Both Membrane-Bound and Cytoplasmic Receptors and Controls Bacterial Pathogenicity," Mol. Cell, vol. 35, no. 2, pp. 143–153, 2009, doi: 10.1016/j.molcel.2009.05.029.

[69] J. Carette, A. Nachtergael, P. Duez, M. El Jaziri, and T. Rasamiravaka, " Natural Compounds Inhibiting Pseudomonas aeruginosa Biofilm Formation by Targeting Quorum Sensing Circuitry ," Bact. Biofilms, no. i, pp. 1–22, 2020, doi: 10.5772/intechopen.90833.

[70] U. Müh, M. Schuster, R. Heim, A. Singh, E. R. Olson, and E. P. Greenberg, "Novel Pseudomonas aeruginosa quorum-sensing inhibitors identified in an ultra-high-throughput screen," Antimicrob. Agents Chemother., vol. 50, no. 11, pp. 3674–3679, 2006, doi: 10.1128/AAC.00665-06.

[71] A. Jahoor et al., "Peroxisome proliferator-activated receptors mediate host cell proinflammatory responses to Pseudomonas aeruginosa autoinducer," J. Bacteriol., vol. 190, no. 13, pp. 4408–4415, 2008, doi: 10.1128/JB.01444-07.

[72] T. Bjarnsholt et al., "Silver against Pseudomonas aeruginosa biofilms," Apmis, vol. 115, no. 8, pp. 921–928, 2007, doi: 10.1111/j.1600-0463.2007.apm-646.x.

[73] S. Ghosh et al., "Phytocompound Mediated Blockage of Quorum Sensing Cascade in ESKAPE Pathogens," Antibiotics, vol. 11, no. 1, pp. 1–22, 2022, doi: 10.3390/antibiotics11010061.

[74] F. M. Husain et al., "Seed Extract of Psoralea corylifolia and Its Constituent Bakuchiol Impairs AHL-Based Quorum Sensing and Biofilm Formation in Food- and Human-Related Pathogens," Front. Cell. Infect. Microbiol., vol. 8, no. October, pp. 1–16, 2018, doi: 10.3389/fcimb.2018.00351.

[75] I. A. Sybiya Vasantha Packiavathy, P. Agilandeswari, K. S. Musthafa, S. Karutha Pandian, and A. Veera Ravi, "Antibiofilm and quorum sensing inhibitory potential of Cuminum cyminum and its secondary metabolite methyl eugenol against Gram negative bacterial pathogens," Food Res. Int., vol. 45, no. 1, pp. 85–92, 2012, doi: 10.1016/j.foodres.2011.10.022.

[76] M. Hentzer et al., "Inhibition of quorum sensing in Pseudomonas aeruginosa biofilm bacteria by a halogenated furanone compound," Microbiology, vol. 148, no. 1, pp. 87–102, 2002, doi: 10.1099/00221287-148-1-87.

[77] M. A. Shah, J. E. Keach, and P. Panichayupakaranant, "Natural Products Chemistry of Global Tropical and Subtropical Plants Antidiabetic Naphthoquinones and Their Plant Resources in Thailand," vol. 66, no. 5, pp. 483–492, 2018.

[78] A. K. Irino, Y. T. Akasuka, A. N. Ishi, S. K. Awabe, and H. Y. Amashita, "Analysis and Functionality of Major Polyphenolic Components of Polygonum cuspidatum (Itadori)," pp. 278–286, 2012.

[79] "REYNOUTRIA JAPONICA Houttuyn, Nat. Hist. 2(8): 640, t. 51(1). 1777."

[80] J. Patocka, Z. Navratilova, and M. Ovando, "(Reynoutria spp .)," vol. 86, no. 1, pp. 17–31, 2017, doi: 10.31482/mmsl.2017.004.

[81] V. K. Tandon, R. V. Singh, and D. B. Yadav, "Synthesis and evaluation of novel 1,4-naphthoquinone derivatives as antiviral, antifungal and anticancer agents," Bioorganic Med. Chem. Lett., vol. 14, no. 11, pp. 2901–2904, 2004, doi: 10.1016/j.bmcl.2004.03.047.

[82] B. B. Shen et al., "Analysis of the Phytochemistry and Bioactivity of the Genus Polygonum of Polygonaceae," Digit. Chinese Med., vol. 1, no. 1, pp. 19–36, 2018, doi: 10.1016/S2589-3777(19)30005-9.

[83] J. Lee ., "Emodin 8-O-glucoside primes macrophages more strongly than emodin aglycone via activation of phagocytic activity and TLR-2/MAPK/NF-KB signalling pathway," Int. Immunopharmacol., vol. 88, no. April, p. 106936, 2020, doi: 10.1016/j.intimp.2020.106936.

[84] W. Peng, R. Qin, X. Li, and H. Zhou, "Botany, phytochemistry, pharmacology, and potential application of Polygonum cuspidatum Sieb.et Zucc.: A review," J. Ethnopharmacol., vol. 148, no. 3, pp. 729–745, Jul. 2013, doi: 10.1016/J.JEP.2013.05.007.

[85] W. Sun et al., "2-Methoxy-6-acetyl-7-methyljuglone (MAM), a natural naphthoquinone, induces NO-dependent apoptosis and necroptosis by H2O2-dependent JNK activation in cancer cells," Free Radic. Biol. Med., vol. 92, pp. 61–77, 2016, doi: 10.1016/j.freeradbiomed.2016.01.014.

[86] J. H. Kim, A. A. K. Khalil, H. J. Kim, S. E. Kim, and M. J. Ahn, "2-methoxy-7-acetonyljuglone isolated from reynoutria japonica increases the activity of nuclear factor erythroid 2-related factor-2 through inhibition of ubiquitin degradation in HeLa cells," Antioxidants, vol. 8, no. 9, pp. 1–10, 2019, doi: 10.3390/antiox8090398.

[87] "Juglone: A Versatile Natural Platform for Obtaining New Bioactive Compounds Author(s): Caroline dos S. Moreira, Thaís B. Santos, Rosana H.C.N.

Freitas, Paulo A.F. Pacheco and David R. da Rocha* Volume 21 , Issue 22 , 2021 Published on: 04 August, 2021."

[88] D. Shrimali ., "Targeted abrogation of diverse signal transduction cascades by emodin for the treatment of inflammatory disorders and cancer," Cancer Lett., vol. 341, no. 2, pp. 139–149, 2013, doi: 10.1016/j.canlet.2013.08.023.

[89] Y. Matsuda et al., "One-year chronic toxicity study of Aloe arborescens Miller var. natalensis Berger in Wistar Hannover rats. A pilot study," Food Chem. Toxicol., vol. 46, no. 2, pp. 733–739, 2008, doi: 10.1016/j.fct.2007.09.107.

[90] G. Lv, Z. Lou, S. Chen, H. Gu, and L. Shan, "Pharmacokinetics and tissue distribution of 2-3-4- tetrahydroxystilbene-2-o-b-d-glucoside from traditional Chinese medicine Polygonum multiflorum following oral administration to rats," J. Ethnopharmacol., vol. 137, no. 1, pp. 449–456, 2011, doi: 10.1016/j.jep.2011.05.049.

[91] X. J. Zhao et al., "Polydatin prevents fructose-induced liver inflammation and lipid deposition through increasing miR-200a to regulate Keap1/Nrf2 pathway," Redox Biol., vol. 18, no. July, pp. 124–137, 2018, doi: 10.1016/j.redox.2018.07.002.

[92] R. P. Li ., "Polydatin protects learning and memory impairments in a rat model of vascular dementia," Phytomedicine, vol. 19, no. 8–9, pp. 677–681, 2012, doi: 10.1016/j.phymed.2012.03.002.

[93] Y. Kimura and H. Okuda, "Effects of Naturally Occurring Stilbene Glucosides from Medicinal Plants and Wine, on Tumour Growth and Lung Metastasis in Lewis Lung Carcinoma-Bearing Mice," J. Pharm. Pharmacol., vol. 52, no. 10, pp. 1287–1295, 2010, doi: 10.1211/0022357001777270.

[94] S. H. Ban, Y. R. Kwon, S. Pandit, Y. S. Lee, H. K. Yi, and J. G. Jeon, "Effects of a bio-assay guided fraction from Polygonum cuspidatum root on the viability, acid production and glucosyltranferase of mutans streptococci," Fitoterapia, vol. 81, no. 1, pp. 30–34, 2010, doi: 10.1016/j.fitote.2009.06.019.

[95] Y. R. Kwon, K. J. Son, S. Pandit, J. E. Kim, K. W. Chang, and J. G. Jeon, "Bioactivity-guided separation of anti-acidogenic substances against Streptococcus mutans UA 159 from Polygonum cuspidatum," Oral Dis., vol. 16, no. 2, pp. 204–209, 2010, doi: 10.1111/j.1601-0825.2009.01636.x.

[96] D. B. Niesen, C. Hessler, and N. P. Seeram, "Beyond resveratrol: A review of natural stilbenoids identified from 2009-2013," J. Berry Res., vol. 3, no. 4, pp. 181–196, 2013, doi: 10.3233/JBR-130062.

[97] B. Salehi et al., "Resveratrol: A double-edged sword in health benefits," Biomedicines, vol. 6, no. 3, pp. 1–20, 2018, doi: 10.3390/biomedicines6030091.

[98] P. Fan, A. Marston, A. E. Hay, and K. Hostettmann, "Rapid separation of three glucosylated resveratrol analogues from the invasive plant Polygonum cuspidatum by high-speed countercurrent chromatography," J. Sep. Sci., vol. 32, no. 17, pp. 2979–2984, 2009, doi: 10.1002/jssc.200900057.

[99] B. Shan, Y. Z. Cai, J. D. Brooks, and H. Corke, "Antibacterial properties of Polygonum cuspidatum roots and their major bioactive constituents," Food Chem., vol. 109, no. 3, pp. 530–537, 2008, doi: 10.1016/j.foodchem.2007.12.064.

[100] L. Paulo, S. Ferreira, E. Gallardo, J. A. Queiroz, and F. Domingues, "Antimicrobial activity and effects of resveratrol on human pathogenic bacteria," World J. Microbiol. Biotechnol., vol. 26, no. 8, pp. 1533–1538, 2010, doi: 10.1007/s11274-010-0325-7.

[101] Z. He, Z. Huang, W. Zhou, Z. Tang, R. Ma, and J. Liang, "Anti-biofilm activities from resveratrol against: Fusobacterium nucleatum," Front. Microbiol., vol. 7, no. JUL, pp. 1–9, 2016, doi: 10.3389/fmicb.2016.01065.

[102] W. B. Wang, H. C. Lai, P. R. Hsueh, R. Y. Y. Chiou, S. Bin Lin, and S. J. Liaw, "Inhibition of swarming and virulence factor expression in Proteus mirabilis by resveratrol," J. Med. Microbiol., vol. 55, no. 10, pp. 1313–1321, 2006, doi: 10.1099/jmm.0.46661-0.

[103] A. A. K. Khalil, W. S. Park, H. J. Kim, K. M. Akter, and M. J. Ahn, "Anti-helicobacter pylori compounds from polygonum cuspidatum," Nat. Prod. Sci., vol. 22, no. 3, pp. 220–224, 2016, doi: 10.20307/nps.2016.22.3.220.

[104] Y. Han, J. Zhang, C. Q. Hu, X. Zhang, B. Ma, and P. Zhang, "In silico ADME and toxicity prediction of ceftazidime and its impurities," Front. Pharmacol., vol. 10, no. APR, pp. 1–12, 2019, doi: 10.3389/fphar.2019.00434.

[105] A. Kacmaz et al., "Synthesis and antiproliferative evaluation of some 1,4-naphthoquinone derivatives against human cervical cancer cells," Open Chem., vol. 17, no. 1, pp. 337–345, 2019, doi: 10.1515/chem-2019-0030.

[106] M. J. Pang, Z. Yang, X. L. Zhang, Z. F. Liu, J. Fan, and H. Y. Zhang, "Physcion, a naturally occurring anthraquinone derivative, induces apoptosis and autophagy in human nasopharyngeal carcinoma," Acta Pharmacol. Sin., vol. 37, no. 12, pp. 1623–1640, 2016, doi: 10.1038/aps.2016.98.

[107] L. Xie, H. Tang, J. Song, J. Long, L. Zhang, and X. Li, "Chrysophanol: a review of its pharmacology, toxicity and pharmacokinetics," J. Pharm. Pharmacol., vol. 71, no. 10, pp. 1475–1487, 2019, doi: 10.1111/jphp.13143.

[108] E. Yuriev and P. A. Ramsland, "Latest developments in molecular docking: 2010-2011 in review," J. Mol. Recognit., vol. 26, no. 5, pp. 215–239, 2013, doi: 10.1002/jmr.2266.

[109] "Rani, G.J., M. Vinoth, and P. Anitha, Molecular docking studies on oxidosqualene cyclase with 4- piperidinopyridine and 4-piperidinopyrimidine as its inhibitors. Journal of Bioinformatics and Sequence Analysis, 2011. 3(3): p. 31-36."

[110] B. K. Shoichet, S. L. McGovern, B. Wei, and J. J. Irwin, "Lead discovery using molecular docking," Curr. Opin. Chem. Biol., vol. 6, no. 4, pp. 439–446, 2002, doi: 10.1016/S1367-5931(02)00339-3.

[111] N. S. Pagadala, K. Syed, and J. Tuszynski, "Software for molecular docking: a review," Biophys. Rev., vol. 9, no. 2, pp. 91–102, 2017, doi: 10.1007/s12551-016-0247-1.

[112] N. C. Reading and V. Sperandio, "Quorum sensing: The many languages of bacteria," FEMS Microbiol. Lett., vol. 254, no. 1, pp. 1–11, 2006, doi: 10.1111/j.1574-6968.2005.00001.x.

[113] P. D. Lister, D. J. Wolter, and N. D. Hanson, "Antibacterial-resistant Pseudomonas aeruginosa: Clinical impact and complex regulation of chromosomally encoded resistance mechanisms," Clin. Microbiol. Rev., vol. 22, no. 4, pp. 582–610, 2009, doi: 10.1128/CMR.00040-09.

[114] E. P. Abraham, "The Antibiotics," Compr. Biochem., vol. 11, no. 4, pp. 181–224, 1963, doi: 10.1016/B978-1-4831-9711-1.50022-3.

[115] E. Déziel et al., "The contribution of MvfR to Pseudomonas aeruginosa pathogenesis and quorum sensing circuitry regulation: Multiple quorum sensing-regulated genes are modulated without affecting IasRI, rhIRI or the production of N-acyl-L-homoserine lactones," Mol. Microbiol., vol. 55, no. 4, pp. 998–1014, 2005, doi: 10.1111/j.1365-2958.2004.04448. x.

[116] R. Le Berre, K. Faure, S. Nguyen, M. Pierre, F. Ader, and B. Guery, "Quorum sensing : une nouvelle cible thérapeutique pour Pseudomonas aeruginosa," Med. Mal. Infect., vol. 36, no. 7, pp. 349–357, 2006, doi: 10.1016/j.medmal.2006.01.008.

[117] Cheng, J., M.J. Sweredoski, and P. Baldi, Accurate prediction of protein disordered regions by mining protein structure data. Data mining and knowledge discovery, 2005. 11(3): p. 213-222.

[118] Wilkins, M.R., ., Protein identification and analysis tools in the ExPASy server. Methods Mol Biol, 1999. 112: p. 531-52.

[119] Oany, A., Computational Structure Analysis and Function Prediction of an Uncharacterized Protein (I6U7D0) of Pyrococcus furiosus COM1. Austin Journal of Computational Biology and Bioinformatics, 2014. 1: p. 1-5.

[120] Protein complex prediction with AlphaFold-Multimer. Evans R, O'Neill M, Pritzel A, et al. bioRxiv 2021. doi: https://doi.org/10.1101/2021.10.04.463034

[121] Pasi, M., et al., xPyder: a PyMOL plugin to analyze coupled residues and their networks in protein structures. Journal of chemical information and modeling, 2012. 52(7): p. 1865-1874.

[122] Punta, M., et al., The Pfam protein families database. Nucleic Acids Res, 2012. 40 (Database issue): p. D290-301.

[123] Butkiewicz, M., et al., Benchmarking ligand-based virtual High-Throughput Screening with the PubChem database. Molecules, 2013. 18(1): p. 735-756.

[124] C. A. Lipinski, "Lead-and drug-like compounds: the rule-of-five revolution," Drug Discovery Today: Technologies, vol. 1, pp. 337-341, 2004.

[125] Punta, M., et al., The Pfam protein families database. Nucleic Acids Res, 2012. 40(Database issue): p. D290-301.

[126] Gschwend, D.A., A.C. Good, and I.D. Kuntz, Molecular docking towards drugdiscovery. Journal of Molecular Recognition: An Interdisciplinary Journal, 1996. 9(2): p. 175-186.

[127] McConkey BJ, Sobolev V, Edelman M. The performance of current methods in ligand-protein docking. Current Science. 2002; 83:845–855.

[128] J. Dundas, Z. Ouyang, J. Tseng, A. Binkowski, Y. Turpaz, and J. Liang, "CASTp: computed atlas of surface topography of proteins with structural and topographical mapping of functionally annotated residues," Nucleic acids research, vol. 34, pp. W116-W118,.

[129] Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 2000 Jan 1;28(1):27-30. doi: 10.1093/nar/28.1.27. PMID: 10592173; PMCID: PMC102409.

[130] Verdonk ML, Cole JC, Hartshorn MJ, Murray CW, Taylor RD. Improved proteinligand docking using GOLD. Proteins. 2003;52:609–23.

[131] J. Kyte and R. F. Doolittle, "A simple method for displaying the hydropathic character of a protein," J. Mol. Biol., vol. 157, no. 1, pp. 105–132, 1982, doi: 10.1016/0022-2836(82)90515-0.

[132] A. Jaiswal, A. Chhabra, U. Malhotra, S. Kohli, and V. Rani, "Comparative analysis of human matrix metalloproteinases: Emerging therapeutic targets in diseases," Bioinformation, vol. 6, pp. 23-30, 2011.

[133] A. Altunkaya et al., "OUP accepted manuscript," Nucleic Acids Res., vol. 45, no. October 2016, pp. 271–281, 2016, doi: 10.1093/nar/gkw1000.

[134] A. Mitchell et al., "The InterPro protein families database: The classification resource after 15 years," Nucleic Acids Res., vol. 43, no. D1, pp. D213–D221, 2015, doi: 10.1093/nar/gku1243.

[135] Z. Zhang, Y. Li, B. Lin, M. Schroeder, and B. Huang, "Identification of cavities on protein surface using multiple computational approaches for drug binding site prediction," Bioinformatics, vol. 27, no. 15, pp. 2083–2088, 2011, doi: 10.1093/bioinformatics/btr331.

[136] M. Cammisa, A. Correra, G. Andreotti, and M. V. Cubellis, "Identification and analysis of conserved pockets on protein surfaces," BMC Bioinformatics, vol. 14, no. SUPPL7, pp. 1–9, 2013, doi: 10.1186/1471-2105-14-S7-S9.

[137] A. Daina, O. Michielin, and V. Zoete, "SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules," Sci. Rep., vol. 7, no. March, pp. 1–13, 2017, doi: 10.1038/srep42717.

[138] Genoni, A., Pennati, M., Morra, G., Zaffaroni, N., and Colombo, G. (2012). Ligand selection from the analysis of protein conformational substates: new leads targeting the N-terminal domain of Hsp90. RSC Advances, 2(10), 4268. doi:10.1039/c2ra00911k.

[139] Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, Han L, He J, He S, Shoemaker BA, Wang J, Yu B, Zhang J, Bryant SH. PubChem Substance and Compound databases. Nucleic Acids Res. 2016 Jan 4;44(D1):D1202-13. doi: 10.1093/nar/gkv951. Epub 2015 Sep 2.

[140] S. S. Azam and S. W. Abbasi, "Molecular docking studies for the identification of novel melatoninergic inhibitors for acetylserotonin-O-methyltransferase using different docking routines," Theor. Biol. Med. Model., vol. 10, no. 1, pp. 1–16, 2013, doi: 10.1186/1742-4682-10-63.

[141] Meiler J, Baker D. Rosettaligand: protein-small molecule docking with full side-chain flexibility. Proteins. 2006;65:538–48.

[142] Y. Liu, M. Grimm, W. tao Dai, M. chun Hou, Z. X. Xiao, and Y. Cao, "CB-Dock: a web server for cavity detection-guided protein–ligand blind docking," Acta Pharmacol. Sin., vol. 41, no. 1, pp. 138–144, 2020, doi: 10.1038/s41401-019-0228-6.

[143] L. RA and S. MB, "LigPlot+: multiple ligand-protein interaction diagrams for drug discovery," J. Chem. Inf. Model., vol. 51, pp. 2778–2786, 2011.

[144] A. Bhinge, P. Chakrabarti, K. Uthanumallian, K. Bajaj, K. Chakraborty, and R. Varadarajan, "Accurate detection of protein:ligand binding sites using molecular dynamics simulations," Structure, vol. 12, no. 11, pp. 1989–1999, 2004, doi: 10.1016/j.str.2004.09.005.

[145] D. E. Pires, T. L. Blundell, and D. B. Ascher, "pkCSM: predicting small molecule pharmacokinetic and toxicity properties using graph-based signatures," Journal of medicinal chemistry, vol. 58, pp. 4066-4072, 20.

[146] Feig, D.I., B. Soletsky, and R.J. Johnson, E ect of allopurinol on blood pressureof adolescents with newly diagnosed essential hypertension: a randomized trial. Jama, 2008. 300(8): p. 924-932.

[147] Perez-Rodr guez, L., F. Mougeot, and C. Alonso-Alvarez, Carotenoidbased-coloration predicts resistance to oxidative damage during immune challenge. Journal of Experimental Biology, 2010. 213(10): p. 1685-1690.

[148] Rao, A.V. and L.G. Rao, Carotenoids and human health. Pharmacol Res, 2007. 55(3): p. 207-216.

[149] Pechanova, O., et al., Effect of chronic N-acetylcysteine treatment on thedevelopment of spontaneous hypertension. Clin Sci (Lond), 2006. 110(2): p. 235-242.

[150] Q. Zhang, S. X. Zeng, and H. Lu, "Determination of maximum tolerated dose and toxicity of Inauhzin in mice," Toxicol. Reports, vol. 2, pp. 546–554, 2015, doi: 10.1016/j.toxrep.2015.02.011.

[151] Klein Geltink, R.I. and E.L. Pearce, The importance of methionine metabolism. Elife, 2019. 8:p.123-127.

[152] L. Z. Benet, C. M. Hosey, O. Ursu, and T. I. Oprea, "BDDCS, the Rule of 5 and drugability," Adv. Drug Deliv. Rev., vol. 101, pp. 89–98, 2016, doi: 10.1016/j.addr.2016.05.007.

[153] S. Crpa, "Carbapenem-resistant Pseudomonas aeruginosa (CRPA) Fact Carbapenem-resistant Pseudomonas aeruginosa (CRPA) Fact".

[154] K. M. Papp-Wallace, A. Endimiani, M. A. Taracila, and R. A. Bonomo, "Carbapenems: Past, present, and future," Antimicrob. Agents Chemother., vol. 55, no. 11, pp. 4943–4960, 2011, doi: 10.1128/AAC.00296-11.

[155] Y. Han, J. Zhang, C. Q. Hu, X. Zhang, B. Ma, and P. Zhang, "In silico ADME and toxicity prediction of ceftazidime and its impurities," Front. Pharmacol., vol. 10, no. APR, pp. 1–12, 2019, doi: 10.3389/fphar.2019.00434.

An Appendix