doi.org/10.36721/PJPS.2024.37.3.REG.651-662.1

Effective inhibition of *Sesbania grandiflora* bioactive compounds against C-di-GMP phosphodiesterase of *Pseudomonas aeruginosa*

Srilakshmi Ravi[1], Subashkumar Rathinasamy[2], Sivaranjini Annamalai[1,2]*, Shoba Gunasekaran[1]*, Balamuralikrishnan Balasubramanian[3], Souparnika Biju Reji[4], Arumugam Vijaya Anand[4], Santhosh Baboo Sethuraman[5] and Shivakumar Bandhumy Lingam[6]

[1]PG Department of Biotechnology, Dwaraka Doss Goverdhan Doss Vaishnav College, Arumbakkam, Chennai, Tamil Nadu, India
[2]Department of Biotechnology, Sri Ramakrishna College of Arts & Science (Autonomous), Coimbatore, Tamilnadu, India
[3]Department of Food Science and Biotechnology, Sejong University, Seoul, South Korea
[4]Department of Human Genetics and Molecular Biology, Bharathiar University, Coimbatore, Tamil Nadu, India
[5]PG and Research Department of Computer Science, Dwaraka Doss Goverdhan Doss Vaishnav College, Arumbakkam, Chennai, Tamil Nadu, India
[6]Department of Computer Science, Sri Ramakrishna College of Arts & Science (Autonomous), Coimbatore, Tamilnadu, India

Abstract: *Sesbania grandiflora* also known as Agasthya has potent antibiofilm activity and its bioactive compounds obtained from the leaves are medicarpin, isoniazid and 4-methyl oxazole. Extra cellular polymeric substances (EPS) created by the bacterium involve the formation of biofilm and this causes the infections such as nosocomial infections, and urinary tract infections. *Pseudomonas aeruginosa* has been linked with high levels of intracellular Cyclic-di-Guanosine Monophosphate (c-di-GMP; PA4781) in biofilm formation. In this study, Human BLAST analysis of c-di-GMP Phosphodiesterase has been carried out and it shows an insignificant result and it is believed to be a possible drug target for UTI infection caused by *P. aeruginosa*. Its protein structure was retrieved from PDB database which was subjected to molecular docking against *S. grandiflora* bioactive compounds and control drug ciprofloxacin. Compounds taken for the study were screened for ADMET properties and drug-likeliness properties. Molecular interaction analysis of c-di-GMP with medicarpin compound shows -6.75 Kcal/mol binding energy with two hydrogen bonds when compared to the control drug with -6.86 kcal/mol binding energy and two hydrogen bonds respectively. Hence, our findings in the current study suggest that medicarpin could be an inhibitor of c-di-GMP and possess anti-biofilm activity, which could be validated experimentally.

Keywords: Antibiofilm activity, bioactive compounds, *Sesbania grandiflora*, C-di-GMP phosphodiesterase, *Pseudomonas aeruginosa* and molecular docking.

INTRODUCTION

Phytomedicines are plant-based materials or formulations containing raw or refined ingredients from any parts of the plants (leaves, flowers etc.,) with a therapeutic benefit and used as nutritional supplements for the prevention of common infections or diseases in different medicinal systems. Due to their extensive pharmacological functions, natural products derived from plants have gained substantial attention in recent years. *Sesbania grandiflora* commonly referred to as Agathi or Agasthya has potent anti-biofilm activity (Abubakar *et al*., 2015; Binte Arfan *et al*., 2016). The phytochemical analysis of the *S. grandiflora* has shown the presence of various vital bioactive compounds among which medicarpin, isoniazid and 4-methyl oxazole seem to possess potent anti-biofilm properties. However, the medicinal role of *S. grandiflora* bioactive compounds is yet to explore against bacterial biofilm-causing infections (Arfan *et al*., 2016; Bhoumik & Dwivedi 2014; Gandhi *et al*., 2017; Bhokre *et al*., 2022). *Pseudomonas aeruginosa* is rod-shaped structure gram-negative bacteria, commonly found in environmental conditions and that can develop chronic biofilm infections such as nosocomial infections, urinary tract infections it is a contagious micro organism that is typically a normal flora or does not affect its host, but when the host's resistance is low, it may cause diseases (Bjarnsholt *et al*., 2011; Hall-Stoodley & Stoodley, 2009; Hall-Stoodley *et al*., 2004). Biofilm is a structural colony of sessile cells (bacteria or fungi) encapsulated in a self-produced extra cellular polymeric substances (EPS), DNA, peptides and other components. EPS have a significant part in the protection of microbial biofilm from adverse environmental factors such as high-temperature, pH, salt concentration and host immune defence and the unique function and mechanism of the bacteria present in the biofilm prohibits efficient use of traditional antibiotics (Chatterjee *et al*., 2016; Czaczyk & Myszka, 2007; Mulcahy *et al*., 2014; Zhang & Bishop, 2003) Initialization of the formation of biofilm in *Pseudomonas aeruginosa* has been associated with greater intracellular c-di-GMP (Cyclic-di-Guanosine Monophosphate) levels.

*Corresponding authors: e-mail: sivaranjinia@dgvaishnavcollege.edu.in; shobag@dgvaishnavcollege.edu.in

This causes adhesions and extracellular matrix components to be formed that allow bacteria to develop a biofilm (Flemming & Wingender, 2010; Ha & O'Toole, 2015; O'Toole *et al*., 2000; Parsek & Singh, 2003). The c-di-GMP is a nucleotide which is present in the *Pseudomonas aeruginosa* and it is a secondary messenger which controls a number of functions such as developmental changes, synthesis of polysaccharides, adhesions, the formation of biofilms, virulence factor synthesis and bacterial virulence (Fazli *et al*., 2014; Lee *et al*., 2007; Xu *et al.*, 2013).

The human pathogen *Pseudomonas aeruginosa* genome encodes two kinds of proteins with the domain HD-GYP and another protein (PA2572) with the alternative key residue domain (YN-GYP) (An *et al*., 2010; Klockgether *et al*., 2013; Ryan *et al.*, 2009). Phosphodiesterase (PDE) is an enzyme with HD-GYP domain that catalyses the hydrolysis reaction of cyclic diguanylate (c-di-GMP) into GMP.

It is a two-step reaction. It hydrolyzes the c-di-GMP through an intermediate pGpG (5'- Phosphoguanylyl (3'>5') guanosine. In vitro, pGpG can be used as an alternative substrate and can be hydrolyzed into GMP (Kulesekara *et al*., 2006; Valentini & Filloux, 2016). The PA4781 (c-di-GMP Phosphodiesterase) mutation leads to a diverse increase of c-di-GMP levels in *P. aeruginosa* and produces a biofilm with little diversity that encased a large percentage of the static surface, including a few structures like mushrooms. PA4781 is a cyclic-di-GMP Phosphodiesterase (c-di-GMP PDE) that controls the synthesis of various virulence factors such as pycocyanin, exos and pyoverdine (Kuchma *et al*., 2007; Newman *et al*., 2015; Rasamiravaka *et al*., 2015; Sauer *et al*., 2004). Targeting C-di-GMP Phosphodiesterase with natural bioactive compounds would be a novel method to curb the progression of biofilm-causing infections. The anti-biofilm feature of plant materials is attributed to the bioactive compounds present in them.

Biological active compounds are secondary plant secondary metabolites which are additional nutritional constituents found in low concentrations in plants that can control both metabolic and intracellular activity in human beings and animals (Rabin *et al.*, 2015; Soto, 2014). Glycosidic bonds are found inside the biofilm exopolysaccharide. Alkaloids present in plant extracts act against bacterial cells by damaging the cohesion of their plasma membrane. For example, H761 is a Quinoline-derived antimicrobial compound that is capable of disrupting the outer layer of bacteria by modulating it and dispersing the intercellular contents at higher or lower concentrations the minimal prohibition rate of the therapeutic agent (Lakshmi *et al*., 2011; Tuon *et al*., 2022; Slobodníková *et al*., 2016). Thus, this current study aims to explore the therapeutic value of *S. grandiflora* bioactive compounds viz., medicarpin, 4- methyl oxazole, isoniazid and control drug ciprofloxacin in targeting the C-di-GMP Phosphodiesterase of *P. aeruginosa* in comparison with Ciprofloxacin through a computational approach to progress further with experimental research.

MATERIALS AND METHODS

C-di-GMP Phosphodiesterase protein retrieval

Crystal structure (3D) of c-di-GMP PDE was reintegrated from RCSB PDB database (4R8Z) (https://www.rcsb.org/pdb). Using Auto Dock (ADT) 1.5.6 software, hydrogen bonds and Kollman charges were added to the protein retrieved from the PDB database (Guedes *et al*., 2014; Sivakumar *et al*., 2020).

Preparation of Ligand and Optimization

Selected ligands of SMILES format (medicarpin, 4-methyl oxazole, isoniazid and the control drug ciprofloxacin) were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/). Using Chemsketch software, the two-dimensional and three-dimensional structures of selected ligands were generated by using their canonical smiles. The ligands were optimized by using the same software. The optimized ligands were saved in mol format and then converted into Protein Data Bank (PDB) format by using Open Babel Molecular Converter Software (Guedes *et al*., 2014; Hutchison *et al.*, 2011).

Molinspiration analysis of the ligands

The pharmacological and physiochemical properties include, topological polar surface area (TPSA), partition coefficient (logP), molecular weight, number of rotatable bonds, hydrogen bond acceptors and donors for medicarpin, isoniazid, 4-methyl oxazole and the control drug ciprofloxacin were assessed by using Molinspiration web server (https://www.molinspiration.com/cgi-bin/properties). The molecular properties of the selected compounds based on the Lipinski's ''Rule of Five'' and the bioactivity scores (GPCR ligand, kinase inhibitor, protease inhibitor, nuclear receptor ligand, Ion channel modulator, and enzyme inhibitor) were assessed (Guedes *et al*., 2014).

Prediction of ADMET properties

The pharmacokinetic properties or ADMET of the selected peptides were predicted with help of the pkCSM web server (http://biosig.unimelb.edu.au/pkcsm/ prediction). pkCSM server is used to assess different parameters with their characteristics that allow us to evaluate the pharmacokinetic property of a drug. pkCSM is a web interface which provides pharmacokinetic properties and toxicity of the drugs by using the five above-mentioned parameters. The absorption of the chosen ligand was interpreted by using the characteristics such as Water solubility, Caco2 permeability, Intestinal absorption, Skin Permeability, P-glycoprotein substrate

and P-glycoprotein inhibitor I and II. Distribution property can be interpreted by the prediction of its characteristics Distribution, BBB permeability and CNS permeability. Cytochrome P450 model was used for the prediction of metabolism and total clearance and renal OCT2 substrate were used for the determination of the excretion property of the selected compounds. Toxicity was studied by using the AMES toxicity test, hepatotoxicity and skin sensitization parameters (Chaluveelaveedu *et al.*, 2016, Harmsen *et al.*, 2010).

Molecular docking interpretation

Molecular docking studies were used to analyze the affinity between medicarpin, isoniazid, 4-methyl oxazole and the control ciprofloxacin against C-di-GMP Phosphodiesterase of *P. aeruginosa* by using AutoDock tool. There are numerous intermediate steps such as .pdbqt, .dlg and .dpf files for the proteins and ligands. The creation of a grid box with a size of grid 126 × 126 × 126 Å and preparation of the grid map was completed using the graphical user source program ADT. The search parameter used in molecular docking was the Genetic algorithm and the output selected was Lamarckian Genetic Algorithm (Guedes *et al*., 2014; Ramalingam *et al*., 2019; Morris & Lim-Wilby, 2008).

Molecular docking visualization

Using Biovia Discovery Studio software, the following parameters were visualized protein-peptide interconnection such as hydrogen bonding, non-bonded energies between medicarpin, isoniazid, 4-methyl oxazole and the control ciprofloxacin against C-di-GMP Phosphodiesterase of *Pseudomonas aeruginosa* (Ramalingam *et al*., 2019; Sivakumar *et al*., 2020).

RESULTS

Structural retrieval of protein

Crystal structure (3D) of C-di-GMP Phosphodiesterase protein from *P. aeruginosa* (PAO1) was retrieved from the Protein Data Bank database and the structure Id of the protein was reported as 4R8Z. In the last stage eradication of water molecules and the combing of hydrogen atoms with the receptor, molecules were successful. The C-di-GMP Phosphodiesterase was visualized by using RASMOL software.

Structural retrieval of ligands

Using ACD Chemsketch software, the optimization of the ligand was accomplished and recovered in an appropriate format using Open Babel Software. table 1 displays the two-dimensional and three-dimensional configurations of the ligands and their smiles format.

Molecular property interpretations

Prediction of bioactivity scores for medicarpin, isoniazid, 4-methyl oxazole and the control ciprofloxacin relying on drug likelihood property and bioactivity score estimates were scored and tabulated in table 2. Elements molecular properties were determined with the help of Lipinski's ''Rule of Five'. For all the chosen compounds, the Polar Surface Area (PSA) value was <140Å, this value indicates higher absorption and promotes its oral biological administration. From the explanation, the selected compound ciprofloxacin and medicarpin indicated good biological activity (>0.20) towards an enzyme inhibitor. Comparing these two medicarpin shows strong bioactivity.

ADMET properties prediction

pkCSM was utilized to speculate the ADMET characteristics of the chosen peptides. The properties were displayed in table 3. The first property, absorption was predicted by using several parameters including CaCo2 penetrability, water solvability, skin permeability, intestinal absorption, P-glycoprotein substrate, P-glycoprotein I inhibitor and P-glycoprotein II inhibitor. For the prediction of CaCo2 permeability, if the compound has a papp value >8x 10^{-6} and (>0.90) the predicted value is deemed to have a greater CaCo2 permeability. In this sense, the selected ligand medicarpin and 4-methyl oxazole displayed good CaCo2 permeability with >0.90 predicted value.

As intestine is the main source of absorption, intestinal absorption parameter was utilized to predict the amount of drug absorbed by the small intestine. A compound with an absorption rate of less than 30% was deemed poorly absorbed. In this context, all the selected compounds reveals higher intestinal absorption with an absorption rate of>30%. Among all the compounds, ciprofloxacin showed greater intestinal absorption with 96.44%.

The water solubility of the chosen ligand was given as the logarithm of molar concentration. ATP binding cassette transporter (ABC) owns the P-glycoprotein. Its main function is to eject contaminants and any other toxic substances from the body and it acts as a biological shield. By using the P-glycoprotein substrate model we can predict whether a selected compound acts as a substrate of P-glycoprotein or not.

The current investigation can be speculated that only ciprofloxacin act as a substrate of Pgp. Inhibitors of P-glycoprotein aid to inhibit the P-glycoprotein and increase the bioavailability of the drug. There are two types of P-glycoprotein inhibitors class 1 & class 2. The P-glycoprotein inhibitor model was used to determine whether the selected drug acts as P-glycoprotein 1 & 2 inhibitors. The current study showed that all the selected ligands were not P-glycoprotein inhibitor classes 1 & 2.

Skin permeability is a major concern for the safety of many consumers and is of importance to the advancement

of drug delivery systems. Skin permeability was determined using the log Kp value. If the compound has a log Kp value >-2.5 is considered that the compound has low skin permeability. In this context, all the selected compounds displayed favourable skin permeability with log Kp value <-2.5 except 4-methyl oxazole with >-2.5. The distribution property of the selected ligands can be predicted by using many parameters.

The volume distribution is one among them. It is used to predict the uniform distribution of the drug in the blood plasma. The compound's volume distribution is deemed low if the predicted value is below 0.71 L/kg (Log VD value <-0.15) and it is high if the value is above 2.81 L/kg (Log VD value >0.45). According to that, all the selected compounds displayed low volume distribution.

Blood Brain Barrier (BBB) protects the brain from foreign particles. LogBB value is utilised to prognosticate BBB permeability. LogBB >0.3 is known to invade the BBB quickly (a drug given), but LogBB <1 molecules are poorly soluble in the brain. The current study shows that except medicarpin all other chosen compounds showed poor BBB penetrability.

LogPS value was used to predict the CNS permeability. Compounds with LogPS value >-2 were deemed to penetrate the CNS whereas compounds with LogPS < -3 were considered as the compounds that were not able to penetrate the CNS. From this study, it was revealed that CNS was penetrated by medicarpin while other compounds were unable to penetrate the CNS.

The metabolism of compounds was predicted by utilizing the Cytochrome P450 model. It is an essential detoxifying enzyme located primarily in the liver of the body. It oxidises foreign substances and promotes their excretion. Cytochrome P450 is involved in drug molecule activation and deactivation.

Therefore, it is essential to determine the ability of the drug to inhibit Cytochrome P450. Five various models of isoforms include CYP1A2, CYP2C19, CYP2C9, CYP3A4 and CYP2D6 for the prediction of Cytochrome P450 inhibitory ability of a drug. From the selected compounds, only medicarpin displayed an inhibitory effect on four isoforms (CYP1A2, CYP2C19, CYP2C9, CYP3A4) while isoniazid, 4-methyl oxazole and ciprofloxacin displayed no inhibitory effect against Cytochrome P450 inhibitor. Cytochrome P450 is necessary for the metabolism of certain drugs.

There are two isoforms (CYP2D6 and CYP3A4) of the Cytochrome P450 substrate used to determine the metabolism of the drug. The present study indicates that from the selected ligands only medicarpin was capable of serving as a CYP3A4 substrate.

Excretion property was predicted using Renal OCT2 substrate and total clearance parameters. OCT2 was used to determine whether the compound acts as an OCT2 substrate. From the current study, none of the compounds acts as an OCT2 substrate. Toxicity was predicted by using the AMES toxicity test, and hepatotoxicity parameters.

Fig. 1: Protein structure visualized in RASMOL

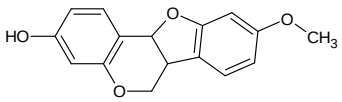
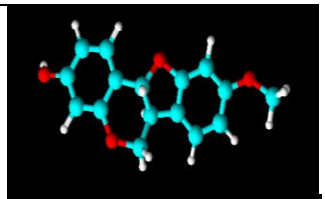
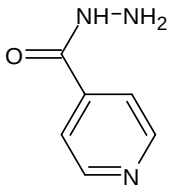
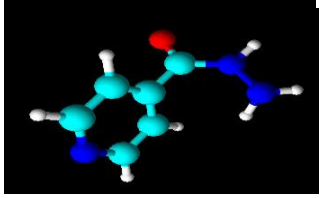
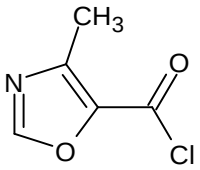
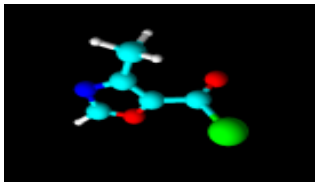
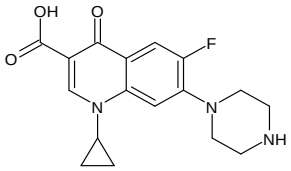
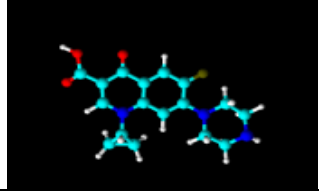
From the selected ligands, medicarpin and 4-methyl oxazole showed AMES toxicity and ciprofloxacin showed hepatotoxicity and the compound 4-methyl oxazole was identified as skin sensitization. Various other toxicity tests such as minnow toxicity here 1 & 2 were negative for all the selected ligands.

Molecular docking analysis

The selected ligands medicarpin, isoniazid, 4-methyl oxazole and the control ciprofloxacin were subjected to molecular docking analysis against C-di-GMP Phosphodiesterase. Using binding energy, the best conformer was chosen. The bond relationships between medicarpin, isoniazid, 4-methyl oxazole and the control ciprofloxacin and the protein C-di-GMP Phosphodiesterase of *P. aeruginosa were* visualized in a stick model by using Biovia Discovery Studio Visualisation software and it is displayed in fig. 2.

Compared to isoniazid with -5.08 Kcal/mol & 1 hydrogen bond and 4-methyl oxazole with -4.87 & 2 hydrogen bonds, the biocompound medicarpin obtained a promising inhibitory activity against C-di-GMP Phosphodiesterase with -6.75 Kcal/mol & 2 hydrogen bonds. The binding energy of the control drug Ciprofloxacin was displayed as -6.86 Kcal/mol & 2 hydrogen bonds. Torsional energy, intermolecular energy, and electrostatic energies for all the selected compounds were displayed in table 3 and Vanderwaal's, alkali interactions, salt bridges etc were displayed in table 4 and fig. 3.

Table 1: 2D and 3D structure of bioactive compounds from leaves of *Sesbania grandiflora*

Compound	Canonical smiles	2D Structure	3D Structure
Medicarpin	COC1=CC2=C(C=C1)C3COC 4=C(C3O2)C=CC(=C4)O		
Isoniazid	C1=CN=CC=C1C(=O)NN		
4-Methyloxazole	CC1=C(OC=N1)C(=O)Cl		
Ciprofloxacin	C1CC1N2C=C(C(=O)C3=CC(=C(C=C32)N4CCNCC4)F)C(= O)O		

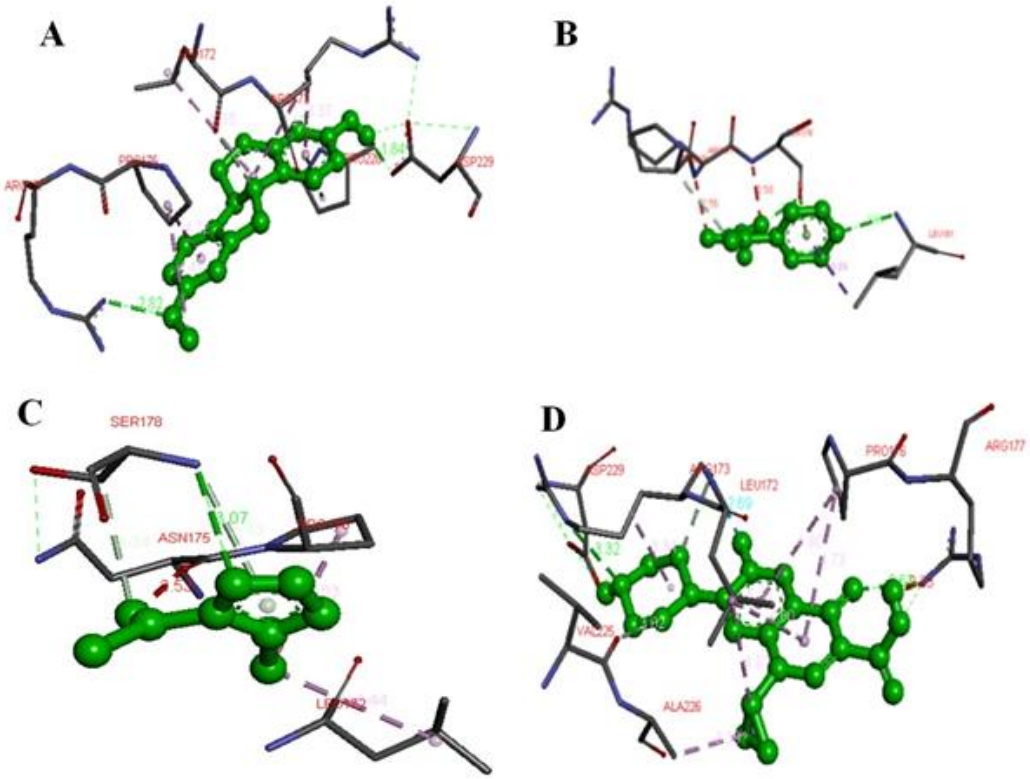


Fig. 2: Molecular Docking Interaction between PA4781 with ligands. a) PA4781 with medicarpin b) PA4781 with isoniazid c) PA4781 with 4-methyl oxazole d) PA4781 with ciprofloxacin interaction visualized by Biovia Discovery Studio Visualizer the ligand is illustrated in stick and ball model and PA4781 residue as a stick model.

Table 2.1: Molinspiration Interpretation

Compound name	Molecular weight	Molecular formula	Hydrogen bond donor	Hydrogen bond acceptor	LogP	Rotatable bonds	TPSA	Volume	N atoms
Medicarpin	270.28	C16H14O4	4	1	2.76	1	47.93	235.76	20
Isoniazid	137.14	C6H7N3O	4	3	-0.97	1	68.01	122.56	10
4- Methyloxazole	145.54	C4H5NO	3	0	1.14	1	43.10	110.53	9
Ciprofloxacin	331.35	C17H18FN3O3	6	2	-0.70	3	74.57	285.46	24

Table 2.2: Molinspiration Interpretation – Druglikeliness prediction and bioactivity score

S. no	Compounds	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1	Medicarpin	0.18	-0.18	-0.09	0.20	-0.36	0.48
2	Isoniazid	-1.39	-1.45	-1.05	-2.33	-1.23	-0.66
3	4- Methyloxazole	-2.52	-2.04	-2.85	-3.15	-3.43	-2.22
4	Ciprofloxacin	0.12	-0.04	-0.07	-0.19	-0.20	0.28

Table 3: Overall Docking Scores

Compound name	Number of Hydrogen Bonds	Binding Energy	Ligand Efficiency	Intermolecular energy	VdW + Hbonb + Desolve	Electrostatic Energy	Torsional Energy	Total Internal unbound
Medicarpin	2	- 6.75	-0.34	-7.35	-6.97	-0.37	0.6	0.05
Isoniazid	1	-5.08	-0.51	-5.67	-5.61	-0.07	0.6	0.08
4- Methyloxazole	2	-4.87	-0.54	-5.17	-5.21	0.04	0.3	-0.23
Ciprofloxacin	2	-6.86	-0.29	-8.06	-5.99	-2.07	1.19	-0.16

Table 4: Protein and Ligand interactions

PA4781 with ligand interactions	Hydrogen bond interactions					Hydrophobic interactions	Other interactions
	Residue	Atom	Ligand Atom	Distance	Docking energy		
PA4781 with Medicarpin	ASP229 ARG177	OD2 HH22	H O	1.84 2.82	-6.75	Alkyl/Pi –Alkyl PRO228 ARG173 PRO176	Carbon Hydrogen Bond LEU172
PA4781 with Isoniazid	Leu181 SER178	NH O	N H	2.85 2.18	-5.08	Nil	Carbon Hydrogen Bond PRO176 Pi-Sigma LEU181 Pi-Lone pair SER178
PA4781 with 4-Methyloxazole	SER178	NH	O	3.07	-4.87	Alkyl/Pi-alkyl LEU172 PRO176	Carbon Hydrogen Bond SER178 Pi-Donor Hydrogen bond SER178
PA4781 with Ciprofloxacin						Alkyl/Pi-alkyl ALA226 LEU172 PRO176	Halogen(Fluorine) ARG173 Carbon Hydrogen Bond VAL225

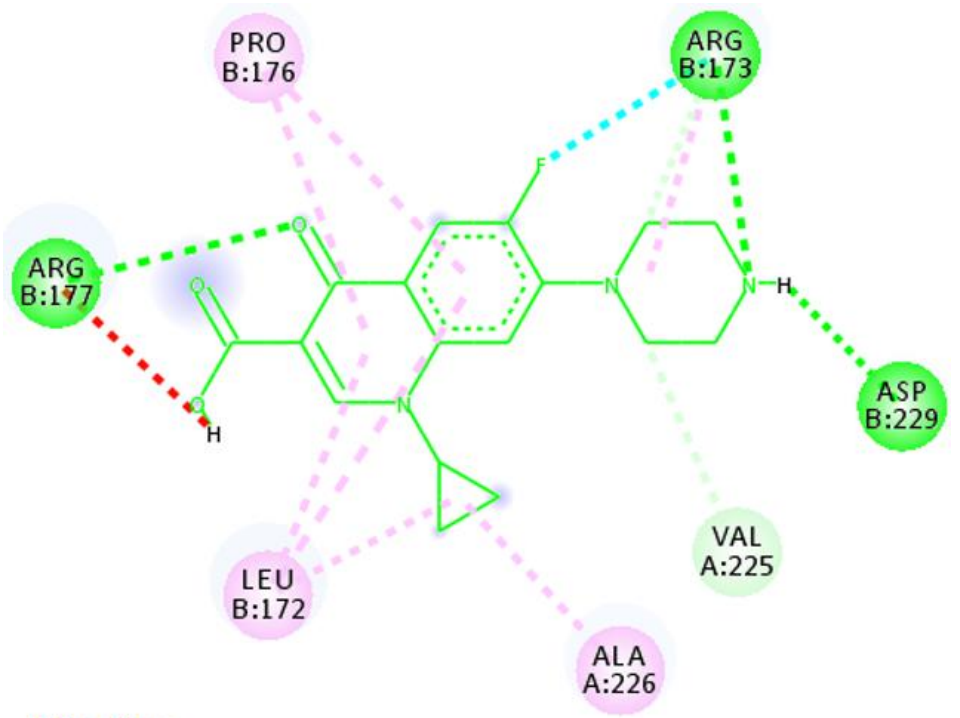
Table 5: ADMET Properties of the ligands

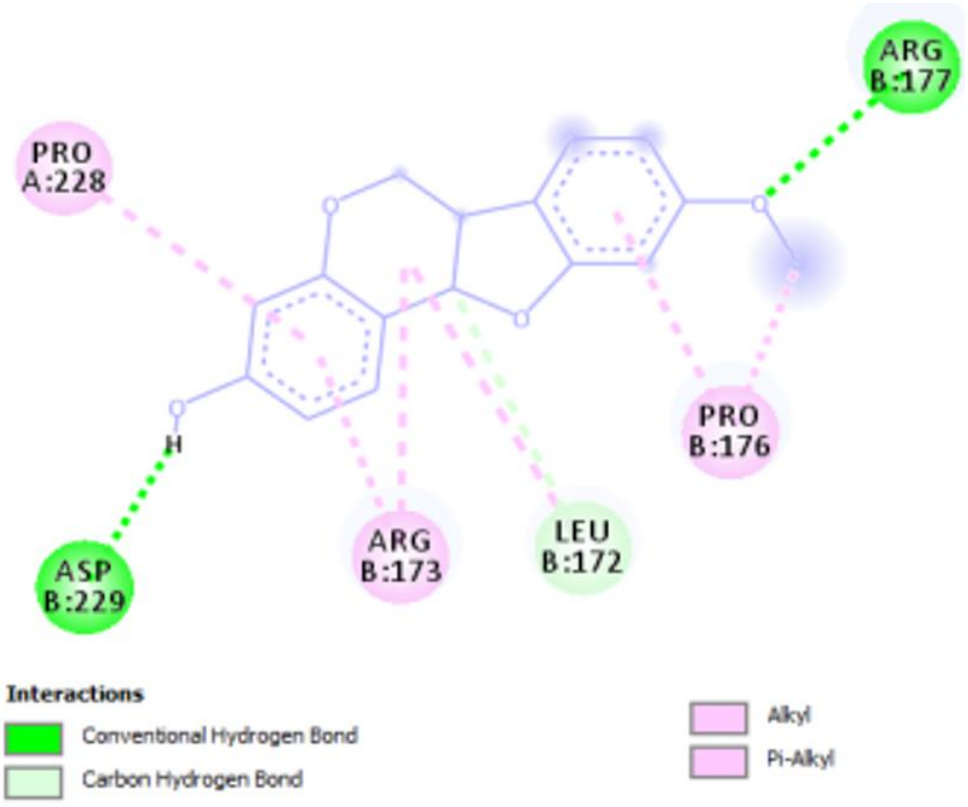
PARAMETERS	Medicarpin	Isoniazid	4-Methyloxazole	Ciprofloxacin
Absorption				
Water solubility	-3.459	-1.6	-0.51	-2.897
Caco2 permeability	1.246	0.52	1.282	0.492
Intestinal absorption	95.188	92.601	96.287	96.466
Skin Permeability	-2.819	-3.351	-2.438	-2.734
P-glycoprotein substrate	No	No	No	Yes
P-glycoprotein I inhibitor	No	No	No	No
P-glycoprotein II inhibitor	No	No	No	No
Distribution				
VDss (human)	0.065	-0.352	-0.36	-0.17
Fraction unbound (human)	0.04	0.728	0.654	0.648
BBB permeability	0.324	0.002	-0.308	-0.587
CNS permeability	-1.838	-3.351	-2.875	-2.999
Metabolism				
CYP2D6 substrate	No	No	No	No
CYP3A4 substrate	Yes	No	No	No
CYP1A2 inhibitor	Yes	No	No	No
CYP2C19 inhibitor	Yes	No	No	No
CYP2C9 inhibitor	Yes	No	No	No
CYP2D6 inhibitor	No	No	No	No
CYP3A4 inhibitor	Yes	No	No	No
Excretion				
Total Clearance	0.273	0.722	0.18	0.633
Renal OCT2 substrate	No	No	No	No
Toxicity				
AMES toxicity	Yes	No	Yes	No
Max. Tolerated dose (human)	-0.102	1.166	1.063	0.924
hERG I inhibitor	No	No	No	No
hERG II inhibitor	No	No	No	No
Oral Rat Acute Toxicity(LD50)	2.512	2.304	2.246	2.891
Oral Rat Chronic Toxicity(LOAEL)	1.875	1.395	2.07	1.036
Hepatotoxicity	No	No	No	Yes
Skin Sensitisation	No	No	Yes	No
T. Pyriformis toxicity	0.688	-0.134	0.254	0.286
Minnow toxicity	0.657	3.12	2.146	1.194

DISCUSSION

Cyclic-di-GMP Phosphodiesterase falls under the hydrolase group playing a vital role in the synthesis of adhesion molecules and exopolysaccharides that allow bacteria to develop biofilms and cause urinary tract infections. Cyclic-di-GMP Phosphodiesterase of *P. aeruginosa* may be regarded as the better target for unique biologically active therapeutic agents in the regulation of UTI (Hall-Stoodley & Stoodley, 2009; Hall-Stoodley *et al*., 2004; Hatt & Rather, 2008). *S. grandiflora* and its bioactive compounds which are phytochemically abundant in alkaloids, flavonoids and tannins are well known for their antibacterial and anti-biofilm ability (Haney *et al*., 2018; Wagh *et al*., 2009). A molecular simulation method is a molecular docking approach used in the current study to accomplish the interaction between bioactive compounds and the target C-di-GMP Phosphodiesterase.

The protein C-di-GMP Phosphodiesterase was recovered from the PDB database as an appropriate target depending on the information stored in the database and it is freely approachable. The current research shows favourable receptor-ligand complexes are formed by compounds such as medicarpin, isoniazid, 4- methyl oxazole, and the control ciprofloxacin. There are two major steps in molecular docking research which include the determination of the actual orientation of the conformers to the better active site residues named pose and then scoring accomplished relationship on the frequency of the target ligand binding. Biovia Discovery Studio is used for the prognosticate bonding relationships among C-di-GMP Phosphodiesterase of *P. aeruginosa* and the selected ligands. It yielded positive results with binding energies and hydrogen bonds. The best match was assessed by the hydrogen bonds involved with enthalpic gain caused by water molecules.


PRO
B:176
ARG
B:173
ARG
B:177
ASP
B:229
VAL
A:225
LEU
B:172
ALA
A:226
Interactions
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Halogen (Fluorine)
Unfavorable Donor-Donor
Alkyl
Pi-Alkyl


ARG
B:177
PRO
A:228
PRO
B:176
ASP
B:229
ARG
B:173
LEU
B:172
Interactions
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Alkyl
Pi-Alkyl

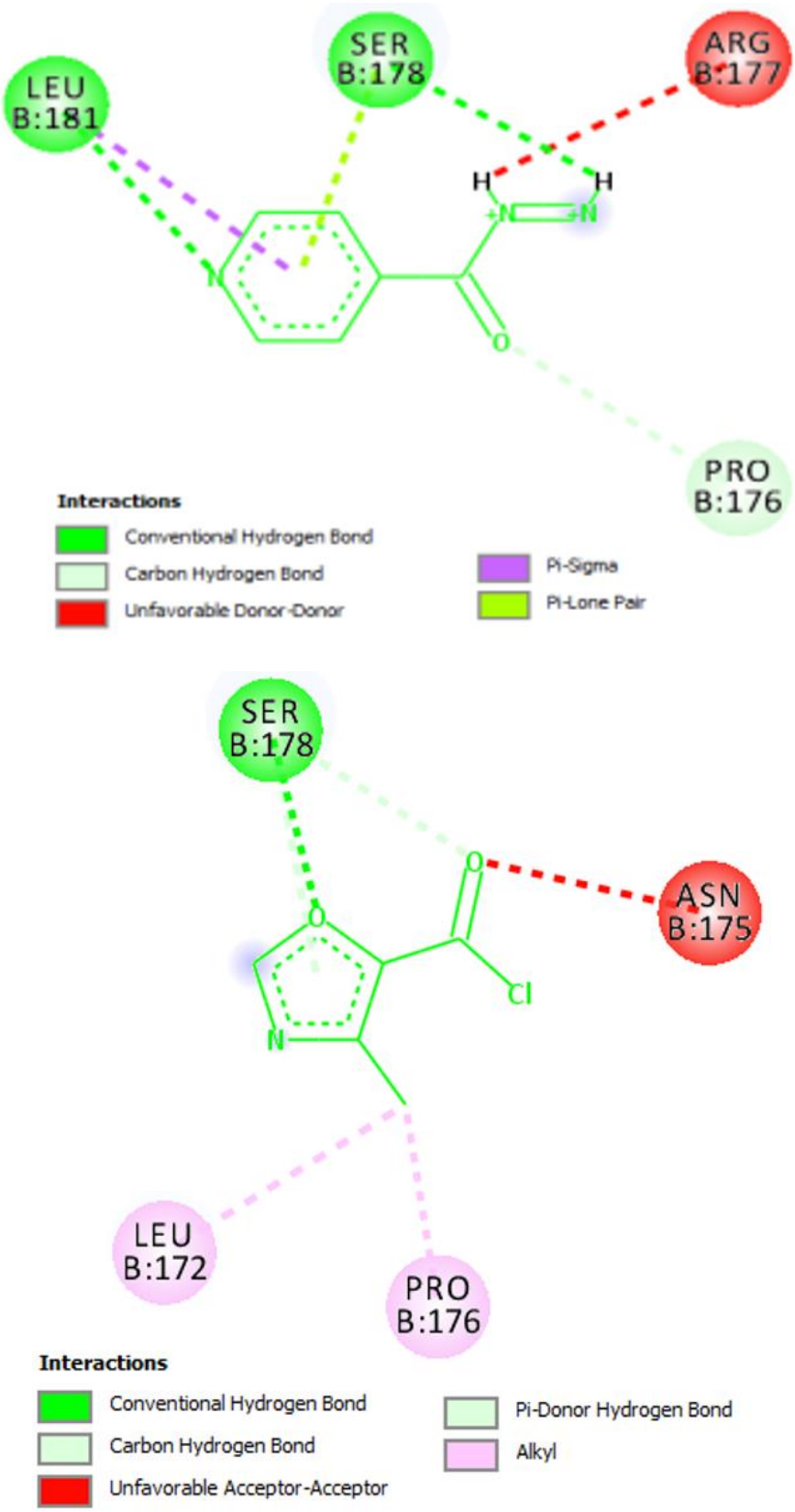


Fig. 3: Two-dimensional structure of molecular docking interaction between protein and ligands; a) PA4781 with medicarpin b) PA4781 with isoniazid c) PA4781 with 4-methyl oxazole d) PA4781 with ciprofloxacin.

Centred on this statement, medicarpin is the strongest inhibitor of C-di-GMP Phosphodiesterase with ionization energy of -6.75 Kcal/mol & 2 hydrogen bonds, whereas ciprofloxacin has a binding energy of -6.86 Kcal/mol & 2 hydrogen bonds (Lloyd *et al.*, 2023; Guedes *et al*., 2014; Reji & Rexin Alphonse, 2013; Zahir Hussain & Kumaresan, 2014).

We performed mol-inspiration calculations to test and determine the drug likelihood properties of the chosen ligands. This is related to the fact that the molecular characteristics including hydrophobicity, biological availability, and membrane permeability were correlated with certain specific molecular parameters such as molecular mass, hydrogen bond acceptor (atom acceptor), hydrogen bond donors (atom donor) and Partition coefficient (logP), that are strongly allocated to the design of novel drugs. In this current study, mol-inspiration studies were found favourable for medicarpin, isoniazid, and 4-methyl oxazole relative to the control drug ciprofloxacin indicating the positive C-di-GMP Phosphodiesterase inhibiting action of the chosen *S. grandiflora* biocompounds (Guedes *et al*., 2014, Ramalingam *et al.*, 2019).

TPSA of a compound is known to be a valuable parameter for the characterization of absorption of the drug and biological availability in mol-inspiration analysis, and the values of topological polar surface area and OH-NH intercommunication reveal the selected peptides medicarpin, isoniazid and 4-methyl oxazole have a smooth and effective bonding to the protein targets. Although, ligand molecules with >140Å TPSA values or higher have poor absorption and (milogP) lipophilicity and has a significant part in the estimation of oral drug availability. In this context, with a TPSA value of <140Å, all the chosen ligands achieve high absorption and better membrane permeability (Guedes *et al*., 2014, Ramalingam *et al.*, 2019).

Pharmacokinetic property is defined as the passage of drugs through the body, into the body and out of the body. It is necessary for drug molecules to exhibit excellent pharmacokinetic properties. So, the pharmacokinetic properties of a potential drug need to be calculated. pkCSM is a web interface that incorporates analytics to understand the pharmacokinetic properties of small molecules. The ADMET properties of the selected ligands were determined through the pkCSM study. The parameter includes Absorption, Distribution, Metabolism, Excretion and Toxicity. From the present investigation, medicarpin and 4-methyl oxazole show high CaCo2 permeability and isoniazid displayed low CaCo2 permeability. All the selected compounds showed greater intestinal absorption. From the selected ligands, only ciprofloxacin acts as a substrate of P-glycoprotein and all the chosen ligands are not P-glycoprotein inhibitor class 1 and 2. Except for 4-methyl oxazole, all the other compounds exhibit favourable skin permeability. All the selected ligands showed low Volume Distribution and exhibit poor BBB permeability and CNS permeability except medicarpin. Cytochrome P450 was utilized to prognosticate the metabolism of chosen compounds. From the selected ligands, medicarpin shows an inhibitory effect on four isoforms (CYP1A2, CYP2C19, CYP2C9, CYP3A4) and also medicarpin was able to act as a substrate for CYP3A4. In the excretion, none of the compounds acts as an OCT2 substrate. In the current study, the toxicity of the selected ligands was assessed. The findings showed that medicarpin and 4-methyl oxazole were identified as AMES toxicity, ciprofloxacin showed hepatotoxicity and the compound 4-methyl oxazole showed skin sensitisation.

In this study, the ADT tool was used, which is regarded as an automatic docking toolset of software to model versatile small molecules such as drug or ligand molecules that bind to receptor proteins with a defined 3D structure. The newer version of ADT 2.0 produces 10 protein-ligand complex conformations (poses), which are shown from the smallest to the largest binding free energy (ΔG). Specific binding affinities for protein C-di-GMP Phosphodiesterase with the ligands medicarpin, isoniazid and 4-methyl oxazole from *S. grandiflora* were predicted by using the computational docking method and structure-inhibitory interactions were observed.

The Lamarckian GA was used in this analysis, to examine the binding structural view of medicarpin, isoniazid and 4-methyl oxazole and the control drug ciprofloxacin. The C-di-GMP Phosphodiesterase docking values showed that a direct correlation occurs between the binding affinity energy and stability. In view of the previous statement, other than from the binding energy, the energy such as torsional interaction, London dispersion energies and intramolecular interaction were displayed greater for medicarpin followed by isoniazid and 4-methyl oxazole. table 3 displayed the overall docking scores. It was also technically clear that medicarpin, isoniazid and 4-methyl oxazole displayed the highest C-di-GMP Phosphodiesterase inhibition activity (Haney *et al.*, 2018, Lloyd *et al*., 2023, Ramalingam *et al.*, 2019, Nadia Al-dawah *et al*., 2015).

CONCLUSION

Urinary tract infection (UTI), is a significant health concern that impacts millions of people per year. It is mostly caused by *Pseudomonas aeruginosa.* The most significant virulent trait of *P. aeruginosa*is biofilm formation, which allows this pathogen to infect the individual and can cause severe UTI by avoiding the defence mechanisms of the host immune system. The current investigation presented experimental evidence

using computational bioinformatics techniques and databases to develop novel drugs from *S. grandiflora*. The targeted *S. grandiflora* ligands tend to have a promising inhibitory action against C-di-GMP Phosphodiesterase and this analysis indicates that *S. grandiflora* biocompounds may have an anti-biofilm activity to fight against UTI infections caused by *P. aeruginosa.*

ACKNOWLEDGMENTS

All the authors are thankful to their respective universities and institutes for their support. The authors extend their appreciation to the researchers supporting project number (RSP2023R261) King Saud University, Riyadh, Saudi Arabia.

DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding authors.

REFERENCES

Abubakar S, Akanbi B, Nasir-Naeem K and Abdulsalam Z (2015). Phytochemical and *In vitro* Antibacterial Activity of the Leaves and Stem Extracts of Sesbania grandiflora (L.) against Some Clinical Isolates. *Br. Microbiol. Res. J.,* **8** (2): 424-433.

Binte Arfan N, Sultana Julie A, Mohiuddin AK, Alam Khan S and Khalid Labu Z (2016). Medicinal properties of the *Sesbania grandiflora* leaves. *In Ibnosina J. Med. Biomed. Sci.,* **8**(6): 271-277.

Arfan NB, Islam T, Julie AS, Mohiuddin AK, Khan SA and Labu ZK (2016). Thrombolytic, membrane stabilizing, antidiarrhoeal, and antimicrobial properties of bioactive compounds isolated from leaves of Sesbania grandiflora naturally growing in Bangladesh. *Iran. J. Pharm. Sci.*, **3**: 12-31.

Bhoumik D and Dwivedi J (2014). A review on pharmacological activity of *Sesbania grandiflora* Linn. *Columbia J. Pharm. Sci.* **1**: 40-43.

Gandhi AD, Vizhi DK, Lavanya K, Kalpana VN, Devi Rajeswari V and Babujanarthanam R (2017). *In vitro* anti-biofilm and anti-bacterial activity of *Sesbania grandiflora* extract against *Staphylococcus aureus*. *Biochem. Biophys. Rep.*, **12**(C): 193-197.

Bhokre C, Gadhe K and Joshi A (2022). Phytochemical analysis and antimicrobial activities of *Sesbania grandiflora* (L) leaf extracts. *Pharma Inno. J.,* **11**(6): 90-94.

Bjarnsholt T, Moser C, Jensen PO and Høiby N (2011). Biofilm infections. Biofilm Infections. Springer. Newyork, USA, p.5.

Hall-Stoodley L and Stoodley P (2009). Evolving concepts in biofilm infections. *Cell Microbiol.,* **11**(7): 1034-1043.

Hall-Stoodley L, Costerton JW and Stoodley P (2004). Bacterial biofilms: From the natural environment to infectious diseases. *Nat. Rev. Microbiol.,* **2**(2): 95-108.

Chatterjee M, Anju CP, Biswas L, Anil Kumar V, Gopi Mohan C and Biswas R (2016). Antibiotic resistance in Pseudomonas aeruginosa and alternative therapeutic options. *Int. J. Med. Microbiol.*, **306**(1): 48-58.

Czaczyk K and Myszka K (2007). Biosynthesis of extracellular polymeric substances (EPS) and its role in microbial biofilm formation. *Pol. J. Environ. Stud.***, 16**(6): 799-806.

Mulcahy LR, Isabella VM and Lewis K (2014). *Pseudomonas aeruginosa* biofilms in sisease. *Microb. Ecol.,* **68**(1): 1-12.

Zhang X and Bishop PL (2003). Biodegradability of biofilm extracellular polymeric substances. *Chemosphere*, **50**(1): 63-69.

Flemming HC and Wingender J (2010). The biofilm matrix. *Nat. Rev. Microbiol.,* **8**(9): 623-33.

Ha DG and O'Toole GA (2015). c-di-GMP and its effects on biofilm formation and dispersion: A *Pseudomonas aeruginosa* review. *Microbiol. Spectr.,* **3**(2): MB-0003-2014.

O'Toole G, Kaplan HB and Kolter R (2000). Biofilm formation as microbial development. *Annu. Rev. Microbiol.,* **54**: 49-79.

Parsek MR and Singh PK (2003). Bacterial biofilms: An emerging link to disease pathogenesis. *Annu. Rev. Microbiol.,* **57**: 677-701.

Fazli M, Almblad H, Rybtke ML, Givskov M, Eberl L and Tolker-Nielsen T (2014). Regulation of biofilm formation in Pseudomonas and Burkholderia species. *Env. Microbiol.,* **16**(7): 1961-1981.

Lee VT, Matewish JM, Kessler JL, Hyodo M, Hayakawa Y and Lory S (2007). A cyclic-di-GMP receptor required for bacterial exopolysaccharide production. *Mol. Microbiol.,* **65**(6): 1474-1484.

Xu Z, Fang X, Wood TK and Huang ZJ (2013). A systems-level approach for investigating *Pseudomonas aeruginosa* biofilm formation. *Plos One.,* **8**(2): e57050.

An S, Wu J and Zhang LH (2010). Modulation of pseudomonas aeruginosa biofilm dispersal by a cyclic-di-gmp phosphodiesterase with a putative hypoxia-sensing domain. *Appl. Env. Microbiol.,* **76**(24): 8160-8173.

Klockgether J, Cramer N, Wiehlmann L, Davenport CF and Tummler B (2011). *Pseudomonas aeruginosa* genomic structure and diversity. *Front. Microbiol.,* **2**: 150.

Ryan RP, Lucey J, O'Donovan K, McCarthy Y, Yang L, Tolker-Nielsen T and Dow JM (2009). HD-GYP domain proteins regulate biofilm formation and virulence in Pseudomonas aeruginosa. *Env. Microbiol*., **11**(5): 1126-1136.

Kulesekara H, Lee V, Brencic A, Liberati N, Urbach J, Miyata S, Lee DG, Neely AN, Hyodo M, Hayakawa Y, Ausubel FM and Lory S (2006). Analysis of

Pseudomonas aeruginosa diguanylate cyclases and phosphodiesterases reveals a role for bis-(3′-5′)-cyclic-GMP in virulence. *Proc. Natl. Acad. Sci. USA.,* **103**(8): 2839-2844.

Valentini M and Filloux A (2016). Biofilms and Cyclic di-GMP (c-di-GMP) signaling: Lessons from *Pseudomonas aeruginosa* and other bacteria. *J. Biol. Chem.,* **291**(24): 12547-12555.

Kuchma SL, Brothers KM, Merritt JH, Liberati NT, Ausubel FM and Toole GAO (2007). BifA, a cyclic-Di-GMP phosphodiesterase, inversely regulates biofilm formation and swarming motility by *Pseudomonas aeruginosa* PA14. *J. Bacteriol.* **189**(22): 8165-8178.

Newman JW, Floyd RV and Fothergill JL (2017). The contribution of *Pseudomonas aeruginosa* virulence factors and host factors in the establishment of urinary tract infections. *FEMS Microbiol. Lett.,* **364**: 15. Rasamiravaka T, Labtani Q, Duez P and El Jaziri M (2015). The formation of biofilms by Pseudomonas aeruginosa: A review of the natural and synthetic compounds interfering with control mechanisms. *BioMed. Res. Int.,* **2015**: 759348.

Sauer K, Cullen MC, Rickard AH, Zeef LAH, Davies D G and Gilbert P (2004). Characterization of nutrient-induced dispersion in Pseudomonas aeruginosa PAO1 biofilm. *J. Bacteriol.,* **186**(21): 7312-7326.

Rabin N, Zheng Y, Opoku-Temeng C, Du Y, Bonsu E and Sintim HO (2015). Biofilm formation mechanisms and targets for developing antibiofilm agents. *Future Med. Chem.* **7**(4): 493-512.

Soto SM (2014). Importance of biofilms in urinary tract infections: New therapeutic approaches. *Adv. Biol.* **2014**: 543974.

Lakshmi T, Geetha RV and Anitha R (2011). *In vitro* evaluation of antibacterial activity of ethanolic and aqueous leaf extracts of *Sesbania grandiflora* (Linn) against clinical pathogens. *Int. J. Drug Develop. Res.,* **3**(3): 217-221.

Tuon FF, Dantas LR, Suss PH and Tasca Ribeiro VS (2022) Pathogenesis of the *Pseudomonas aeruginosa* biofilm: A review. *Pathogens,* **11**(3): 300.

Slobodníková L, Fialová S, Rendeková K, Kováč J and Mučaji P (2016). Antibiofilm activity of plant polyphenols. *Molecules,* **21**(12): 1717.

Hatt JK and Rather PN (2008). Role of bacterial biofilms in urinary tract infections. *Curr. Top. Microbiol. Immunol.,* **114**(5): 1285-1293.

Haney EF, Brito-Sánchez Y, Trimble MJ, Mansour SC, Cherkasov A and Hancock REW (2018). Computer-aided discovery of peptides that specifically attack bacterial biofilms. *Sci. Rep.* **8**: 1871.

Wagh VD, Wagh KV, Tandale YN and Salve SA (2009). Phytochemical, pharmacological and phytopharmaceutics aspects of *Sesbania grandiflora* (Hadga): A review. *J. Pharm. Rese.,* **2**(5): 889-892.

Lloyd C, Wong MWK, Sin LJ, Manickavasagam PP, Shoba G, Yue SR and Girija AS (2023). Antimicrobial potential of *Chlorella sorokiniana* on MRSA-An *in vitro* study and an in silico analysis on ClpP protease. *J. King Saud Univ. Sci*., **35**(5): 102668.

Guedes IA, de Magalhães CS and Dardenne LE (2014). Receptor-ligand molecular docking. *Biophys. Rev.,* **6**(1): 75-87.

Reji AF and Rexin Alphonse N (2013). Phytochemical study on *Sesbania grandiflora*. *J. Chem. Pharm. Res*., **5**(2): 196-201.

Zahir Hussain A and Kumaresan S (2014). GC-MS studies and phytochemical screening of *Sesbania grandiflora* L. *J. Chem. Pharm. Res.,* **6**(9): 43-47.

Ramalingam AK, Girija S and Selvi A (2019). Targeting prolyl tripeptidyl peptidase from *Porphyromonas gingivalis* with the bioactive compounds from *Rosmarinus officinalis. Asian Biomed*., **13**(5): 197-203.

Morris GM and Lim-Wilby M (2008). Molecular docking. *Meth. Mol. Biol.,* **443**: 365-382.

Nadia Al-dawah KJ, Al-safi S, Aboktifa M, Al-zeiny S, Al-shimmary B, Al-bayati MTN (2014). Comparative of phytochemical and antimicrobial of *Sesbania grandiflora* leaves extract. *Med. J. Babylon.* **11**(3): 2014.

Sivakumar S, Girija ASS and Priyadharsini JV (2020). Journal of King Saud University - Science evaluation of the inhibitory effect of caffeic acid and gallic acid on tetR and tetM efflux pumps mediating tetracycline resistance in Streptococcus sp., using computational approach. *J. King Saud Univ. Sci.,* **32**(1): 904-909.

Hutchison GR, Morley C, James C, Swain C, Winter H De and Vandermeersch T (2011). Open Babel Documentation Release 2.3.1. *Open Babel,* pp.1-151.

Chaluveelaveedu MN, Ashwini K, Prateek N, Nityasha G, Chitrangda S, Timir T and Awanish K (2016). Molecular Docking and *In silico* ADMET Study Reveals Acylguanidine 7a as a Potential Inhibitor of β-Secretase. *Adv. Bioinform.,* 9258578: 1-6.

Harmsen M, Yang L, Pamp J, Tolker-nielsen T and Tolker-nielsen CT (2010). An update on Pseudomonas aeruginosa biofilm formation, tolerance, and dispersal. *FEMS Immunol. Med. Microbiol*., **59**(3): 253-268.