In Silico Efficacy of Oleic Acid, n-Hexanoic acid, and Epiglobulol Obtained from Conocarpus Lancifolius against Class D β-lactamases

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KEYWORDS
Plant-derived products, Antimicrobial resistance, Docking, Oleic acid, OXA-48 β-lactamases.

ABSTRACT
Introduction: Antimicrobial resistance poses an escalating global challenge, compounded by the sluggish pace of new antibiotic discovery. Objectives: This study undertook an in silico analysis of phytochemicals derived from Conocarpus lancifolius, a local plant in the Jazan region of Saudi Arabia, to investigate potential antibacterial agents. Methods: The 3D crystallographic structure of OXA-48 was sourced from the RCSB protein databank (PDBID: 3HBR) and pre-processed. The Lib Dock algorithm facilitated the analysis of optimal binding orientations and potential interactions between compounds and the 3HBR. Results: The docking analysis outcome revealed Oleic acid as the most active compound and the binding mode exhibited that the -OH group of the terminal carboxylic acid moiety formed hydrogen bonds with Thr197 and Ala207, and the oxygen atom showed bonding with Lys116. Moreover, N-hexanoic acid also demonstrated promising binding interactions with the 3HBR, displaying hydrogen bond interactions with Lys208, Tyr211, and Ser70, along with hydrophobic alkyl interactions with Lys208 and Met115 at the protein's active sites. Unfavourable donor-donor and acceptor-acceptor bindings were also observed with Ser70 and Arg250 residues. Conclusions: The OXA-48 and NDM-1 predominate in countries of the Arabian Peninsula. Our findings suggest oleic acid as a promising candidate for combating OXA-48 β-lactamase, pending further assessment of its atomic structure via X-ray crystallography.

1. Introduction

The emergence of antimicrobial drug resistance represents a pressing global challenge, with the ongoing development of new antibiotics failing to adequately address this escalating problem. Consequently, medical procedures such as surgery, cancer chemotherapy, organ transplant, caesarean section, hip replacement, and others face increased risks, while also bearing the brunt of associated economic burdens. The World Bank has estimated that it could result in additional healthcare costs of USD 1 trillion by 2050, and 1-3.4 USD gross GDP loss/year by 2030 (1) https://www.worldbank.org/en/topic/health/publication/drug-resistant-infections-a-threat-to-our-economic-future).

Initiated by the World Health Organization (WHO) in 2015, the Global Antimicrobial Resistance and Use Surveillance System (GLASS) serves as a comprehensive program aimed at monitoring antimicrobial resistance (AMR) in bacteria responsible for common human infections. Over the years, GLASS has broadened its scope to encompass surveillance of antimicrobial consumption (AMC), invasive fungal infections, and a One Health surveillance model pertinent to human health. By the close of 2022, 127 countries, territories, and areas were actively engaged in GLASS participation. The recently released fifth GLASS report, developed in collaboration with Member States, provides a summary of 2020 data on AMR rates in common bacteria from diverse regions. The GLASS 2022 report indicates a troubling rise in resistance rates among commonly encountered bacterial pathogens. Additionally, this report introduces novel elements, including assessments of population testing coverage and AMR trends. Notably, it presents 2020 data on antimicrobial consumption at the national level for the first time.

In summary, GLASS plays a crucial role in understanding and addressing antimicrobial resistance, essential for effective infection control and public health strategies worldwide.

The major concern was third-generation cephalosporin-resistant Escherichia coli and methicillin-resistant

Staphylococcus aureus where the median reported rate in 76 countries was 42% in the 3GC-resistant E. coli and 35% in the S. aureus that are resistant to methicillin. Recent reports indicate that UTI-causing E. coli strains exhibit reduced susceptibility to standard antibiotics such as fluoroquinolones, ampicillin, and co-trimoxazole, thereby limiting the effectiveness of treatment options.

Similarly, Klebsiella pneumoniae also presented a higher level of resistance against crucial antibiotics, which in turn leads to increased utilization of the last resort of antibiotics namely carbapenems. The escalating global antibiotic resistance crisis poses a substantial threat, undermining the effectiveness of commonly used antibiotics in combating widespread bacterial infections (Antimicrobial resistance (www.who.int) (2).

There is an increase in Carbapenem resistance in clinical isolates of Enterobacterales and P. aeruginosa worldwide (3–7). Recently, WHO classified carbapenem-resistant and ESBL-producing Enterobacterales and carbapenem-resistant P. aeruginosa as critical, priority 1 pathogens (8) and CDC lists carbapenem-resistant Enterobacterales as an urgent threat to public health (9). Carbapenemases (serine carbapenemases and MBLs) are pervasive resistance mechanisms in carbapenem-resistant-Enterobacterales. Currently, the total number of beta-lactamase enzymes identified is 8171. Out of which, 1950 belong to Class A, 606 to Class B1, 24 to Class B2, and 3811 were found to be categorised as Class C. While 1274 belong to Class D beta-lactamase (10).

OXA-48 is a naturally occurring beta-lactamase that belongs to class D (Ambler classification) and serine beta-lactamases. It hydrolyzes its substrates by forming an acyl intermediate through the active site serine and has oxacillin as its preferred substrate. This group of beta-lactamase vary greatly in its substrate spectrum as well as sequence. Since the initial description of the OXA-48 enzyme, multiple variants have been identified, collectively constituting the 'OXA-48-like' subfamily. OXA-48-like β-lactamases, including OXA-48 itself, play a crucial role as they enzymatically hydrolyze carbapenems. These enzymes are readily transmitted among a broad spectrum of Enterobacterales (11). Remarkably, these β-lactamases often co-occur with other resistance mechanisms and have been sporadically detected in Pseudomonas aeruginosa and Acinetobacter baumannii (12, 13). Over the past two decades, OXA-48 and 'OXA-48-like' have become the dominant carbapenem-hydrolyzing enzymes among enterobacteria in most European countries and the MENA region (Middle East and Northern Africa).

OXA-48-like enzymes pose a significant challenge in detection due to their tendency to exhibit only minimal in vitro resistance to carbapenems. Consequently, the actual burden of these enzymes is likely underestimated. Nevertheless, they are linked to treatment failures involving carbapenems. A highly conserved IncL plasmid scaffold frequently harbours the bla_{OXA-48} gene and may also carry other antimicrobial resistance genes. This combination results in multidrug resistance, significantly limiting available treatment options. When dealing with infections caused by multidrug-resistant bacteria carrying bla_{OXA-48} and other resistance genes on IncL plasmids, exploring alternative treatment options is crucial. Here are some strategies:

Novel Antibiotics: Consider using recently developed antibiotics that specifically target resistant bacteria. Some examples include:

Meropenem/vaborbactam, Plazomicin Ceftazidime/avibactam, Eravacycline, Ceftolozane/tazobactam (14).

Host-Directed Therapeutics: These approaches focus on modulating the host's immune response to enhance its ability to combat infections. Strategies may involve: Boosting immune cell function, and modifying disease pathology.

Medicinal Plants: Explore the potential of natural compounds from medicinal plants. Some secondary metabolites possess antimicrobial properties and could play a role in treatment.

Nanotechnology: Investigate nanotechnology-based approaches for combating multidrug-resistant bacteria. Nanoparticles can be designed to target specific pathogens and enhance drug delivery.

Combination Therapies: Combine different antibiotics or therapeutic agents to improve efficacy and prevent resistance development.

Centuries ago, herbs and medicinal plants served as primary remedies for treating various diseases across the globe. Plants yield a plethora of natural compounds boasting varied structures and bioactive attributes, particularly secondary metabolites, which find extensive applications in the pharmaceutical and food sectors (15). Unlike conventional antimicrobials, the structural variability of phytochemicals allows them to function through various mechanisms and affect multiple biochemical pathways. Plants produce thousands of structurally

distinct compounds such as polyphenols, terpenoids, phenolic acids, essential oils, lectins, polypeptides, and alkaloids, each exhibiting diverse biological properties (16). This serves as the scientific rationale behind the utilization of herbs in traditional medicine across numerous ancient societies (17). Most of these compounds constitute secondary metabolites, characterized by their heterogeneous biosynthetic and structural properties. The presence or absence of certain specific functional groups contributes to the diversity of these compounds (18).

In the Gulf Cooperation Council (GCC) region, Conocarpus is commonly referred to as "Damas." This evergreen tree is notable for its ability to withstand high temperatures and salt, as well as its resilience to drought. The two primary species of the Conocarpus genus, C. erectus and C. lancifolius, are found in Gulf countries such as the UAE, Yemen, and KSA (19).C. erectus originates from the mangrove forest ecosystem of Florida in North America, whereas C. lancifolius is indigenous to the coastal and river regions of Somalia and Yemen. It is a tree with greenish foliage that typically reaches heights of 10 to 30 meters. It is widely distributed in the Jazan region, located in the southwestern part of the Kingdom of Saudi Arabia. C. lancifolius, also known as the Conocarpus lancifolius, exhibits remarkable adaptability to various environmental conditions. C. lancifolius is highly drought-tolerant. It can withstand prolonged periods of water scarcity, making it suitable for arid and semi-arid regions. The species demonstrates impressive salinity resistance. It can thrive even when exposed to high levels of salt in irrigation water (up to 18,000 ppm salinity). Moreover, C. lancifolius excels in withstanding high summer temperatures. When other plant species suffer from extreme heat (even exceeding 50°C), this tree remains resilient. In summary, C. lancifolius is a hardy tree that withstands drought, salinity, and high temperatures, making it a valuable species for challenging environmental contexts (20). It has already been reported for its medicinal value with special reference to anti-diabetic and cytotoxicity properties (21). Its application and use in treating diabetes mellitus, anaemia, flu, fever, skin ulcers, and syphilis have also been reported (22, 23). The hot methanolic extract (HMEL) of Conocarpus lancifolius leaves contains several bioactive compounds, including 1-(3-Methoxy-2-nitrobenzyl) isoquinoline, Hexadecanoic acid, 2,3-dihydroxypropyl ester, Caryophyllene oxide, Epiglobulol, Campesterol, Oleic acid, and eicosyl ester (Fig. 1). Palmitate (commonly known as Hexadecanoic acid) has previously been recognized for its antibacterial and antifungal properties (24). Additionally, the alkaloidal extract from the leaves of C. lancifolius demonstrated antibacterial effects.

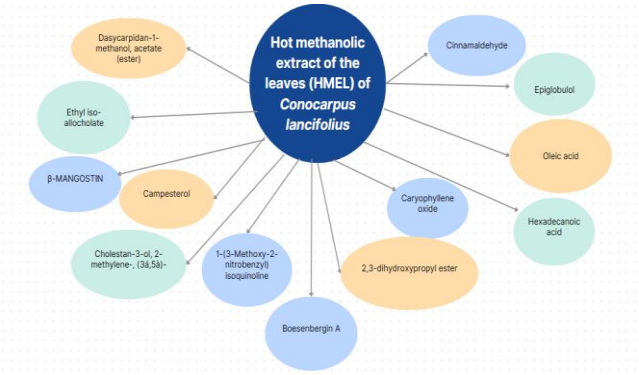


Fig 1: Schematic representation of the extracts isolated from Conocarpus lancifolius

Molecular docking, a crucial technique for in silico drug design, evaluates the binding affinity between a protein and a ligand. It quantifies this interaction through binding energy calculations using computer algorithms. Lower binding energy indicates a higher affinity of the ligand for the target (26). Furthermore, the relative orientation of interacting molecular entities can influence the type of signal generated, for example, agonism versus antagonism. Molecular docking plays a crucial role in predicting both the potency and specific signalling outcomes. It remains a widely utilized technique in structure-based drug design due to its ability to forecast the binding conformation of small molecule ligands to their appropriate target sites.

Understanding and characterizing the binding behaviour is crucial for rational drug design and for unravelling fundamental biochemical processes (27). Recognizing the fundamental principles governing interactions—such as van der Waals forces, hydrogen bonding, and electrostatic interactions—between ligands and their protein or nucleic acid targets provides a framework for designing highly potent and specific drugs. These insights are crucial for addressing specific therapeutic targets (28).

2. Objectives

There is inadequate research and development for newer antibiotics amid the rising resistance level. There is an urgent need to search for other potential alternatives that possess antimicrobial properties. Exploring phytochemicals and bacteriophage could be a suitable solution for this issue. Considering the need to explore potential antibacterial agents, this study was intended to perform molecular docking analysis of various phytochemicals obtained from an indigenous plant Conocarpus lancifolius against Class D β-lactamases.

3. Methods

i. Selection of the ligands: All phytochemical molecules were selected as ligands from the existing literature to target the proteins OXA-48 β-lactamase (29).

ii. Molecular Docking of Identified Compounds with 3HBR

Preparation of proteins: The 3D crystallographic structure of the OXA-48 β-Lactamase (3HBR) was retrieved from the RCSB Protein Data Bank (PDB) database (PDB ID: 3HBR). Later, the retrieved structure was pre-processed. This process involves removing water molecules from the cavity, stabilizing charges, reconstructing missing residues, and adjusting side chains based on the available parameters. Moreover, amendments in protein structure involve various jobs, comprising the addition of misplaced atoms into incomplete residues, mislaid loop section modelling, deletion of assertions substitution, proton titration residues, eliminating heteroatoms, insertion of hydrogen atoms and giving standardized atomic names.

Preparation of Ligands: Ligands can be obtained from various databases like ZINC and PubChem, or they can be manually sketched.

The CHARMM (Chemistry at HARvard Macromolecular Mechanics) force field-based molecular dynamics algorithm was used to perform the preparation of the desired protein.

The CHARMM program is a widely used set of force fields for molecular dynamics and then analysing a wide range of molecular simulations. The most basic kinds of simulation are minimizing a given structure and production runs of a molecular dynamics' trajectory (30). ChemDraw Professional 15.1 was used to draw the structure of the desired compounds or ligands. The resulting files were saved in MOL format.

Protein-ligand docking: To analyse the possible binding orientations and a better understanding of the probable interactions of the desired compounds with the OXA-48 β-Lactamase (3HBR) receptor, a molecular docking study that utilizes the LibDock algorithm was used (Accelrys Discovery Studio Version 2.0 software). This algorithm utilizes the physicochemical properties of the ligands to guide docking to the corresponding features in the protein binding sites by matching a triplet of ligand atoms to a triplet of protein hot spots. Each configuration is the combined score of Vander Waals forces, H-bonds, pi interactions and other parameters and is referred to in terms of the LibDock/Docking score. A higher LibDock score means a high chance of ligand-protein binding.

The Ligand was docked alongside the protein and the interactions were analysed (31). The "Best" conformation technique was utilized, which involves the application of a poling algorithm to generate a varied set of confirmations with low- energy. The current study involved the analysis of the top 10 poses of the compounds docked into the active site of the targeted proteins to determine the type of interactions they exhibited.

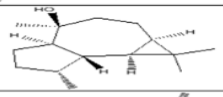
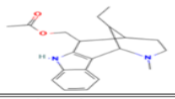
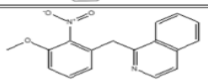
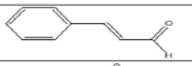
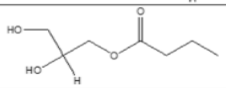
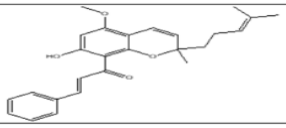
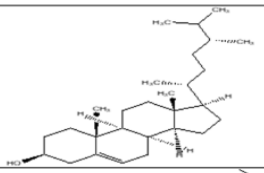
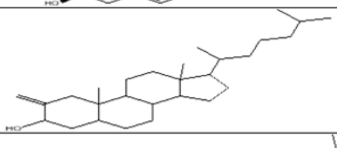
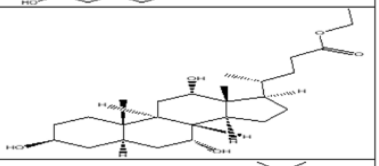
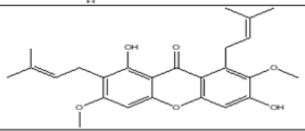
iii. Docking Assessment: Using a docking ensemble with a scoring function allows for efficient screening of extensive databases of potential drugs in silico. This approach identifies molecules that are likely to bind to a specific protein of interest Available at [Lead Optimization - Creative Biolabs (creative-biolabs.com)].

4. Results

Binding interactions between the selected compounds and OXA-48 β-Lactamase (3HBR) protein:

To investigate how the 3HBR receptor interacts with various compounds, molecular docking was conducted using specific chemical compounds derived from C. lancifolius. The compounds were labeled as samples or extracts with codes (1), (2), (3), (5), (6), (7), (8), (9), (10), (11), and (12) as detailed in Table 1. This approach helps in understanding the binding affinities and interactions between the receptor and the compounds.

Table 1: Chemical compounds and their structures obtained from C. lancifolius

Chemical Compounds	Code for the chemical compound	Structures
Oleic acid	(1)	
Hexadecanoic acid	(2)	
Epiglobulol	(3)	
Caryophyllene oxide	(4)	
Dasycarpidan-1-methanol, acetate	(5)	
1-(3-Methoxy-2-nitrobenzyl) isoquinoline	(6)	
Cinnamaldehyde	(7)	
2,3-dihydroxypropyl ester	(8)	
Boesenbergin A	(9)	
Campesterol	(10)	
Cholestan-3-ol, 2-methylene-, (3á,5à)-	(11)	
Ethyl iso-allocholate	(12)	
β-MANGOSTIN	(13)	

Binding affinity (docking scores) and hydrogen bonding catalytic residue for all the chosen compounds against OXA-48 β-Lactamase (3HBR) protein (PDB identification: 3HBR) are shown in Table 2.

Table 2: Docking scores of various phytochemicals obtained from Conocarpus lancifolius.

COMPOUND	DOCKING SCORE	AMINO ACID RESIDUES	H-BOND DISTANCE
Oleic acid	107.795	H- Lys116 C- Lys208 H- Thr197 O- Ala207 C- Val120	2.09 5.11 2.35 2.55 5.49
n- Hexanoic acid	96.634	O- Lys208 C- Lys208 C- Met115 N- Tyr211 O- Ser70 H- Arg250	2.86 4.68 4.71 2.59 2.94 2.65
Epiglobulol	87.947	C- Lys116 C- Lys208 H- Met195 C- Met115 C- Trp222	4.03 4.33 3.09 3.44 4.85
Caryophyllene oxide	86.817	O-Thr209 O- Lys208 C- Lys116 C- Trp222 H- Met115	2.01 2.19 3.65 4.66 2.79
Dasycarpidan-1-methanol	74.977	C- Lys116 C- Lys208 C- Met115	4.52 4.65 5.27
1-(3-methoxy-2-nitrobenzyl) isoquinoline	79.748	O- Thr209 C- Thr197 O- Lys116 O- Lys208 C-Met115	1.96 2.73 2.81 3.74 3.82
Cinnamaldehyde	62.148	C- Lys116 C- Lys208 C- Met195 C- Met115 O- Gln251	5.31 5.19 4.82 5.61 2.64

These outcomes revealed Oleic acid as the most active compound and the binding mode exhibited that the hydroxyl group of the terminal carboxylic acid moiety formed hydrogen bonds with Thr197 and Ala207, and the oxygen atom showed bonding with Lys116. Additionally, the long alkene chain of Oleic acid also displayed alkyl interactions with the Val120 and Lys208 on the active sites of the OXA-48 β-Lactamase receptor (Fig. 2).

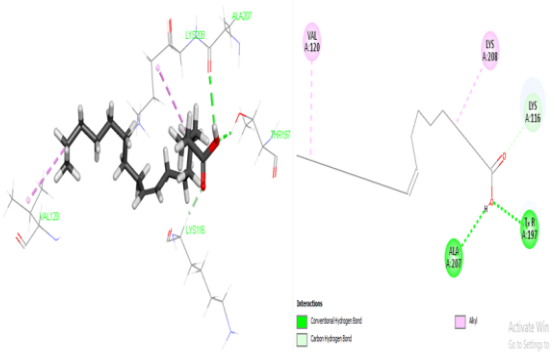


Figure 2: Docking analysis of Oleic acid

The binding acquaintances of n-Hexanoic acid with OXA-48 β-Lactamase receptor also seems to be promising as it displayed hydrogen bond interactions with Lys208, Tyr211 and Ser70, as well as hydrophobic alkyl interactions with Lys208 and Met115 active sites of the protein. Moreover, unfavourable donor-donor and acceptor-acceptor bindings are also observed with Ser70 and Arg250 residues (Fig. 3).

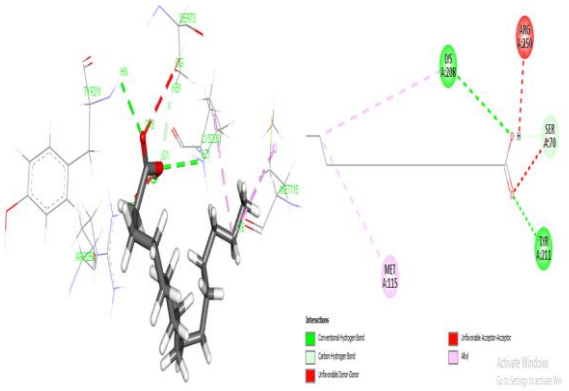


Figure 3: Docking analysis of n- Hexanoic acid

Surprisingly, the binding affinities of Epiglobulol, Caryophyllene oxide and Dasycarpidan-1-methanol were almost similar as they demonstrated hydrogen bonding with Met115, Thr209, Lys208 and Met195.

Moreover, alkyl and pi-alkyl groups of these compounds exhibited promising hydrophobic interactions with Lys116, Lys208, Try222 and Met115. Likewise, the aromatic ring structures of the above isomers showed strong pi-sigma interaction with Lys116 on the active sites of the OXA-48 β-Lactamase receptor (Fig. 4).

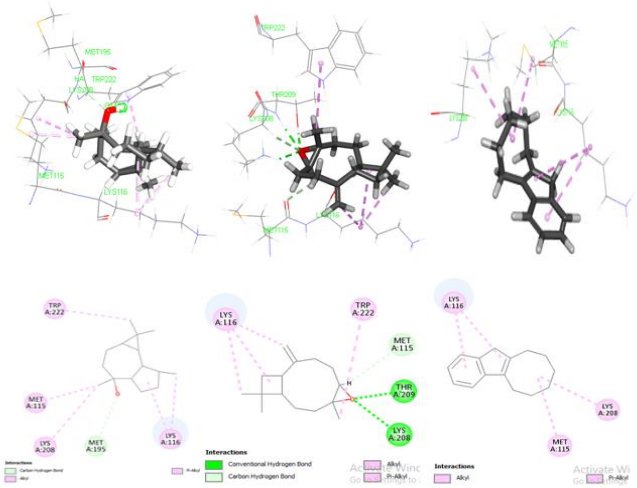


Fig. 4: Docking analysis of Epiglobulol, Caryophyllene oxide and Dasycarpidan-1-methanol

Besides, after analysing the docking results we observed that the nitro group of 1-(3-methoxy-2-nitrobenzyl) isoquinoline showed hydrogen bonding with Thr209, Lys208 and Thr197. This compound also displayed other hydrophobic interactions with Lys116, Met115, and Lys208 active sites of the receptor including pi-sigma, pi-lone pair, alkyl and pi-alkyl interactions (Fig. 5).

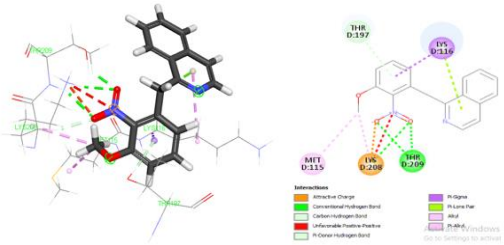


Fig. 5: Docking analysis of 1-(3-methoxy-2-nitrobenzyl) isoquinolone

The -CHO group of Cinnamaldehyde also revealed hydrogen bonds and Van der Waals interactions with Gln251 and Leu196 residues of OXA-48 β-Lactamase protein. Additionally, these compounds displayed hydrophobic pi-alkyl and pi-sulphur bond interaction with Lys116, Lys208 and Met115 on active sites of the given receptor

(Fig. 6).

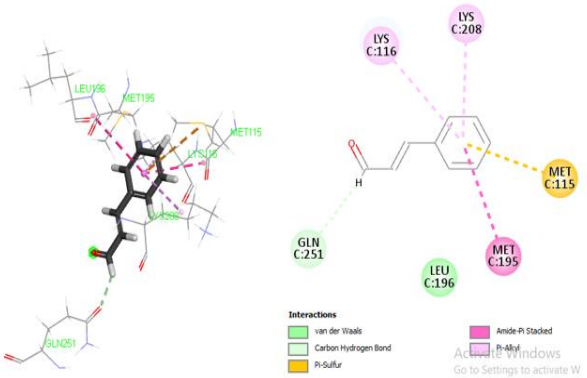


Figure 6: Docking analysis of Cinnamaldehyde

Additionally, no binding interactions were observed between OXA-48 protein and the other chemical compounds namely 2,3-dihydroxy propyl ester, boesenbergin A, campesterol, cholestan-3-ol,2-methylene-(3á,5à)-, ethyl iso-allocholate and β-mangostin.

5. Discussion

The OXA-48 β-lactamase displays a dimeric configuration that is highly resembling that of OXA-10 (35), OXA-13 (36), and OXA-46 (37). The enzyme's active site resides within a narrow crevice, featuring three motifs characteristic of Class D β-lactamase. Additionally, the carbamylated side chain of Lys73 contributes to its functional properties (37).
The enzyme's active site is situated within a narrow crevice, featuring three characteristic motifs commonly found in Class D beta-lactamases, along with the carbamylated side chain of Lys73 (38). Notably, Arg214, working in tandem with these motifs, serves a pivotal function in substrate recognition and catalytic facilitation (34, 39). Furthermore, OXA-48-like enzymes exhibit a distinctive b-5–b-6 loop conformation that alters the charge distribution and narrows the active site as it extends toward the outer portion of the active site cleft (35, 40).

The actual reservoir of bla_{OXA-48} was proved to be Shewanella oneidensis (a waterborne Gram-negative saprophyte), which harbours a chromosomal β-lactamase gene, bla_{OXA-54}. The upstream and downstream genetic components of bla_{OXA-54} and bla_{OXA-48} are the same, and its product has 92% amino acid identity to the plasmid-associated OXA-48 (41).

More recently, ST383 isolates harbouring bla_{OXA-48} and bla_{NDM-5} were reported in Lebanon and the United Kingdom (42). Published data shows a predominance of bla_{OXA-48} and bla_{NDM-1} enzymes in countries of the Arabian Peninsula (43).

Plasmids are used as the primary vehicle for the transmission and proliferation of bla_{OXA-48}-like genes. Additionally, various plasmid types like IncL, IncA/C, IncF, ColKP3, ColE2, IncX3, IncN1, and IncT have been reported to host bla_{OXA-48}-like genes (44). Surprisingly, bla_{OXA-48}-like genes are not associated with class 1 integrons in contrast to many other oxacillinase determinants (45, 46).

The treatment options to treat infections due to carbapenemase producers were mostly polymyxins, tigecycline, aminoglycosides, and fosfomycin, sometimes in combination and often together with carbapenems. However, the emergence of OXA-48 like beta-lactamase poses a threat to the treatment options.

Throughout history, plant resources have proven to be extraordinary reservoirs for the creation of medicinal treatments. Nonetheless, the medicinal potential of C. lancifolius remains largely untapped. In a study conducted by Moni et al. in 2021, they analyzed the hot methanolic extract of C. lancifolius leaves (HMEL) using GC-MS. Their findings revealed the presence of several bioactive compounds, including: 1-(3-Methoxy-2-nitrobenzyl) isoquinoline, Morphin-4-ol-6,7-dione, 1-bromo-N-methyl-Phytol, Hexadecanoic acid, 2,3-dihydroxypropyl ester, 2,2':4',2"-terthiophene, Ethyl iso-allocholate, Caryophyllene oxide, Campesterol, Epiglobulol, Cholestan-3-ol, 2-methylene-, (3á,5à)-Dasycarpidan-1-methanol, acetate (ester), Oleic acid, eicosyl ester (47). Phytol, found in essential oils extracted from various plants, is recognized as a notable constituent. These oils are

renowned for their potent antimicrobial effects (48). Hexadecanoic acid, also known as palmitate, has been documented for its antibacterial and antifungal properties (49). Furthermore, Ethyl iso-allocholate, identified in the hot methanolic extract of C. lancifolius leaves, has demonstrated antibacterial activity (50). Epiglobulol belongs to the class of compounds referred to as 5,10-cycloaromadendrane sesquiterpenoids. These compounds have also been detected in the hot methanolic extract of C. lancifolius leaves (HMEL), which has been associated with antimicrobial activity (51). The range of antibacterial activity is linked to the existence of various compounds such as phytol, hexadecanoic acid, 2,3-dihydroxy propyl ester, dasycarpidan-1-methanol, and acetate (ester). Previous research demonstrated that the alkaloidal extract derived from the leaves of C. lancifolius displayed antibacterial properties (52).

Molecular docking offers a suite of valuable tools for drug design and analysis. Visualizing molecules and accessing structural databases are essential components of this process. After systematically screening the target, ligand, and docking technique, the probable docking method is determined. While ligand flexibility poses minimal challenges, enhancing protein mobility remains crucial. Successful application examples show that computational approaches have the potential to screen hits from a huge database and design novel small molecules. However, the realistic interactions between small molecules and receptors still rely on experimental technology. Accurate and low computational cost scoring functions may bring docking applications to a new stage.

In a study conducted by Gupta et al. in 2020, researchers employed a structure-based screening approach to identify non-beta-lactam inhibitors targeting class D beta-lactamases. Their methodology involved molecular dynamics and docking techniques. Significantly, they suggest that M1593 and M2680 represent novel non-β-lactam inhibitors that complement the active site of particular OXA variants. These inhibitors interact with conserved residues crucial for β-lactam recognition and hydrolysis. The suggested inhibitors engage with the active site via noncovalent interactions, including hydrogen bonding and hydrophobic interactions, making them suitable for use as reversible-competitive inhibitors. Additionally, they propose that the presence of the valine residue within the active site of numerous OXA variants could be crucial for the binding of inhibitors or antibiotics to OXA enzymes. This insight could facilitate the development of targeted inhibitors aimed at inhibiting the enzyme's activity (53). The present structure-based drug development study is the maiden to report the inhibition of OXA-24 from A. baumannii by honey bee venom-derived AMP melittin and a synthetic AMP RP-1 exhibiting higher binding affinity (compared to the tazobactam standard) resulting in stable protein-AMP complex formation. Moreover, the GAPW mechanics in DFT calculation using CP2K that involved atom-centred Gaussian orbitals combined with plane waves containing regular grid approaches facilitated the convergence of the QM/MM simulation for the drug-design perspective towards inhibition of OXA-24 β-lactamase by melittin and RP-1. Consequently, the present findings set the foundation for AMP-based drug discovery with melittin and RP-1 to combat A. baumannii β-lactam resistance, since there is a definite need for an effective β-lactamase inhibitor to fill the therapeutic gap (53).

A recent investigation in 2024 by Cheng et al. explored the inhibitory capabilities of α-mangostin against OXA-48. α-mangostin exhibited an IC50 value of 0.52 μM. Enzyme activity assessments indicated that α-mangostin reversibly hindered OXA-48 through a non-competitive and dosage-dependent mode of inhibition. Docking analysis revealed that the 7-hydroxyl group of α-mangostin established hydrogen bonds with Thr197 and Trp222, whereas the 5-hydroxyl group and the 4-carbonyl group interacted with Lys116 and Met115. The study proposes that α-mangostin effectively inhibits OXA-48 and displays potential as a lactamase inhibitor (54).

The present study aimed to develop phytochemical derivatives of an indigenous plant Conocarpus lancifolius as potential drug candidates against Class D carbapenemases (Serine beta-lactamases), OXA-48. Our findings revealed that oleic acid could be a potential drug against OXA-48 β-lactamase, however, the atomic structure will be assessed by X-ray crystallography later. Moreover, the oleic acid can be screened against β-lactamases of other classes to be used against microbes co-exhibiting different β-lactamases. The binding of n-hexanoic acid with OXA-48 beta-lactamase receptor also seems to be promising as it displayed hydrogen bond interaction with Lys208, Tyr 211, and Ser 70, but there were unfavourable donor-donor and acceptor-acceptor bindings with Ser70 and Arg 250 residues. It could be further investigated against other classes of beta-lactamase.

The ligands that exhibit satisfactory docking performance are recommended for additional wet laboratory investigation. Since the study relied on a computational approach, further in vitro analysis is necessary to validate their activities. These compounds hold the potential to perform even better and could be considered

potential inhibitors against OXA-48 beta-lactamase.

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