Virtual screening and Simulation study exploring the Phytochemical profile of *Woodfordia fruticosa* to identify potential *A. baumannii* OXA-23 antagonists

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SUBMITTED BY

SHREYA KAPOOR
2K21/MSCBIO/49

Under the supervision of

DR. NAVNEETA BHARADVAJA

DEPARTMENT OF BIOTECHNOLOGY

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi college of Engineering)

Bawana Road, Delhi-110042

MAY, 2023

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi college of Engineering)

Bawana Road, Delhi-110042

CANDIDATE'S DECLARATION

I, Shreya Kapoor, 2K21/MSCBIO/49 of MSc. Biotechnology, hereby declare that the project Dissertation titled "Virtual screening and Simulation study exploring the Phytochemical profile of *Woodfordia fruticosa* to identify potential *A. baumannii* OXA-23 antagonists" which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi. in partial fulfilment of the requirement for the award of the degree of Master of Science is an authentic record of my own carried out work under the supervision of Dr. Navneeta Bharadvaja. The matter presented in this report has not been submitted by me for the award of any other degree of this or any other Institute/University. The work has been accepted in IEEE conference with the following details:

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DEPARTMENT OF BIOTECHNOLOGY

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi college of Engineering)

Bawana Road, Delhi-110042

CERTIFICATE

I hereby certify that the Project Dissertation titled "Virtual screening and Simulation study exploring the Phytochemical profile of *Woodfordia fruticosa* to identify potential A. baumannii OXA-23 antagonists" which is submitted by Shreya Kapoor, 2K21/MSCBIO/49, Delhi Technological University Delhi, in partial fulfilment of the requirement for the award of the degree of Masters in Science, is a record of the project work carried out by the students under my supervision. To the best of my knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

DR. NAVNEETA BHARADVAJA

In-charge, Plant Biotechnology Lab

Department of Biotechnology

Delhi, India 110042

Supervisor

PROF. PRAVIR KUMAR

Head of the Department

Department of Biotechnology

Delhi, India 110042

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ABSTRACT

Owing to its remarkable resistance capabilities and ability to cause tough-to-treat infections associated with high mortality rates, CRAb poses a significant threat to mankind worldwide. Over and above, it can potentially serve as repository for the dissemination of the resistance genes to other pathogens existing in hospital environment. OXA-23 protein plays a crucial role in driving carbapenem resistance in *A. baumannii*. Currently, the spectrum of effective therapeutics targeting this notorious pathogen is exceedingly limited thus indicating the dire need to explore new compounds with enhanced broad-spectrum activity and less likelihood of development of resistance. In this regard, repurposing of the naturally derived compounds offers additional benefits of minimal cost and side effects. Utilising a computational approach, the present work explores the potential of phytochemicals derived from *W. fruticosa*. Among the screened compounds, 5 molecules with binding affinity lesser than -8.0 kcal/mol were identified. Masilinic acid being the most potent phytochemical with binding energy -9.4 kcal/mol. Following this, MD simulation analysis revealed the acceptable stability of the protein in the presence of two different ligands Masilinic acid and Oleanolic acid as evident from the observed plots of RMSD, RMSF, and Radius of gyration. The findings of this study possess the likelihood to assist in identifying potentially effective OXA-23 antagonists to combat Carbapenem resistant *Acinetobacter baumannii*.

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LIST OF ABBREVIATIONS

1. CRAb: Carbapenem resistant *Acinetobacter baumannii*
2. OXA: Oxallinases
3. AMR: Antimicrobial Resistance
4. CarO: Carbapenem-associated outer membrane protein
5. ABC: ATP-binding cassette
6. MF: major facilitator
7. MBLs: Class B beta lactamases
8. RO5: Rule of five
9. NRL: Nuclear receptor ligand
10. ICM: Ion Channel Modulator
11. GPCR: G-protein coupled Receptor
12. EI: Enzyme Inhibitor
13. KI: Kinase Inhibitor
14. RND: resistance-nodulation-division)
15. GES: Guiana extended-spectrum β-lactamase;
16. NDM: New Delhi metallo-β-lactamase
17. CHDLs: Carbapenem hydrolysing class D beta-lactamases
18. SAR: Structure activity relationship
19. PDB: Protein Databank
20. IMPPAT: Indian Medicinal Plants, Phytochemicals and Therapeutics
21. ML: Machine Learning
22. MD: Molecular Dynamics
23. ADME: Absorption, Distribution, Metabolism, Excretion
24. SMILES: Simplified Molecular Input Line Entry System
25. RMSD: Root-mean-square Deviation
26. CDDEP: Centre for Disease Dynamics, Economics & Policy
27. RMSF: Root mean square fluctuations
28. WHO: World Health organization
29. AHLs: Acyl homoserine lactones
30. LPS: Lipopolysaccharide
31. VAP: Ventilator associated pneumonia
32. PK: pharmacokinetics
33. PD: Pharmacodynamics
34. ADRs: Adverse Drug Reactions
35. MGL: Molecular Graphic Library
36. AgNPs: Silver Nanoparticles

CHAPTER 1: INTRODUCTION

Antimicrobial Resistance (AMR) has led to one of the biggest medical catastrophes of all time as it has rendered current healthcare system and therapeutics substantially inactive thus leading to increased death rates and economic loss. Nearly 1.27 million individuals lost the battle to drug-resistant superbugs, and it is estimated that by 2050, 10 million people may die from AMR alone, which is more than cancer and road traffic accidents [1]. According to the reports published by CDDEP in 2015, India consumes more antibiotics than any other country (12.9 billion units of antibiotics were consumed in 2010 [2]. Moreover, India has one of the highest rates of resistance to antimicrobial agents used both in humans and livestock. The imprudent use of antimicrobials and inadequate wastewater treatment (from farms, pharmaceutical companies, homes etc.) are essential in promoting AMR in India [1]. Infections involving multidrug-resistant pathogens increase the severity of illness and healthcare costs that are accompanied by prolonged hospital stays [2]. The drug-resistant superbugs threaten immunocompromised patients undergoing surgeries, dialysis, organ transplant, and chemotherapy [3]. Hence, physicians are compelled to use drugs like carbapenems and polymyxins for their treatment, which can further cause a severe imbalance of the gut-microbiome, creating a platform for the proliferation of drug-resistant strains of *Clostridioides difficile* (formerly known as *Clostridium difficile*) [4]. These issues warrant investigations into the discovery of alternative strategies for warding off this global crisis before it can consummate mankind.

The anticipated worldwide occurrence rate of *A. baumannii* infections has been reported to be over 1000 000 cases per year, half of which demonstrate resistance to multiple antibiotics [5]. The first identification of carbapenem-resistant *A. baumannii* was in 1991. Thereafter, the world witnessed a considerable surge in the prevalence of *A. baumannii* strains that have acquired resistance genes. Owing to its remarkable resistance capabilities and ability to cause tough-to-treat infections associated with mortality rates as high as 76%, CRAb poses a significant threat to mankind e worldwide. Over and above, it can potentially serve as repository for the dissemination of resistance-conferring genes to other pathogens commonly found in healthcare facilities [6]. In light of these factors, it is recognised as a high priority pathogen by WHO. Initially, in 2013 according to WHO,

CRAb was regarded as a "serious threat" pathogen however owing to the ever-growing incidence of resistance strains, it was recently categorised as a "Critical priority pathogen". The occurrence of both NDM belonging to class B beta-lactamases (MBLs) and OXA genes belonging to class D beta lactamases (CHDLs) in *A. baumannii* strains confers formidable resistance capabilities to this highly perilous to pathogen. These genes play a pivotal role in conferring resistance to carbapenem antibiotics, which are typically considered the last resort therapeutic options against multidrug-resistant bacterial infections [7], [8].

Given this trend of rapidly emerging strains of multi drug resistant bacteria and development of new resistance mechanisms against the available antibiotics, computational technologies can play a significant role in screening large number of molecules in a limited timeframe and assist in prioritizing the ones with most promising properties for in-vitro and in-vivo experimentation.

In silico drug screening owing to its unmatched capabilities including but not limited to reduced cost, exploration of a large library of compounds in limited timeframe has emerged as a highly imperative and reliable technology for drug design, optimization and development. With the advent of simulated methodologies, there has been a substantial and pervasive upheaval in drug development strategies [9]. It utilises a multitude of data analysis algorithms and predictive models for the identification, evaluation and optimization of the lead compounds. It provides better understanding of the complex interaction and assists in analysing the binding mechanisms of the test compound towards the target, stability of the complex in a simulated environment and their dynamic behaviour [10]. It additionally enables the researchers to optimize pharmacokinetic properties of the lead compounds and achieve desirable drug likeliness behaviour viz., mitigate side effects, increase efficacy. So, simulated technologies work as a magic wand in the field of medical research. These methods substantially reduce the wastage of valuable resources and as well as financial investments.

Molecular docking is a type of simulative method for the analysis and regulation of the favoured orientation between two molecules (ligand and receptor). It relies on a multitude of complex algorithms and scoring functions for the identification and analysis of

preferred binding conformers and affinity [10]. Molecular dynamics simulation. The central motive behind simulation studies is to have a better understanding of structural, dynamic and thermodynamic characteristics of the molecular complex. It mimics the realistic and dynamic environment for protein as in their native environment proteins aren't static and rather exhibit different types of motions like vibrations. Besides, proteins undergo numerous conformational changes [11].

Objectives of this study:

1. To assess the antagonistic potential of phytocompounds towards OXA-23 protein of Carbapenem resistant *Acinetobacter baumannii*

2. Pharmacokinetic analysis and prediction of drug likeliness properties of the selected compounds

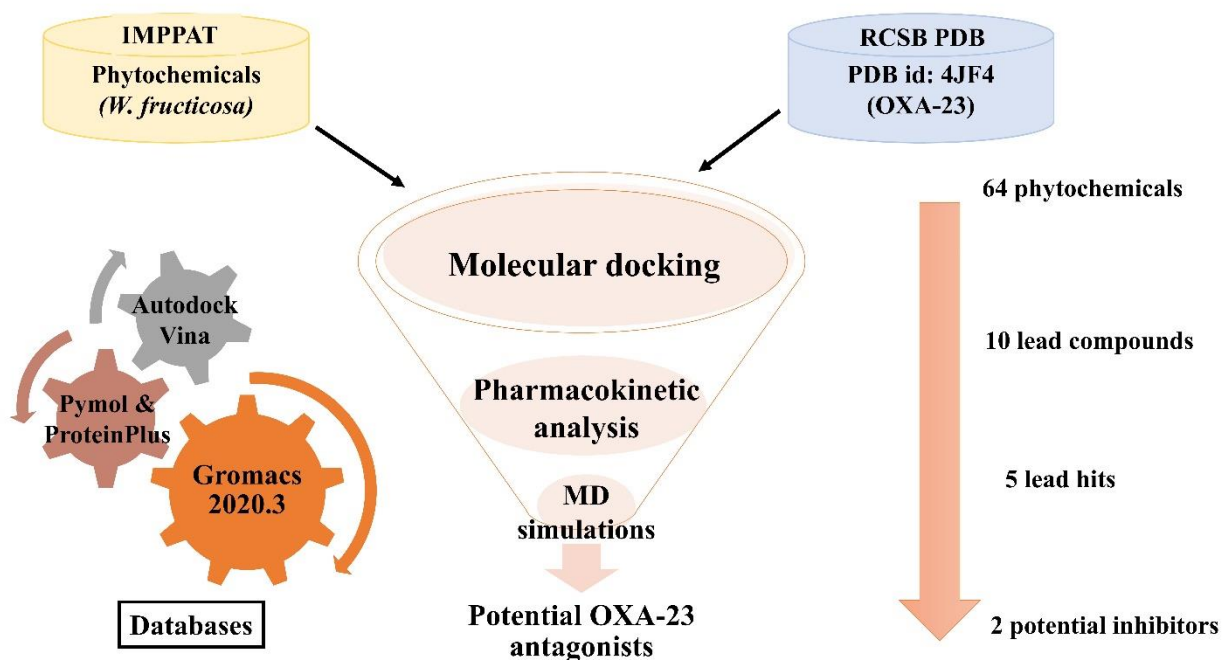3. To evaluate the structural stability of the protein in presence of the lead hit molecules

Fig. 1 Overview of workflow of the study

CHAPTER 2: LITERATURE REVIEW

2.1 *Acinetobacter baumannii* and its pathogenesis

Acinetobacter baumannii is a gram-negative aerobic, and bacillus. It is an opportunistic bacteria associated with hospital acquired infections and primarily infects individuals with compromised immune systems [5]. Owing to its ability to survive in a diverse range of environmental conditions for prolonged periods of time, it is responsible for causing outbreaks of infections. Though the population at risk is not very large but the mortality rates associated with this bacterium are very high.

The presence of particular virulence factors inside *Acinetobacter baumannii* has a significant impact on the pathogenesis of the bacterium [12] . About 16 gene islands linked to virulence have been found in recent research [13]. Porins, which are outer membrane proteins encoded by ompA, attaches to the surface of host cells. It localises in the mitochondria and nuclei of cells, where it causes apoptosis, ultimately resulting in cell death. OmpA promotes bloodstream dispersion and aids in the development of biofilms. By permitting the extrusion of antibiotics from the periplasmic area through the outer membrane in concert with inner membrane efflux mechanisms, OmpA also contributes to the development of antimicrobial resistance [14].

The presence of capsular polysaccharides and LPS is a crucial virulence component. The function of these elements is to shield the bacterium against antibodies. The bacterial capsule acts as a defence against phagocytosis and stresses from the environment. Notably, it has been discovered that some antibiotics cause the bacterial surface to produce capsular antigens, which increases antibiotic resistance [15].

In addition to LPS and capsules, some enzymes—particularly lipolytic enzymes like phospholipases—perform virulence functions in *Acinetobacter baumannii*. The phospholipids that are present in the cell membranes of afflicted cells can be broken down by these enzymes. They also impair immunological responses by interfering with signalling pathways [13]. Another enzyme discovered in *Acinetobacter* is CpaA, an glycan specific adamalysin-like protease. The coagulation pathway's factor XII is inhibited by CpaA, which lowers the production of thrombi and promotes the

transmission of germs to other sites [16]. The transfer of microbial effector molecules into host cells is thought to occur via outer membrane vesicles (OMVs), which include DNA, RNA, periplasmic proteins, and LPS. They enable interactions between host cells and numerous other virulence factors without bacterial and host cell contact. Cytotoxicity are a result of this process [13].

It is one of many bacterial species that have chaperone or usher pilus systems that are controlled by the BfmRS two-component system. This pathway contributes to the development of an attachment pili and a protective capsule around the bacterium [17]. Under stressful circumstances, the GacSA system—another system—is involved in the creation of biofilms. When the bap gene is present, the biofilm develops AHLs, which are necessary for the formation of biofilms and the control of motility in *Acinetobacter*, are produced as a result of autoinducers in quorum sensing in this organism [18].

In the pathogenesis of Acinetobacter, the acquisition of micronutrients plays a critical role in virulence. The ability of these bacteria to obtain and store vital micronutrients including iron, zinc, and manganese will ensure their survival during times of micronutrient scarcity Proteins like FecA and FecI are in responsible for absorbing iron for the formation of heme, while siderophores, which are iron chelators, assist iron uptake [19]. The ZnuABC transporter and the ZigA GTPase, which are both zinc scavenging systems in Acinetobacter, are both involved in metabolism. The former is responsible for uptake of zinc, while the latter is for metabolism. The resistance-associated macrophage protein (NRAMP), in addition, assists in the absorption of manganese in the presence of calprotectin [20].

There are multiple protein secretion systems, with the latest being the type II secretion system. This system facilitates the movement of various substances from the periplasmic space to the extracellular environment, specifically outside of the cell. The substrates targeted by this system include lipases and the metallopeptidase CpaA. Additionally, the type IV secretion system allows the transfer of toxins from one bacterium to another through direct contact, aiding in polymicrobial infections. These systems are commonly found in individuals with weakened immune systems and can produce toxins such as nucleases and toxins that affect cell membranes [21].

Acinetbactor baumanii also possesses penicillin-Binding Protein 7/8 (PBP7/8) and β-Lactamase PER, the former provides resistance to beta lactam antibiotics whereas later affects structure of peptidoglycan and also associated with cell adhesions [22].

Furthermore, this bacterium has a special protein called CipA that can attach to complement proteins and trigger the activation of plasminogen. CipA thus increases serum resistance by aiding in the conversion of plasminogen into active fibrin and complement C3b [21].

2.2 Clinical manifestations of CRAb

CRAb's capacity to colonise inanimate objects and survive there for extended periods of time makes it capable of infecting a variety of different organ systems. CRAb primarily affects respiratory system explicitly lungs. The remarkable ability of *A. Baumannii* to form persistent biofilms on the endotracheal tube accounts for extensive infestation of lower respiratory systems. So, pneumonia is the most predominant and common clinical presentation of *A. Baumannii* contributing to more than 50 percent of all infections. Ventilator associated pneumonia is the most frequently encountered complications of CRAb infections and is a major concern among patients or other people in health-care as it is typically linked with lengthened stay in the hospitals and if left unattended, it can lead to high mortality rates [23]. The casualty of CRAb induced VAP is more than that of VAP induced by other gram-negative bacilli because of extended dissemination and restricted treatment choices. *A. Baumannii* was one of the major intruders leading to large number of deaths during COVID times as supported by an observational study that showed the presence of CRAb strains in more than 19% of patients admitted to ICUs during corona. This bacterium upon infecting these patients further increased the mortality rates to ~ 65% [24]. Subsequent manifestations of CRAb include catheter-associated complications, bacteremia and opthalmic infections. Prolonged catheterization is usually associated with *A. Baumannii* induced urinary tract infections; however, these are rarely fatal as only 2% of UTIs caused by this bacterium are known for their invasiveness. Bacteremia is an indirect manifestation of CRAb infections that usually occurs as a result of poor prognosis. It has a very limited spectrum and is thus associated with heightened death tolls. Intravascular catheters serve as the principal sources for the development of bacteremia. Besides, *A. Baumannii* is one of the main etiological agents for ophthalmic infections specifically post-surgery corneal ulcers. Apart from this, soft

tissues and bone-related infections due to this bacterium have also been reported. These usually occur as a result of colonization of the prosthetic organs or during surgical procedures [6].

Though very rare but highly fatal outcome of CRAb infection are endocarditis and meningitis. Former is developed as a result of the bacteria colonizing native or prosthetic valve, while latter occurs because of post-surgical CSF-leak or Neurosurgical procedures like ventriculostomy [5]. The ability of CRAb or any other bacterium to colonize skin, tissues, organs is primarily attributed to the break in anatomical barrier

One of the most common modes of transmission of CRAb are the tainted medical devices like surgical instruments, Velcro on blood pressure cuffs, and contaminated fomites like furniture, door knobs and gloves. Additionally, there have been occurrences of cross-contamination between the patients and environment via air droplets as unveiled by Pulsed-field gel electrophoresis (PFGE) analysis (Jiang). Immunocompromised patients, especially those on ventilators, bearing open wounds, admitted to ICUs, having medical devices like prosthetic valves and catheters are at higher risk of acquiring CRAb infections. [25].

2.3 Current therapeutics available for *A. baumannii*

Multiple antibiotics viz Aminoglycosides, Cephalosporin, and Penicillin have been futile in treating *A. baumannii* infections. Carbapenems were the frontline therapy for MDR *A. baumannii,* but their indiscriminate use has caused an upsurge in carbapenems resistance over the past few years [23]. Owing to the perpetually expanding carbapenem-resistant strains of *A baumannii*, and pharmacokinetic constraints of the drugs viz., high toxicity and low plasma levels, the existing therapeutic options for CRAb are proven to be ineffectual. It is worth noting that, there have been indications of the emergence of strains demonstrating resistance to existing therapies because of exploitation or overutilisation. Over and above, there is sparsity of reliable, randomized studies to support the antimicrobial efficacy of the potential candidates showing favourable in-vitro activity [5]. Generally, carbapenem-resistant strains are treated using a combination of two or more drugs. Combination therapies are designed to achieve better clinical outcomes by targeting multiple resistance mechanisms simultaneously. The plausible candidates currently being used to mitigate CRAB infections are outlined on next page.

Polymyxins are the cationic polypeptides, considered as salvage treatments for of CRAB infections. These exhibit concentration-dependent inhibitory activity against gram-negative bacteria and are the last resort antibiotics to combat MDR pathogens. The amphipathic nature owing to the presence of hydrophobic fatty acyl chain and hydrophilic cationic peptide ring, is responsible for the associated bactericidal activity [26]. As a consequence of its bipolar nature, polymyxin can easily integrate itself into the bacterial membrane, disrupting its structural stability, ultimately leading to cell death. Nevertheless, due to the subsequent drawbacks, polymyxins are not completely reliable. There have been reports of the effective inhibition of OXA-23 gene by polymyxin in combination with Minocycline [27]. The limitations of these antibiotics include: a) Site specific responsiveness e.g., suboptimal activity in pulmonary epithelial cells b) dosage complications as non-tolerable dosages are required to achieve desirable PK/PD targets c) Reported cases of nephrotoxicity and neurotoxicity d) likelihood of development of resistance mechanisms e.g., generation of mcr-1 gene and e) limited global availability [28].

Tetracyclines: Tetracyclines are broad-spectrum, easy to administer antibiotics, targetting the bacterial protein synthesis. These have exhibited inhibitory activity against ~60% of CRAb clinical isolates. Different types of antibiotics belong to this class. Tigecycline exhibiting maximum activity followed by minocycline and tetracycline. Despite their promising in-vitro and clinical efficacy, these antibiotics possess certain limitations such as low blood plasma levels that diminish their resilience. The lower blood plasma levels of tetracyclines can be attributed to its high volumes of distribution and increased protein binding [8]. Besides, tigecycline is known to exhibit unfavourable PK in lungs and blood (major zones associated with *A. baumannii* infections). Furthermore, there have been reported cases of toxicities and ADRs e.g., severe coagulopathy and reduced plasma fibrinogen concentration [29].

Sulbactam: sulbactam is a commonly used penicillinate sulfone belonging to Ambler Class A beta-lactamases. It demonstrates high binding affinity towards penicillin binding proteins of *A. baumannii*. However, there are reported cases of nephrotoxicity associated with its consumption. Additionally, it is inefficient against CRAb isolates harbouring blaOXA-23 genes. Recent studies have suggested the potential of avibactam in combination with sulbactam to combat OXA-23 carrying CRAb isolates [30].

Aforementioned drugs are currently being used to treat CRAb infections. However, in-vitro activity of several other drugs like Cefiderocol (siderophore-cephalospotin antibiotic), Eravacycline has also been reported. Diverse in-silico and in-vitro studies have reported the anticipated potential of several other compounds against CRAb. These include Cefiderocol (siderophore-cephalosporin antibiotic) [31]. Eravacycline (fluorocycline), trimethoprim–sulfamethoxazole, Durlobactam (diazabicyclooctanone) [32] in combination with Sulbactam. Lastly, the combinatorial therapy consisting of polymyxin B, tigecycline and fosfomycin have exhibited promising clearance and effectivity rates [8]. When considering the functionality of prospected and available therapeutics, their in-silico and in-vitro activity is often more favourable and reliable than actual clinical outcomes, notably because of their limited pharmacokinetic abilities. Thus, the rational use of these available antibiotics along with the exploration of new compounds are essential to combat CRAb.

2.4 Underlying Mechanism for carbapenem resistance

Drugs Resistance in bacteria is the result of the cumulative and simultaneous engagement of diverse mechanisms such as impaired membrane permeability, efflux pumps, inactivation of the antibiotics, horizontal acquisition of foreign resistance determinants, and target site modifications. Out of all the major causes Table I discusses the most prevalent and significant mechanisms conferring carbapenem resistance.

CHDLs also known as oxacillinases, are the most frequently encountered factors contributing to the development of carbapenem resistance in *A. baumannii.* These are characterized by the presence of serine residue in their active site, that serves as an attacking nucleophile and covalently interacts with beta-lactam ring forming an acyl-intermediate leading to the cleavage of beta-lactam ring thereby rendering the antibiotic ineffective [33]. These are the most potent beta-lactamases against which even EDTA and clavulanic acid are proven to be futile. CHDLs hold substantial clinical significance as they are remarkably active bacterial mechanisms that make them highly virulent and exacerbates the ability of existing drugs to counter highly lethal *A baumannii*. These are further divided into phylogenetic sub groups which can either be naturally occurring for ex OXA-51-like, or plasmid-acquired viz. OXA-58-like, OXA-143-like, OXA-23-like, OXA-235-like and OXA-24/40-like β-lactamases [34].

OXA-23 is a potent carbapenem hydrolysing enzyme, which can also degrade other beta-lactam antibiotics including aminopenicillins, piperacillin, oxacillin, and aztreonam thus leading to broad spectrum resistance [13] OXA-23 was the first oxacillinase to be identified in *A. baumannii* isolate in Scotland. It is thought to be originated from *Acinetobacter radioresistens* that spread to other Acinetobacter species via plasmid transfer [35].

Table I. Major causes of carbapenem Resistance in *A. baumannii* [References: [5], [36]–[38]

Characteristic	Description	Example
Loss of function of outer membrane proteins	These membrane proteins are essential for the uptake of antibiotics. Alternation in their function or structure leads to decreased membrane permeability	Omp; CarO
Enhanced expression of Efflux Pumps	Efflux pumps are membrane-associated proteins that flush out drugs from the bacterial cells thereby reducing the cellular drug accumulation. 6 different families of efflux pumps are known to exist: ABC, RND, MATE, SMR, PACE, MF	Tet(A), Tet(B), AbaF, AdeABC
Intrinsic expression or acquisition of Carbapenemases	Inactivation of carbapenems; Among 4 different Ambler classes of beta-lactamases, Class A, B and D are known for carbapenemases activity; are highly transmissible	GES (Class A), NDM (Class B), OXA (Class D)

It is a highly efficient hydrolytic enzyme which can alone confer resistance to carbapenems without the synergistic effect of other resistance mechanisms. This was supported by a report demonstrating that in *A. baumannii* strains carrying only bla$_{OXA}$-23 genes, MIC of carbapenems was as high as 16 μg ml^{-1} surpassing the susceptibility threshold. However, the additional presence of efflux pumps in A. baumannii strains

further elevated the MIC to 32 µg ml^{-1} thus indicating that in the presence of other concurrent mechanisms resistance reaches substantial levels [34], [39]. Furthermore, it has been reported that the presence of an upstream promoter namely ISAba1, facilitates a substantial elevation in the expression of OXA-23. In the absence of this promoter, the OXA-23 carrying *A. baumannii* isolates exhibited mild resistance towards carbapenems and strong resistance towards beta-lactams, however its presence makes the bacterium highly resistant towards carbapenems in addition to clavulanic acid, ampicillin, ticarcillin and amoxicillin. Its gene is highly transmissible and has been disseminated globally, as it can be horizontally transferred from one species to another via conjugative plasmids harbouring transposons e.g., pABTJ1 containing Tn2009, repAci6 containing Tn2006/ Tn2008 [35]. Over that, OXA-23 is the most prevalent of all CHDLs. Over 80% of clinical isolates of CRAB have been reported to be the carriers of OXA-23 [12]. Its predominance is higher in East, Africa, Southeast Asia, South America, and Southeast Asia, The present scenario suggests that, the crisis caused by OXA-23 is likely to exacerbate owing to its high diversity, prevalence, transmission, and hydrolytic efficiency. Nonetheless, taking into account the extensive range of evolved OXA-23 genes, it is strongly probable that certain OXA-23 variants are already resistant to novel inhibitors or will swiftly acquire resistance overtime. Furthermore, CRAb isolates harbouring OXA-23 have exhibited an exceptional capability to survive in healthcare settings for prolonged periods and trigger upsurges of infections and deaths [40]. Notably, OXA-23 is associated with increased death rates and morbidities in conjunction with extended periods of hospitalization making it clinically significant. In line with the aforementioned characteristics of OXA-23, it is a promising therapeutic target to tackle highly pathogenic CRAb [41].

2.5 Potential health benefits of *Woodfordia fruticosa*

W. fruticosa is an esteemed therapeutic plant traditionally identified as Dhataki and typically recognized as Fire flame bush. It is known for its abundant propagation in tropical and subtropical regions including Sri Lanka, Indonesia, India, Malaysia, China, Japan and Pakistan. It serves as a crucial element for the preparation of ayurvedic components viz., Asavas known for their total-body vitality benefits and Aristhas that best serve as health revitalizers. Out of 18 existing aristhas, this plant provides 17 of them. Its entire plant body is of notable therapeutic potential, peculiarly flowers as evidenced

by the literatures of yunani and other traditional systems of medicine [42], [43]. Several studies have reported its contemplated function in successfully managing a variety of diseases ranging from general fever and dysentery to leprosy and haemothermia. In vitro studies have shown elevated stimulation of bone marrow cells in the presence of methanolic extract thus indicating its immunomodulatory roles. Hepatoprotective activity against several toxic compounds viz., phenytoin, acetaminophen, diclofenac sodium and carbon tetrachloride has also been reported. In-vivo studies have demonstrated anti-diabetic effect of the ethanolic extract of this plant. As upon administration of the plant preparation, the fasting blood sugar level was seen to reduce significantly while insulin levels were observed to rise [43], [44]. The significant therapeutic role of bio-extracts of this plant are due to the Diverse Phytochemical spectrum including but not limited to flavonoids, anthraquinone, glycosides polyphenols, and hecogenin (non-phenolic).

The phenolics compounds are the major contributors for their broad spectrum anti-bacterial activity. The plant extracts (petroleum ether extract, alcoholic extracts etc.) have been reported to show significantly inhibitory activity against a variety of gram-positive bacteria e.g., *S. aureus, B. cereus* and as well as gram negative bacteria viz., *E. coli, K. pneumonia*. Methanolic extracts have been explicitly shown their inhibitory potential against *Pseudomonas pseudoalcaligenes*. Over that, the efficacy was found to surpass that to commonly used drug namely ciprofloxacin as depicted by disc diffusion assay. Besides, studies have reported the use of the plant extract for the biogenic synthesis of silver nanoparticles that exhibited a synergistic anti-microbial activity [45]. The generated AgNPs were shown to have better inhibition efficacy than the available therapeutic options [46].

CHAPTER 3: MATERIALS AND METHODOLOGY

Several Bioinformatics methods and tools were employed to conduct in-silico studies for the identification of potential phytochemicals with favourable binding affinities towards *A. baumannii* OXA-23.

3.1 Identification of key interacting residues

Literature review was conducted to identify the catalytic amino acid residues within the protein OXA-23. Herein, the interactions of the protein with the current inhibitors viz., Durlobactam, avibactam, meropenem ligands and other co-crystallised ligands were taken into consideration. 16 amino acid residues as S79, L82, N85, F110, W113, G120, K124, L125, S126, K216, T217, W219, M221, D222, R259 and N260.

3.2 Retrieval of protein structure and receptor preparation

RCSB-PDB was used to retrieve the crystal structure of the protein: OXA-23, available under the pdb id: 4JF4. The chosen structure had a resolution of 2.14 Å. The analysis of the primary structure of protein revealed the presence of 273 amino acids in each of the two homo-dimeric chains. It was complexed with a ligand molecule bound to meropenem. The protein, in the provided state was not fit for molecular docking and required few refinements.

For the purpose of modifications MGL (Molecular Graphic Library) tools 1.5.7 were used. It is a software package built by The Scripps Institute that enables visualization of the protein molecule, preparation of protein and ligand molecules for molecular docking, generation of the grid box and the analysis of vina results. The several modifications made to the protein molecule included, removal of one of the chains of the homodimer, exclusion of water molecules, the bound ligand and as well as heteroatoms. These modifications were made as the mentioned components not being the integral part and the key functional component of the protein, if present would have hindered the docking process.

Following this, the Kollman charges and polar hydrogens were added to further stabilize the protein-ligand complexes as the former assist in establishing electrostatic interactions between the ligand and the protein and the latter aids in stabilizing the protein-ligand interactions by favouring H-bond interactions. The central rationale behind these modifications was to increase the efficacy and accuracy of the entire process and make it less energy-intensive.

3.3 Binding site detection and Grid box preparation

Grid box is generated to define a 3D confined space in which the docking algorithms can be conducted. Generating a grid box offers multiple advantages over blind docking including but not limited to reduced energy consumption thus lesser computational expenses and less time consuming as only a specific region is being scrutinized.

In this study, the parameters were defined to be 8.881, 0.395, 13.250 (X, Y, Z axis) for Grid box centre and 76, 96, 84 (X, Y, Z) for the coordinates. These parameters were stringently defined to include all the identified amino acids.

All the phytochemicals (64 in number) of the chosen plant *W. fruticosa* were obtained in the pdb format using IMPPAT (Indian Medicinal Plants Phytochemistry and Therapeutics) followed by the ligand library preparation using MGL tools. Besides, PubChem was utilised to retrieve the 3D conformer of the control i.e., Avibactam. However, this conformer was available in .sdf format so it was converted to pdbqt format using Open Babel GUI interface, a well-known open-access tool that facilitates effortless conversion of chemicals between various file formats.

3.4 Molecular docking analysis and visualisation

Molecular docking is a simulative method for the analysis and regulation of the favoured orientation between two molecules (ligand and receptor). It relies on a multitude of complex algorithms and scoring functions for the identification and analysis of preferred binding conformers and affinity.

Autodock Vina developed in 2010 by Oleg Trott in Scripps Research Institute, is an open access, user-friendly tool that utilises multiple algorithms to assess protein-ligand interactions [47]. It takes into account multiple factors to identify favourable interaction

profiles and generate a stable protein-ligand adduct e.g., chemical and structural properties of the receptor and ligand, steric clashes, hydrophobic interactions, h-bond interactions and empirical force fields [48].

Using Autodock Vina 1.7.5 [49], we docked multiple phytochemicals against OXA-23 protein of carbapenem resistant *A. Baumannii* were used to dock the selected compounds against the target protein. The grid parameters were defined as mentioned above followed by fixating energy range and exhaustiveness to be 4 and 10 respectively. Former outlines the maximum permissible variation between the different conformations of ligand and protein while latter describes the number of molecular conformations that were explored during molecular docking.

Following this, the protein ligand interactions were visualized in 3-dimensional and 2-dimensional space using Pymol and ProteinPlus respectively.

3.5 Pharmacokinetic analysis

Pharmacokinetic analysis is done to identify whether the test compounds can potentially act as drug molecules by assessing the presence of drug likeliness properties. To achieve so, following assessments were done

RO5 analysis: Bioavailability of the compounds can be assessed on the basis of their compliance with RO5 i.e., Lipinski's rule of five. It was done by using an open access tool: *SCIFBio* [50].

ADMET analysis: this was performed by using SWISSADME. For this, the canonical smiles for each molecule were obtained and were used as inputs for this tool and bioavailability radars were obtained [51].

Bioactivity prediction: It is done to assess the ability of the selected compounds to interact with several different human cell receptors like GPCRs. An open-access web-based tool namely Molinispiration was used for this purpose.

3.6 Molecular Dynamics (MD) Simulations

To analyse the conformational stability and flexibility of the protein-ligand complex of the top lead molecules exhibiting highest binding affinity among all the docked phytochemicals molecular dynamic simulations were conducted. The fundamental

motive behind simulation studies is to have a better understanding of structural, dynamic and thermodynamic characteristics of the molecular complex. The purpose behind this was to study the effect of binding of lead compounds with the proteins.

In the current study, to GROMACS version 2020.3 was used. It is a software suite designed with a rich set of comprehensive algorithms that provides the insights into the dynamics of the complex on the basis of Newton's equations of motion. It relies on force field mechanics for generating the inter-atomic interactions within the molecular system and takes into consideration different types of bonded interactions viz., covalent bonds, dihedral angles, and non-bonded interactions like van der waal forces [52]. However, all these parameters must be defined before runs in topology (.top) and gromacs (.gro) files. Ultimately, it provides outputs as trajectories which can be analysed by making use of a wide range of its pre-defined commands and tools.

The biomolecular system was prepared for MD simulations using web-based, graphical user interface namely CHARMM (Chemistry at Harvard Macromolecular Mechanics)-GUI [53], The input file was generated in .tgz file format. The length of the simulation run was specified to be 50 ns for each compound. The trajectory files obtained post MD runs were analysed using several different gromacs commands viz., gmx rms, and 'gmx rmsf' were used for measuring the Root Mean Square deviation and root mean square fluctuation of the protein ligand complex, while 'gmx gyrate' was used to find the radius of gyration of protein.
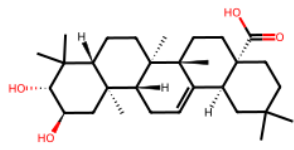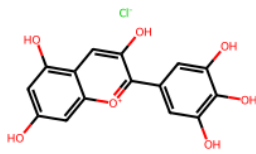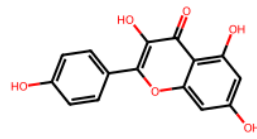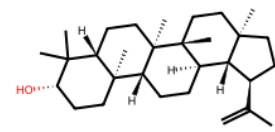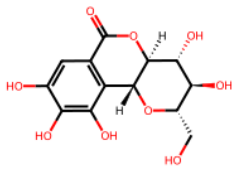
Maslinic acid
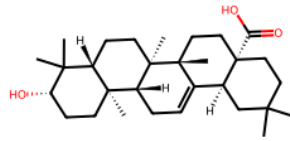
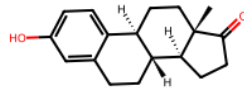Oleanolic acid
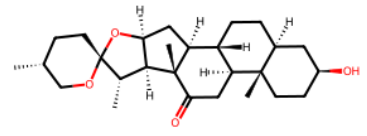
Hecogenin

Delphinidin

Kaempferol
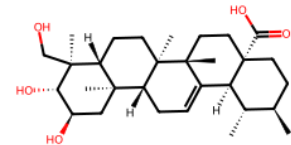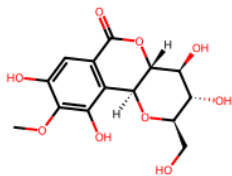
Lupeol

Norbergenin

estrone

Asiatic acid

Bergenin

Fig. 2: Structure of selected phytochemicals from *Woodfordia fruticosa*

CHAPTER 4: RESULTS

4.1 Molecular docking analysis

The docking studies revealed over 30 bioactive compounds with binding energy lower than the standard inhibitor (Avibactam) used in this study i.e., -6.4 kcal/mol. Table II shows the lead compounds obtained for phytochemical profile of *W. fruticosa* along with their corresponding binding energies and the key interacting residues as revealed by visualization tools. Lower binding energy than the standard molecule is indicative of higher binding affinity of the test compounds towards the target protein i.e., OXA-23. Among phytochemicals, Masilinic acid displayed strongest binding affinity followed by oleanolic acid, hecognenin, delphinidin and kaempferol.

The data obtained is supported by previous studies reporting in-vitro or in silico antimicrobial activities of these compounds. Masilinic acid alongwith oleanolic acid (the second top lead molecule identified in this study) have been reported to exhibit significant inhibitory activity against oral pathogens [54]. Oleanolic acid is additionally reported to possess inhibitory potential against *E. coli, E. aerogenes* EA27 and *Providencia stuartii* PS2636

Hecogenin, a spirostane aglycone exhibited binding affinity of ~8.6 kcal/mol is well known for its anti-inflammatory role. Its inhibitory activity against *Escherichia coli* (*E. coli*), *Salmonella enterica, Pasteurella multocida*, and *Physalospora piricola* has been reported.

Delphinidin, an anthocyanidin, commonly found in epidermal tissues of flower exhibited Binding energy of -8.2. Several Investigations have suggested its deterring potential against a wide range of bacteria including *P. aeruginosa, S. aureus, P. gingivalis, T. forsythia, T. denticola*, *Clostridium histolyticum, Serratia mascences*, *Clostridium sporogenes, Escherichia coli, Klebsiella pneumoniae, Bacillus cereus* and *Pseudomonas fluorescence* [37].

Kaempferol, a flavonoid with known anti-oxidant, anti-inflammatory and, cardioprotective roles showed binding energy of -8.0 kcal/mol. Studies have reported its inhibitory activity against *Shigella, E. coli,* Multidrug resistant *S. aureus, Mycobacterium,*

P. aeruginosa, and *V. cholerae*. It has been suggested that it impedes DNA gyrase activity in *E. coli* and MRSA while in rest of the mentioned bacteria, it blocks the biosynthesis of fatty acids thereby obstructing cell envelop function and biofilm formation [55], [56] [57]. In *S. aureus*, it is additionally known to inhibit DNA helicases, in particular SAPriA. Results obtained from this molecular docking analysis revealed 10 molecules with favourable binding affinities as shown in Table II.

Table II. The binding affinities of the top 10 lead compounds along with the key interacting residues.

| Compound	Key interacting residues	BE(kcal/mol)
Avibactam	Ser79, Ser126, Gln132, Trp219	-6.4
Masilinic acid	Phe110, Ser126, Thr217, Trp219, Met 221, Arg259	-9.4
Oleanolic acid	Phe110, Ser126, Thr217, Arg259	-9.0
Hecogenin	Phe110, Trp113, Trp219	-8.6
Delphinidin	Ser79, Thr217, Trp219, Arg259	-8.2
Kaempferol	Ser79, Phe110, Trp113, Thr217, Trp219	-8.0
Lupeol	Phe110, Leu125, Thr217, Asn260	-7.5
Norbergenin	Ser79, Trp113, Thr217, Trp219, Arg259	-7.4
Estrone	Ser79, Leu125, Trp219	-7.3
Asiatic acid	Phe110, Trp113, Ser257	-7.2
Bergenin	Ser79, Ser126, Thr217, Trp219, Arg259	-7.0
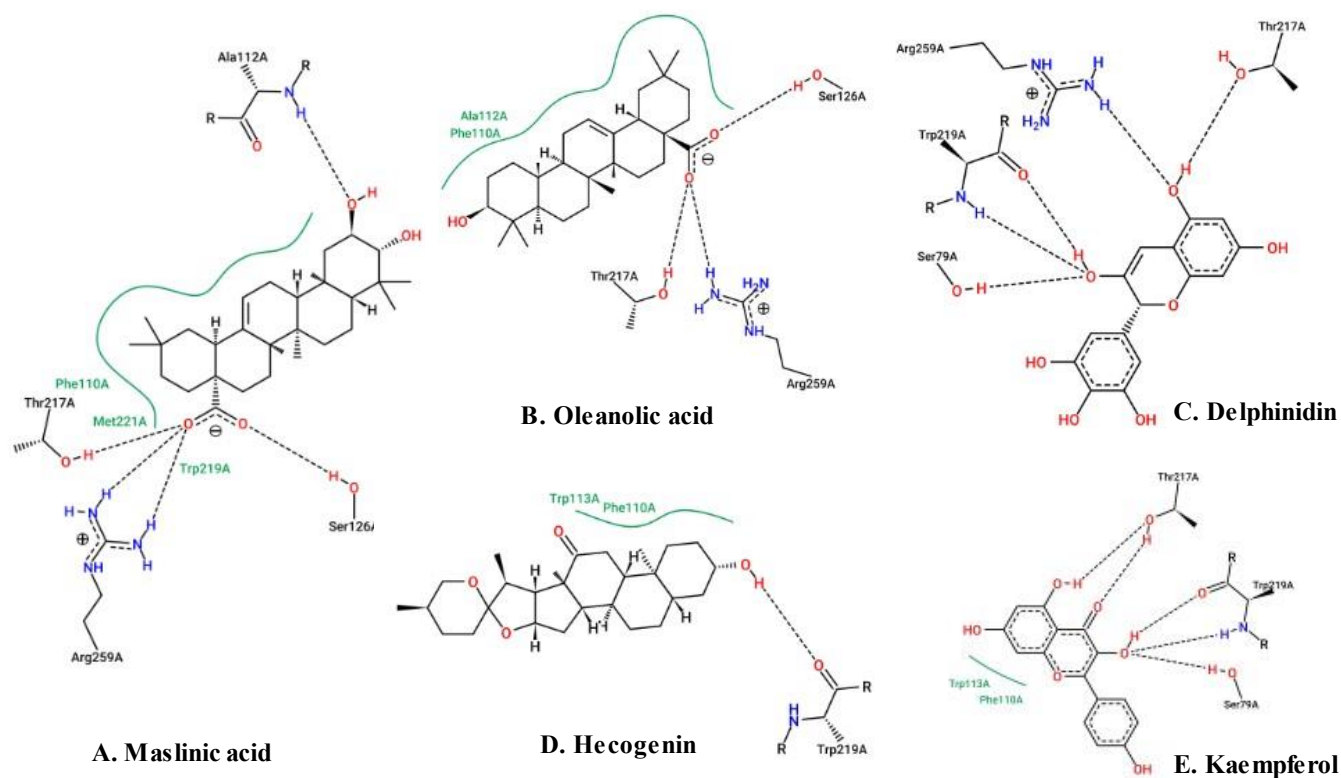
Fig. 3 2-D plots showing interaction of A. Oleanolic acid B. Hecogenin with OXA-23. Using PoseView at the ProteinPlus web portal https://proteins.plus/

4.2 Pharmacokinetic Analysis

RO5 analysis for top 5 lead molecules for each set of ligand library is given table 4.3. This analysis assesses drug likeliness properties for the test compounds. The rule states that the compounds molar Mass $\leq$ 500 Da; H-bond donor $\leq$ 5; H-bond acceptor $\leq$ 10; High lipophilicity (LogP) $\leq$ 5; and Molar refractivity (MR) around 40-130 cm3/mol are likely to be orally bioavailable [50].

The findings revealed that majority of the plant compounds possess favourable drug likeliness properties as majority of the lead compounds showed zero or single violation to the rule. Compounds showing zero violation were: Hecogenin, and Kaempferol

Table III. RO5 Analysis for the bioactive compounds with favourable binding energies towards *A. baumannii* OXA-23

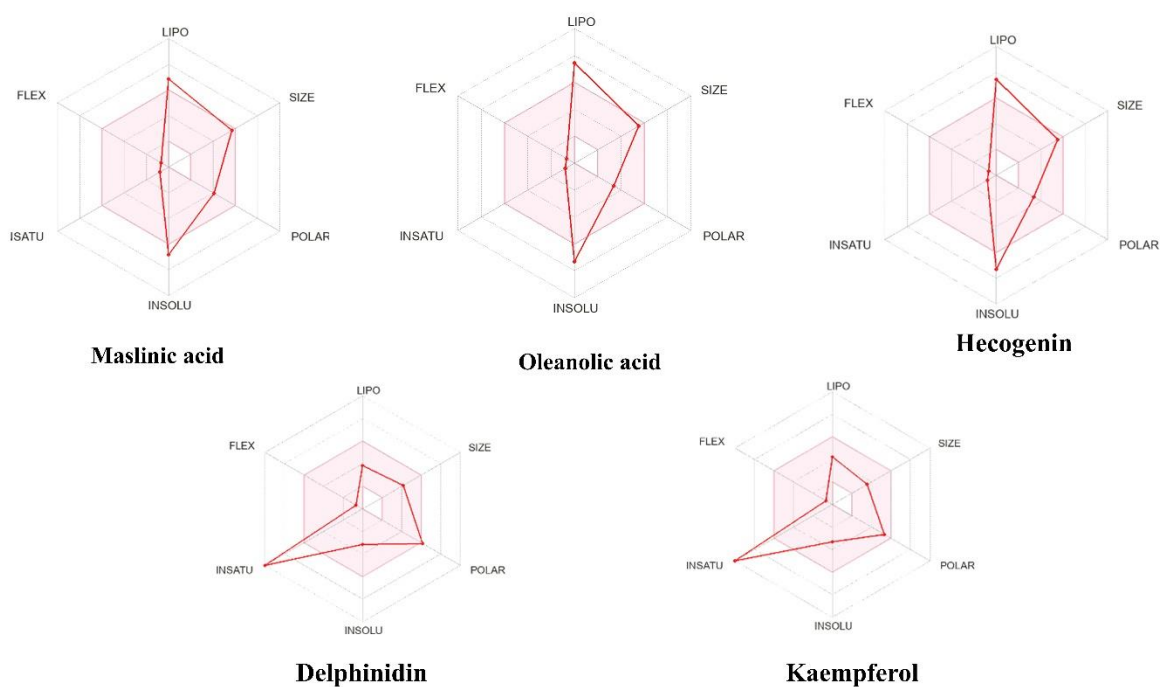| Compounds	Violation	HBA	HBD	LogP	Mass	MR
Maslinic acid	1	4	3	6.204	472	134.071
Oleanolic acid	1	3	2	7.234	456	132.681
Hecogenin	0	4	1	4.973	430	118.115
Delphinidin	1	7	6	0.889	338	74.872
Kaempferol	0	6	4	2.305	286	72.386

Fig. 4 Bioavailability RADAR showing the drug-likeliness behaviour of top lead compounds. Pink regions depict area of standard range for each characteristic.

SWISS ADME evaluation showed that all the top lead molecules from the *W. fruticosa* ligand library exhibited moderate to high bioavailability score. With oleanolic acid displaying highest i.e., 0.85. Bioavailability radars obtained as shown in Fig 4 provide more clear depiction of the drug-like attributes of the lead compounds. These computationally generated plots account for certain physiochemical traits of the compounds viz, polarity, saturation etc., [51].

Bioactivity predictions are done on the basis of scores assigned to each compound by a web-based tool viz., Molinspiration. The compounds having positive score are considered to be highly active while the compounds with scores less than -5.0 are categorised as inactive. However, those with bioavailability within in this range are considered to be moderately active. Like the previous results, the bioactivity for the phytochemicals is predicted to be more favourable than that of algal compounds.

Table IV. Bioactivity scores of top 5 lead molecules: Affinity of the given ligand towards common human cell receptors

Compounds	GPCR	ICM	KI	NR	PI	EI
Maslinic acid	0.24	-0.15	-0.41	0.81	0.2	0.62
Oleanolic acid	0.28	-0.06	-0.4	0.77	0.15	0.65
Hecogenin	0.05	0.04	-0.57	0.47	0.08	0.61
Delphinidin	-0.12	-0.09	0.05	0.07	-0.24	0.04
Kaempferol	-0.1	-0.21	0.21	0.32	-0.27	0.26

4.2 Molecular Dyanamics Simulation

Root mean square deviations (RMSD) serve as a measurement for the stability of protein-ligand complex during the simulations by analysing the time dependent motions of the structure. Though it must be noted that several different factors affect the stability of the complex and RMSD takes into account only structural changes w.r.t the deviation of atoms within the simulated structure. Generally, a higher value of RMSD is indicative of structural or conformational changes in the complex thus suggesting its flexibility and dynamics, whereas lower RMSD values are indicative of stabilization. However, the scale varies from system to system and the value of high or low RMSD, is not generalized [58].
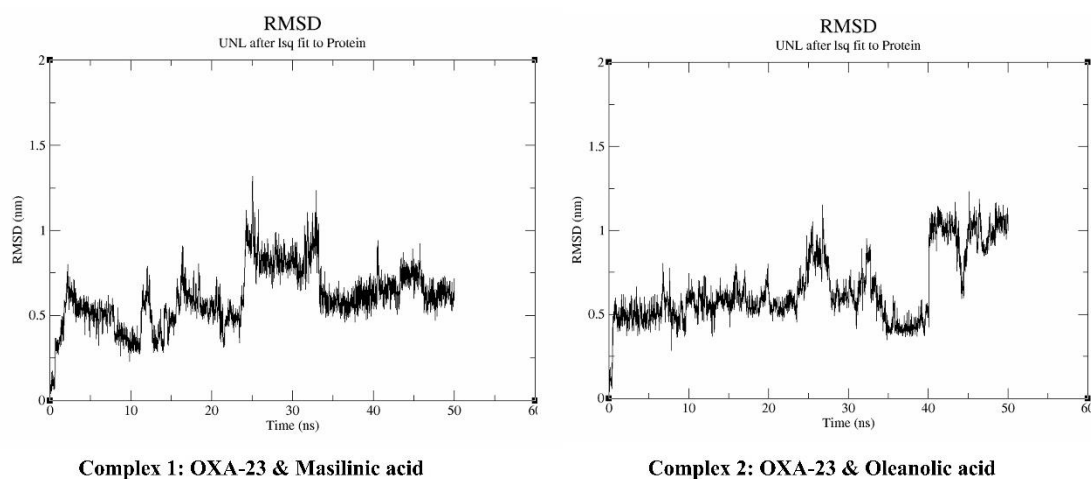

Complex 1: OXA-23 & Masilinic acid   **Complex 2: OXA-23 & Oleanolic acid**

Fig. 5 RMSD plots for the lead compounds during 50 ns run. 1) OXA-23 with Masilinic acid and 2) OXA-23 with Oleanolic acid

Complex 1. In the early stages of the simulation in between 0-26 ns, equilibration phase is observed. The complex stays stable till ~12ns. Following equilibration, plateaus can be seen in the following regions: ~26-32 ns, ~34-43 ns, and 46-50 ns. The plateaus are suggestive of a stable state of the system as during this phase, minimum structural changes occur. Simulation length and system's stability significantly influence the plateau length. The plateaus observed in this case indicate the stability of the system.

In this graph major structural changes are seen at 12 ns, 24 ns, 32 ns, 42 ns. These could potentially occur because of ligand binding or unbinding or conformational changes. The substantial fluctuations in the graph or large spikes are seen when the system undergoes

any conformational change or there occurs ligand binding/unbinding. Inclusively, the RMSD plot observed in this case shows that throughout the simulation, the RMSD values are relatively constant and there are minor fluctuations throughout the system.

Complex 2: According to the RMSD plot of complex between OXA-23 and oleanolic acid as shown in Fig 5. the equilibration phase lasts from 0-23 ns. Relatively, more Plateaus regions are observed in the beginning of the simulation. Regions showing plateaus: 1-23 ns, 28-32 ns, 36-40 ns, 41-44 ns. Significant RMSD Changes are observed in the span: 6-7 ns, 24 ns, 32ns, 41ns, 45 ns.

In both graph, there are regions of constant stability and fluctuations. Former being relatively more are indicative of the structurally stable system as in either of graphs there are no instances of sudden increase in RMSD values.
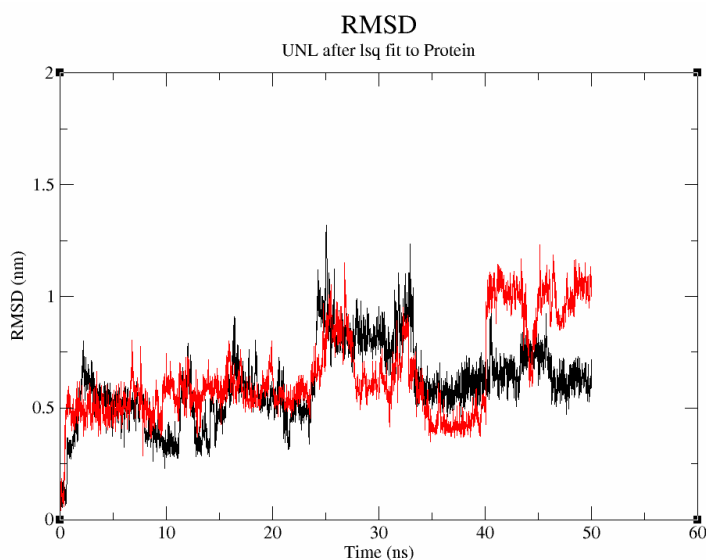

Fig. 6 Combined RMSD plots for both the complexes for comparative analysis. Red: OXA-23 with Oleanolic acid; Black: OXA-23 with Masilinic acid

Root-mean-square fluctuation describes the local changes within the protein structure during simulations and aids in identifying the flexibility and Mobility of the residues. It basically measures the fluctuation of atoms from their mean structure.

RMSF of OXA-23-Masilinic acid complex   RMSF of OXA-23-Oleanolic acid complex
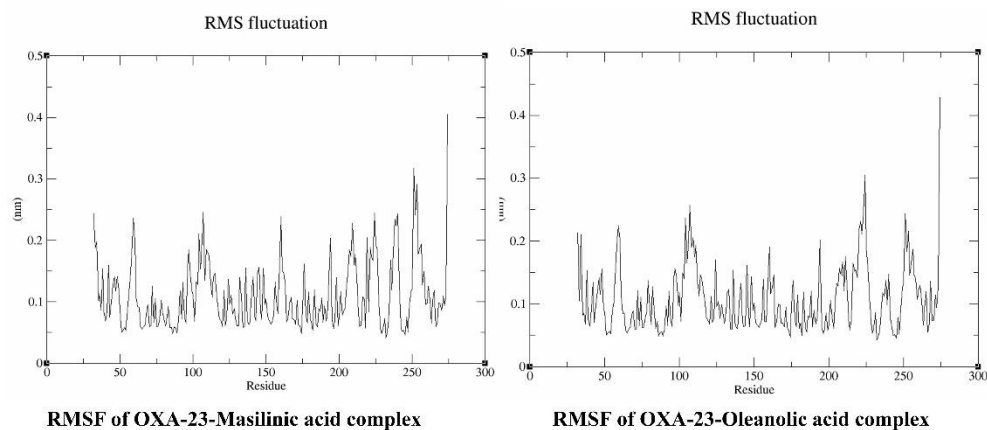
Fig. 7 RMSF plots for the lead compounds during 50 ns run. 1) OXA-23 with Masilinic acid and 2) OXA-23 with Oleanolic acid

The graphs for both the complexes (Complex 1: Masilinic acid-OXA-23 and Complex 2: Oleanolic acid-OXA-23) are relatively same. Residues with high RMSF values are : 55, 95, 106, 108, 158, 175, 192, 208, 209, 210, 220, 225, 237, 250. These regions are significant for ligand binding, or conformational changes.
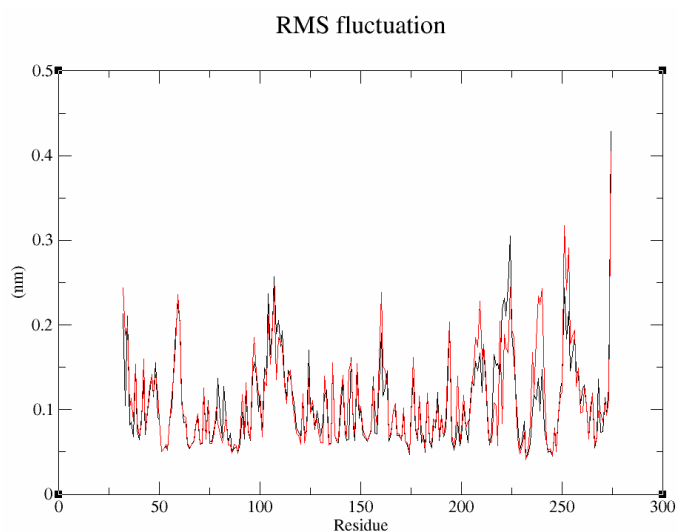
Fig. 8 Combined RMSF plots for Complex 1 and 2 for a comparative analysis run. Red: OXA-23 and Masilinic acid Black: Oleanolic acid

4.3 Radius of gyration provides an idea of compactness. That is correlated with the compactness and flexibility of the complex during simulations. It is an additional measure for the stability of protein-ligand complex. Lower the radius of gyration, greater is the compactness that corresponds to stable interaction between the ligand and the protein. Binding of a ligand molecule to the protein changes its structural conformation thereby changing its radius of gyration. An overall decreasing trend in graph may indicate a more compact or stable complex, while an increasing trend may suggest increased flexibility or conformational changes. Rg values are calculated with respect to three axis X, Y, Z providing insights on three directional distribution of the system.

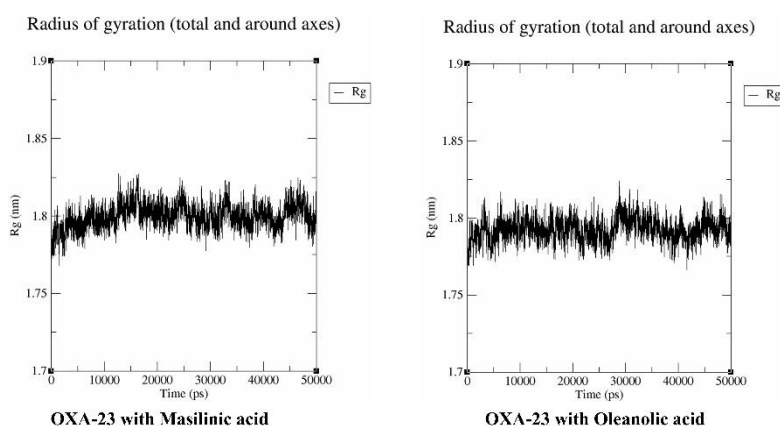**Fig. 9** Radius of gyration for protein-ligand complex: OXA-23 with Masilinic acid and OXA-23 with oleanolic acid

The Rg value for both the complexes is in the range of 1.77 to 1.79 at 0 ps as depicted in Fig. 9. Average radius of gyration for the individual complex is calculated along the three axis X, Y, Z.

Rg for complex 1 starts from 1.78 at 0 ps and lies in the range of 1.78nm to 1.83 nm for the duration until 11000 ps. A peak in Rg value (1.83 nm) is observed for complex 1 at 12500 ps. Which confers to fluctuation in complex upon binding of ligand to specific protein residue. Following this, the gyration slightly decreases for around 2000 ps and then again a peak is observed at 1.82 nm. Between 15000 to 20000 ps the Rg values are slightly decreased however, and a plateau is observed in this region indicating that the complex attained a stable state. Through out the entire simulation period there has not been any sudden increase or decrease in the Rg vales which are observed to slightly

fluctuate within the range of 1.77 nm to 1.83 nm thus indicating the equilibrium conformations and stability of the system.

For, complex 2, at 0 ps, radius of gyration is 1.77 nm and the first peak of 1.81 nm appears corresponding to 5000 ps following which there occurs sudden decrease indicating that the system is adjusting itself. And then a peak of 1.82 nm is observed corresponding to 8000 ps. Following this, a plateau region is observed at the time span of 10000 ps. This plateau is maintained over a relatively long period of time. Corresponding to 30000ps, highest peak of 1.82 is observed, following which slight fluctuations can be seen wherein the radius of gyration decreases gradually. In the time span between 41000 to 50000ps fluctuation are relatively higher than previous fluctuations, and the value corresponding to these fluctuations range between 1.76 nm to 1.82 nm indicating that the during this time frame the complex is undergoing certain conformational changes or structural rearrangement.

The overall results show that a narrow range of Rg value which can confer to stable conformations. Constants plateau in the graph are reflective of equilibrium conformations and stability of the complexes. In case of complex 2, thought fluctuations occur towards the end of the simulation run but is relatively very stable in the beginning. In line with observed results, sustained compactness of the system can be conferred.

CHAPTER 5: DISCUSSION

Employing a simulated methodology, this study identifies Maslinic acid, Oleanolic acid, Hecogenin, Delphinidin and Kaempferol obtained from *Woodfordia fruticosa* as potential inhibitors for OXA-23 protein of *A. baumannii* as the molecular docking studies. These compounds exhibited binding energy greater than -8.0 kcal/mol when docked against OXA-23 protein while Avibactam, the control used in this study exhibited relatively high binding affinity of around -6.0 kcal/mol thereby indicating that the lead compounds have more binding affinity towards the target protein i.e. OXA-23 than the standard control. Visualization of the best docked poses via Pymol and Protein plus identified Ser79, Phe110, Ser126, Thr217, Trp219, and Arg259 as the common key catalytic residues. These results are comparable to previously reported study that identified the role of SER126, THR217, TRP219, and ARG259 in stabilizing the complex of OXA-23 with a meropenem analogue thus validating the present study's finding and supporting its relevance [39].

In an in-vitro and in-silico study conducted in 2022, the OXA-23-antagonistic potential of phytochemicals like Epigallocatechin 3-gallate and Quercetin has been suggested [5]. The binding energies obtained for Epigallocatechin 3-gallate (-5.82 kcal/mol) and Quercetin (-5.77 kcal/mol) were comparable to but more than the standard inhibitor (Avibactam) [41]. In present study, sixteen molecules with binding energies significantly higher than the same standard inhibitor were identified. Though few of them were then excluded because of lack of favourable bioavailability.

Following Molecular docking, pharmacokinetic analysis revealed that the majority of the compounds had favourable bioavailability and bioactivity scores. Additional majority of them were in compliance with lipinski's rule of five thus indicating the drug likeliness properties of the test compounds.

The RMSD, RMSF and Rog plots obtained via MD simulation studies suggested that both the ligands Masilinic acid and Oleanolic acid bind with OXA-23. RMSD plots revealed that throughout the entire system there has not been any sudden changes in the RMSD values indicating that the subjects have taken up relatively stable conformation. While RMSF of the complexes obtained showed the common regions exhibiting mobile behaviour. Though the current MD simulation results do not provide a comparative

analysis of the OXA complex with any standard molecule that was because of the incompatibility of the ligand structure with CHARMM GUI interface. As of now, the MD simulation results obtained are indicative of the ligand's ability to bind with the OXA-23 complex, as following equilibration phase in the RMSD graphs of both the complexes, we can see the plateau regions and as well as the regions with minor fluctuations. Additionally, the RMSF analysis provides us the insights of residues showing maximum mobility and flexibility during the simulations, that were almost similar in both the ligands.

In accordance with available literature, in-vitro activity of several other drugs like Cefiderocol (siderophore-cephalospotin antibiotic), Eravacycline has also been reported. Diverse in-silico and in-vitro studies have reported the anticipated potential of several other compounds against CRAb. These include Cefiderocol (siderophore-cephalosporin antibiotic) [31]. Eravacycline (fluorocycline), trimethoprim–sulfamethoxazole, Durlobactam (diazabicyclooctanone) [32] in combination with Sulbactam. Lastly, the combinatorial therapy consisting of polymyxin B, tigecycline and fosfomycin have exhibited promising clearance and effectivity rates [8]. When considering the functionality of prospected and available therapeutics, their in-silico and in-vitro activity is often more favourable and reliable than actual clinical outcomes, notably because of their limited pharmacokinetic abilities. Thus, the rational use of these available antibiotics along with the exploration of new compounds are essential to combat CRAb.

CHAPTER 6: CONCLUSION

Countries across the globe have been the victims of negative ramifications of antimicrobial drug resistance. Rapidly emerging resistance mechanism and unending inability of the currently available therapeutics necessitates the exploration of alternative strategies and development of novel drug candidates. This work exploits bioinformatics tools and strategies to discern the potential inhibitors of OXA-23 protein of Carbapenem Resistance *Acinetobacter Baumannii* from the phytochemical repertoire of *Woodfordia fruticosa*.

The virtual screening analysis undertook molecular docking to assess the binding affinities of the chosen compounds towards OXA-23 protein, pharmacokinetic studies to predict the drug likeliness properties of the molecules exhibiting strong binding affinities towards the target protein lastly molecular dynamic simulations to understand Structure-activity relationships for the top lead molecules. However, there is a need to conduct further in-vitro and in-vivo studies to validate this data. Though, existing literature suggests the bioactive role of phytochemicals but target specific activity of the identified compounds can only be confirmed with in-vitro and in-vivo studies. This work significantly serves as the preliminary research that will ultimately aid in discovering cost-effective and reliable therapeutic options against Carbapenem resistant *Acinetobacter baumannii*.

The findings of the present work highlight the potential of phytochemicals to replace the current therapeutic systems. The indigenous medicines have substantially relied upon the therapeutic properties of the herbal plants for curing several different ailments. The remarkable bio-functional properties in conjunction with negligible side effects and versatile range of influence makes them well-suited for drug development.

REFERENCES

[1]   C. Manyi-Loh, S. Mamphweli, E. Meyer, and A. Okoh, "Antibiotic Use in Agriculture and Its Consequential Resistance in Environmental Sources: Potential Public Health Implications," *Molecules*, vol. 23, no. 4, p. 795, Mar. 2018, doi: 10.3390/molecules23040795.

[2]   T. M. Wozniak, A. Dyda, and X. Lee, "The Increased Length of Hospital Stay and Mortality Associated With Community-Associated Infections in Australia," *Open Forum Infect Dis*, vol. 9, no. 5, May 2022, doi: 10.1093/ofid/ofac133.

[3]   A. A. DeNegre, M. L. Ndeffo Mbah, K. Myers, and N. H. Fefferman, "Emergence of antibiotic resistance in immunocompromised host populations: A case study of emerging antibiotic resistant tuberculosis in AIDS patients," *PLoS One*, vol. 14, no. 2, p. e0212969, Feb. 2019, doi: 10.1371/journal.pone.0212969.

[4]   D. V. Patangia, C. Anthony Ryan, E. Dempsey, R. Paul Ross, and C. Stanton, "Impact of antibiotics on the human microbiome and consequences for host health," *Microbiologyopen*, vol. 11, no. 1, Feb. 2022, doi: 10.1002/mbo3.1260.

[5]   M. Nguyen and S. G. Joshi, "Carbapenem resistance in *Acinetobacter baumannii* , and their importance in hospital-acquired infections: a scientific review," *J Appl Microbiol*, vol. 131, no. 6, pp. 2715–2738, Dec. 2021, doi: 10.1111/jam.15130.

[6]   C. A. Smith *et al.*, "Structural Basis for Carbapenemase Activity of the OXA-23 β-Lactamase from Acinetobacter baumannii," *Chem Biol*, vol. 20, no. 9, pp. 1107–1115, Sep. 2013, doi: 10.1016/j.chembiol.2013.07.015.

[7]   R. Beigverdi, A. Sattari-Maraji, M. Emaneini, and F. Jabalameli, "Status of carbapenem-resistant Acinetobacter baumannii harboring carbapenemase: First systematic review and meta-analysis from Iran," *Infection, Genetics and Evolution*, vol. 73, pp. 433–443, Sep. 2019, doi: 10.1016/j.meegid.2019.06.008.

[8]   J. C. Abdul-Mutakabbir, N. C. Griffith, R. K. Shields, F. P. Tverdek, and Z. K. Escobar, "Contemporary Perspective on the Treatment of Acinetobacter baumannii Infections: Insights from the Society of Infectious Diseases Pharmacists," *Infect Dis Ther*, vol. 10, no. 4, pp. 2177–2202, Dec. 2021, doi: 10.1007/s40121-021-00541-4.

[9]   B. Shaker, S. Ahmad, J. Lee, C. Jung, and D. Na, "In silico methods and tools for drug discovery," *Comput Biol Med*, vol. 137, p. 104851, Oct. 2021, doi: 10.1016/j.compbiomed.2021.104851.

[10]   Y. Chang, B. A. Hawkins, J. J. Du, P. W. Groundwater, D. E. Hibbs, and F. Lai, "A Guide to In Silico Drug Design," *Pharmaceutics*, vol. 15, no. 1, p. 49, Dec. 2022, doi: 10.3390/pharmaceutics15010049.

[11]   M. De Vivo, M. Masetti, G. Bottegoni, and A. Cavalli, "Role of Molecular Dynamics and Related Methods in Drug Discovery," *J Med Chem*, vol. 59, no. 9, pp. 4035–4061, May 2016, doi: 10.1021/acs.jmedchem.5b01684.

[12] C.-R. Lee *et al.*, "Biology of Acinetobacter baumannii: Pathogenesis, Antibiotic Resistance Mechanisms, and Prospective Treatment Options," *Front Cell Infect Microbiol*, vol. 7, Mar. 2017, doi: 10.3389/fcimb.2017.00055.

[13] C. Ayoub Moubareck and D. Hammoudi Halat, "Insights into Acinetobacter baumannii: A Review of Microbiological, Virulence, and Resistance Traits in a Threatening Nosocomial Pathogen," *Antibiotics*, vol. 9, no. 3, p. 119, Mar. 2020, doi: 10.3390/antibiotics9030119.

[14] Y. Smani, A. Fàbrega, I. Roca, V. Sánchez-Encinales, J. Vila, and J. Pachón, "Role of OmpA in the Multidrug Resistance Phenotype of Acinetobacter baumannii," *Antimicrob Agents Chemother*, vol. 58, no. 3, pp. 1806–1808, Mar. 2014, doi: 10.1128/AAC.02101-13.

[15] E. Geisinger and R. R. Isberg, "Antibiotic Modulation of Capsular Exopolysaccharide and Virulence in Acinetobacter baumannii," *PLoS Pathog*, vol. 11, no. 2, p. e1004691, Feb. 2015, doi: 10.1371/journal.ppat.1004691.

[16] U. Waack *et al.*, "CpaA Is a Glycan-Specific Adamalysin-like Protease Secreted by Acinetobacter baumannii That Inactivates Coagulation Factor XII," *mBio*, vol. 9, no. 6, Dec. 2018, doi: 10.1128/mBio.01606-18.

[17] C. Greene, J. Wu, A. H. Rickard, and C. Xi, "Evaluation of the ability of *Acinetobacter baumannii* to form biofilms on six different biomedical relevant surfaces," *Lett Appl Microbiol*, vol. 63, no. 4, pp. 233–239, Oct. 2016, doi: 10.1111/lam.12627.

[18] M. Whiteley, S. P. Diggle, and E. P. Greenberg, "Progress in and promise of bacterial quorum sensing research," *Nature*, vol. 551, no. 7680, pp. 313–320, Nov. 2017, doi: 10.1038/nature24624.

[19] F. C. Morris, C. Dexter, X. Kostoulias, M. I. Uddin, and A. Y. Peleg, "The Mechanisms of Disease Caused by Acinetobacter baumannii," *Front Microbiol*, vol. 10, Jul. 2019, doi: 10.3389/fmicb.2019.01601.

[20] L. J. Juttukonda, W. J. Chazin, and E. P. Skaar, "Acinetobacter baumannii Coordinates Urea Metabolism with Metal Import To Resist Host-Mediated Metal Limitation," *mBio*, vol. 7, no. 5, Nov. 2016, doi: 10.1128/mBio.01475-16.

[21] N. M. Elhosseiny and A. S. Attia, "*Acinetobacter* : an emerging pathogen with a versatile secretome," *Emerg Microbes Infect*, vol. 7, no. 1, pp. 1–15, Dec. 2018, doi: 10.1038/s41426-018-0030-4.

[22] G. D. Repizo, "Prevalence of *Acinetobacter baumannii* strains expressing the Type 6 secretion system in patients with bacteremia," *Virulence*, vol. 8, no. 7, pp. 1099–1101, Oct. 2017, doi: 10.1080/21505594.2017.1346768.

[23] C. Bartal, K. V. I. Rolston, and L. Nesher, "Carbapenem-resistant Acinetobacter baumannii: Colonization, Infection and Current Treatment Options," *Infect Dis Ther*, vol. 11, no. 2, pp. 683–694, Apr. 2022, doi: 10.1007/s40121-022-00597-w.

[24] G. Montrucchio *et al.*, "The Burden of Carbapenem-Resistant Acinetobacter baumannii in ICU COVID-19 Patients: A Regional Experience," *J Clin Med*, vol. 11, no. 17, p. 5208, Sep. 2022, doi: 10.3390/jcm11175208.

[25] S. Santajit *et al.*, "Phenotypic and Genotypic Investigation of Carbapenem-Resistant Acinetobacter baumannii in Maharaj Nakhon Si Thammarat Hospital, Thailand," *Antibiotics*, vol. 12, no. 3, p. 580, Mar. 2023, doi: 10.3390/antibiotics12030580.

[26] C. Lyu, Y. Zhang, X. Liu, J. Wu, and J. Zhang, "Clinical efficacy and safety of polymyxins based versus non-polymyxins based therapies in the infections caused by carbapenem-resistant Acinetobacter baumannii: a systematic review and meta-analysis," *BMC Infect Dis*, vol. 20, no. 1, p. 296, Dec. 2020, doi: 10.1186/s12879-020-05026-2.

[27] X. Qu *et al.*, "Polymyxin B Combined with Minocycline: A Potentially Effective Combination against blaOXA-23-harboring CRAB in In Vitro PK/PD Model," *Molecules*, vol. 27, no. 3, p. 1085, Feb. 2022, doi: 10.3390/molecules27031085.

[28] B. Isler, Y. Doi, R. A. Bonomo, and D. L. Paterson, "New Treatment Options against Carbapenem-Resistant *Acinetobacter baumannii* Infections," *Antimicrob Agents Chemother*, vol. 63, no. 1, Jan. 2019, doi: 10.1128/AAC.01110-18.

[29] M. E. Falagas, K. Z. Vardakas, A. Kapaskelis, N. A. Triarides, and N. S. Roussos, "Tetracyclines for multidrug-resistant Acinetobacter baumannii infections," *Int J Antimicrob Agents*, vol. 45, no. 5, pp. 455–460, May 2015, doi: 10.1016/j.ijantimicag.2014.12.031.

[30] Y. Deng, L. Chen, M. Yue, X. Huang, Y. Yang, and H. Yu, "Sulbactam combined with tigecycline improves outcomes in patients with severe multidrug-resistant Acinetobacter baumannii pneumonia," *BMC Infect Dis*, vol. 22, no. 1, p. 795, Oct. 2022, doi: 10.1186/s12879-022-07778-5.

[31] M. Falcone *et al.*, "Cefiderocol as Rescue Therapy for *Acinetobacter baumannii* and Other Carbapenem-resistant Gram-negative Infections in Intensive Care Unit Patients," *Clinical Infectious Diseases*, vol. 72, no. 11, pp. 2021–2024, Jun. 2021, doi: 10.1093/cid/ciaa1410.

[32] R. R. Watkins and R. A. Bonomo, "Sulbactam-durlobactam: A Step Forward in Treating Carbapenem-Resistant *Acinetobacter baumannii* (CRAB) Infections," *Clinical Infectious Diseases*, vol. 76, no. Supplement_2, pp. S163–S165, May 2023, doi: 10.1093/cid/ciad093.

[33] S. Ibrahim, N. Al-Saryi, I. M. S. Al-Kadmy, and S. N. Aziz, "Multidrug-resistant Acinetobacter baumannii as an emerging concern in hospitals," *Mol Biol Rep*, vol. 48, no. 10, pp. 6987–6998, Oct. 2021, doi: 10.1007/s11033-021-06690-6.

[34] M. H. Wong, B. K. Chan, E. W. Chan, and S. Chen, "Over-Expression of ISAba1-Linked Intrinsic and Exogenously Acquired OXA Type Carbapenem-Hydrolyzing-Class D-ß-Lactamase-Encoding Genes Is Key Mechanism Underlying Carbapenem Resistance in Acinetobacter baumannii," *Front Microbiol*, vol. 10, Dec. 2019, doi: 10.3389/fmicb.2019.02809.

[35] S. J. Nigro and R. M. Hall, "Structure and context of *Acinetobacter* transposons carrying the *oxa23* carbapenemase gene," *Journal of Antimicrobial Chemotherapy*, vol. 71, no. 5, pp. 1135–1147, May 2016, doi: 10.1093/jac/dkv440.

[36] M. Hamidian and S. J. Nigro, "Emergence, molecular mechanisms and global spread of carbapenem-resistant Acinetobacter baumannii," *Microb Genom*, vol. 5, no. 10, Oct. 2019, doi: 10.1099/mgen.0.000306.

[37] S. Abdel-Shafi *et al.*, "Antimicrobial Activity and Chemical Constitution of the Crude, Phenolic-Rich Extracts of Hibiscus sabdariffa, Brassica oleracea and Beta vulgaris," *Molecules*, vol. 24, no. 23, p. 4280, Nov. 2019, doi: 10.3390/molecules24234280.

[38] S. Li, X. Duan, Y. Peng, and Y. Rui, "Molecular characteristics of carbapenem-resistant Acinetobacter spp. from clinical infection samples and fecal survey samples in Southern China," *BMC Infect Dis*, vol. 19, no. 1, p. 900, Dec. 2019, doi: 10.1186/s12879-019-4423-3.

[39] B. Ramachandran, S. Muthupandian, J. Jeyaraman, and B. S. Lopes, "Computational exploration of molecular flexibility and interaction of meropenem analogs with the active site of oxacillinase-23 in Acinetobacter baumannii," *Front Chem*, vol. 11, Feb. 2023, doi: 10.3389/fchem.2023.1090630.

[40] I. Kyriakidis, E. Vasileiou, Z. D. Pana, and A. Tragiannidis, "Acinetobacter baumannii Antibiotic Resistance Mechanisms," *Pathogens*, vol. 10, no. 3, p. 373, Mar. 2021, doi: 10.3390/pathogens10030373.

[41] E. Aydemir, E. Sarıyer, E. Akyıldız, A. Özad Düzgün, Y. Camadan, and A. Saral Sarıyer, "In vitro and in silico evaluation of some plant extracts and phytocompounds against multidrug-resistant Gram-negative bacteria," *Advances in Traditional Medicine*, vol. 22, no. 4, pp. 749–759, Dec. 2022, doi: 10.1007/s13596-021-00602-6.

[42] J. R. Shubha, P. Tripathi, B. S. Somashekar, and P. Bhatt, "Gut microbiota and metabolic changes towards improved gut health with supplementation of Woodfordia fruticosa, a medicinal plant: An in vitro study," *Innovative Food Science & Emerging Technologies*, vol. 75, p. 102896, Jan. 2022, doi: 10.1016/j.ifset.2021.102896.

[43] S. Thakur, H. Kaurav, and G. Chaudhary, "A Review on Woodfordia fruticosa Kurz (Dhatki): Ayurvedic, Folk and Modern Uses," *Journal of Drug Delivery and Therapeutics*, vol. 11, no. 3, pp. 126–131, May 2021, doi: 10.22270/jddt.v11i3.4839.

[44] M. A. Tayab *et al.*, "Antioxidant-Rich Woodfordia fruticosa Leaf Extract Alleviates Depressive-Like Behaviors and Impede Hyperglycemia," *Plants*, vol. 10, no. 2, p. 287, Feb. 2021, doi: 10.3390/plants10020287.

[45] S. Giri, G. Dey, R. Sahu, P. Paul, G. Nandi, and T. K. Dua, "Traditional Uses, Phytochemistry and Pharmacological Activities of Woodfordia fruticosa (L) Kurz: A Comprehensive Review," *Indian J Pharm Sci*, vol. 85, no. 1, 2023, doi: 10.36468/pharmaceutical-sciences.1062.

[46] D. Venkatachalam, A. K, J. A, S. P, K. V, and S. P, "A Comparative Study on Inhibition Efficacy of Solvent Extracts of Woodfordia Fruticosa and Phyto-Synthesized Silver Nanoparticles Against Certain Bacteria," *SSRN Electronic Journal*, 2023, doi: 10.2139/ssrn.4351238.

[47] M. M. Jaghoori, B. Bleijlevens, and S. D. Olabarriaga, "1001 Ways to run AutoDock Vina for virtual screening," *J Comput Aided Mol Des*, vol. 30, no. 3, pp. 237–249, Mar. 2016, doi: 10.1007/s10822-016-9900-9.

[48] I. Azad, "Molecular Docking in the Study of Ligand-Protein Recognition: An Overview," 2023. doi: 10.5772/intechopen.106583.

[49] O. Trott and A. J. Olson, "AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading," *J Comput Chem*, p. NA-NA, 2009, doi: 10.1002/jcc.21334.

[50] C. A. Lipinski, "Lead- and drug-like compounds: the rule-of-five revolution," *Drug Discov Today Technol*, vol. 1, no. 4, pp. 337–341, Dec. 2004, doi: 10.1016/j.ddtec.2004.11.007.

[51] 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. 1, p. 42717, Mar. 2017, doi: 10.1038/srep42717.

[52] M. J. Abraham *et al.*, "GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers," *SoftwareX*, vol. 1–2, pp. 19–25, Sep. 2015, doi: 10.1016/j.softx.2015.06.001.

[53] J. Lee, M. Hitzenberger, M. Rieger, N. R. Kern, M. Zacharias, and W. Im, "CHARMM-GUI supports the Amber force fields," *J Chem Phys*, vol. 153, no. 3, p. 035103, Jul. 2020, doi: 10.1063/5.0012280.

[54] N. Blanco-Cabra *et al.*, "Novel Oleanolic and Maslinic Acid Derivatives as a Promising Treatment against Bacterial Biofilm in Nosocomial Infections: An in Vitro and in Vivo Study," *ACS Infect Dis*, vol. 5, no. 9, pp. 1581–1589, Sep. 2019, doi: 10.1021/acsinfecdis.9b00125.

[55] F. B. Tessema *et al.*, "Flavonoids and Phenolic Acids from Aerial Part of Ajuga integrifolia (Buch.-Ham. Ex D. Don): Anti-Shigellosis Activity and In Silico Molecular Docking Studies," *Molecules*, vol. 28, no. 3, p. 1111, Jan. 2023, doi: 10.3390/molecules28031111.

[56] P. Nandhini *et al.*, "In-Silico molecular screening of natural compounds as a potential therapeutic inhibitor for Methicillin-resistant Staphylococcus aureus inhibition," *Chem Biol Interact*, vol. 374, p. 110383, Apr. 2023, doi: 10.1016/j.cbi.2023.110383.

[57] A. Periferakis *et al.*, "Kaempferol: Antimicrobial Properties, Sources, Clinical, and Traditional Applications," *Int J Mol Sci*, vol. 23, no. 23, p. 15054, Nov. 2022, doi: 10.3390/ijms232315054.

[58] B. Ramachandran, S. Muthupandian, J. Jeyaraman, and B. S. Lopes, "Computational exploration of molecular flexibility and interaction of meropenem analogs with the active site of oxacillinase-23 in Acinetobacter baumannii," *Front Chem*, vol. 11, Feb. 2023, doi: 10.3389/fchem.2023.1090630.

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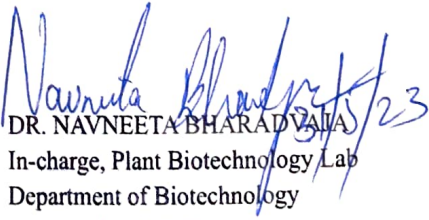
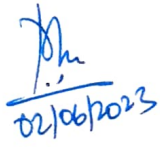# DEPARTMENT OF BIOTECHNOLOGY

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CERTIFICATE

I hereby certify that the Project Dissertation titled "Virtual screening and Simulation Study exploring phytochemical profile of *Woodfordia fruticosa* to identify potential *A. baumannii* OXA-23 antagonists", which is submitted by Shreya Kapoor, 2K21/MSCBIO/49, Delhi Technological University Delhi, in partial fulfilment of the requirement for the award of the degree of Masters in Science, is a record of the project work carried out by the students under my supervision. To the best of my knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

DR. NAVNEETA BHARADVAJA /23
In-charge, Plant Biotechnology Lab
Department of Biotechnology
Delhi, India 110042
Supervisor

02/06/2023

PROF. PRAVIR KUMAR
Head of the Department
Department of Biotechnology
Delhi, India 110042

III