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A Computational Approach for The Use of Phytocompounds as a Drug Candidate Against Carbapenemase Producing *Klebsiella pneumoniae*

Aliyu[1], A., David[1], S. M., Olufadi-Ahmed[1], H. Y., Aroyehun[1], M. A., Ibrahim[1], A., Mohammed[2], N., Olowo-okere[3], A.

[1]Department of Pharmaceutical Microbiology & Biotechnology, University of Ilorin, Nigeria
[2]Department of Microbiology, Federal University Gusau, Nigeria
[3]Department of Pharmaceutics and Pharmaceutical Microbiology, Usmanu Danfodiyo University, Sokoto Nigeria

Abstract

There is a rapid evolution and spread of carbapenem resistant bacteria through horizontal gene transfer of the genes encoding production of carbapenemases leading to the colonization of resistant Klebsiella pneumoniae (K. pneumoniae) in human. This is one of the major causes of hospital-acquired infections that are difficult to treat due to the production of carbapenamases that destroys the carbapenem antibiotics. Therefore, there is an urgent need to accelerate the discovery of antimicrobials for the effective treatment of these diseases. In silico studies are proffered as novel approaches in drug discovery to curb and alleviate diseases by identifying and characterizing potential lead compounds. This study utilized this approach to characterize carbapenemase inhibitors from natural compounds. Molecular docking (MD) and molecular dynamics and simulations (MDS) were used to profile 105 natural compounds against the K. pneumoniae carbapenemase (KPC-2). The compounds (ligands), were docked to KPC-2 (PDB ID: 2OV5) as the receptor. After the virtual screening, 24 of these phytocompounds had better inhibitory activity against KPC-2 than the co-crystallized ligand (Bicine) while 18 had higher binding affinity than the Imipenem that was used as the reference antibiotic. Hypericin, WithaferinA, Eriodictyol-7-O-glucoside and Glabidrin had the best docking scores of -10.6, -9.5, -8.8 and -8.5 Kcal/Mol respectively. Only WithaferinA, Eriodictyol-7-O-glucoside and Glabidrin bound to the active amino acid residues of the KPC-2 while ADMET studies favoured Glabridin with good profiles. MDS of the Glabridin also proved good inhibition of KPC-2 at the active site. Glabridin would be a useful drug candidate in the design of novel KPC inhibitors. Withaferin A and Eriodictyol-7-O-glucoside could also be optimized for better ADMET properties. Naturally occurring phytocompound could serve as solution to the effective treatment of resistance caused by carbapenem resistant Klebsiella pneumoniae. Further in-vitro studies are required to evaluate and confirm the potential of phytocompounds as anti-KPC-2 inhibitors.

Keywords: Antimicrobial Resistance, In-silico technique, Klebsiella pneumoniae, Carbapenemase, Drug discovery.

1. Introduction

The exponential increase of antibiotic resistance observed in recent decades is a cause for significant concern. Antibiotics that were once effective in the treatment of specific bacterial illnesses have lost their efficacy. Antimicrobial Resistance (AMR) has been recognized by the World Health Organization (WHO) as a significant global health concern necessitating

Corresponding Author: Aliyu, A.

Email: aliyu.a@unilorin.edu.ng

urgent attention and collaboration (Salam *et al*., 2023). New resistance mechanisms are arising and spreading throughout the world, posing a danger to our ability to treat common infectious diseases especially the hospital-acquired infections (Nwobodo *et al.*, 2022). The declining efficacy of antibiotics has resulted in an expanding array of infections, such as pneumonia, tuberculosis, blood poisoning, gonorrhea, and foodborne diseases, which are becoming increasingly challenging to treat (WHO, 2023).

The pipeline of novel antimicrobials in clinical trials is depleted. Currently, the number of antibiotics in clinical development is alarmingly low, with only six out of the 32 antibiotics in clinical development that address the WHO list of priority pathogens were designated as novel by WHO in 2019. This scarcity of high-quality antibiotics of broad spectrum continues to be a key problem. Antibiotic shortages are hurting countries at all stages of development, particularly in the healthcare sector (Diamantis *et al*., 2022).

Antibiotic resistance exacerbates healthcare costs, prolongs hospitalization periods, and elevates mortality rates. The increasing resistance to carbapenems, namely among members of the Enterobacteriaceae family, is a concerning manifestation of AMR (De-Oliveira *et al.*, 2020). Carbapenem antibiotics were only used for the clinical treatment of highly resistant gram-negative bacterial pathogens, such as Enterobacteriaceae (e.g. Klebsiella pneumoniae, Escherichia coli) and Pseudomonas aeruginosa, that did not respond to other treatments (Aurilio *et al.*, 2022). The emergence and spread of novel β -lactamases (which hydrolyses the carbapenem antibiotics), particularly class B and D carbapenemases necessitated the search for new inhibitors (Vázquez-Ucha *et al.*, 2020). As such, this is an indication of the urgent need for novel and innovative β-lactamases inhibitors, ideally those that are not based on the existing β-lactam paradigm, due to the tremendous diversity of β-lactamases and their widespread hazard (Grigorenko *et al.*, 2017).

However, many medicinal plants have been reported to contain compounds with antimicrobial activities, some of which are: Azadirachta indica (Alzohairy, 2016), Piper nigrum (Habtemariam *et al.*, 1993), Glycyrrhiza glabra (Pastorino *et al*., 2018), Arnica montana (Hotea *et al.*, 2022), Allium sativa (Perrett *et al.*, 1995) and Curcuma longa (Mendoza *et al.*, 1997). These natural phytocompounds could be exploited to derive a clinically effective antimicrobials to curb AMR.

There is increased emphasis on the synergy between in vivo and in silico study to facilitates identification of new compounds (Ndagi *et al*., 2020). In silico approaches have made the drug discovery against antibiotic resistant organisms and lead compound optimization a lot easier (Mandal and Das, 2017).

Conventional drug discovery ideally requires a minimum investment of US $1 billion and takes 12 to 15 years from discovery to the approved medicine. (Deore *et al.*, 2019). However, in-silico studies have emerged as an innovative method in drug discovery, resulting in reduced time and cost. Since the beginning of the 20th century, the advancement of in-silico methodologies has elevated the quality of medical research by enabling precise predictions (Moradi *et al.*, 2022). Elias *et al.* (2022) reported that with in-silico methods, the development of carbapenemase-inhibitors will not only be sped up significantly but will also be cost-effective. This study therefore aims to utilize in-silico techniques to discover natural

compounds with antimicrobial property that can act as non-β-lactam inhibitors of carbapenemases as potential lead drug candidates for the management of AMR in Klebsiella pneumoniae.

2. Materials and Methods

Receptor (Protein) Selection and Preparation

The 3-dimensional (3D) X-ray crystallographic structure of KPC-2 Carbapenemase (PDB ID:2OV5) with high resolution (1.85 Å) was retrieved from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) (https://www.rcsb.org/structure/2OV5). The protein was prepared (separated from the heteroatoms including co-crystalised ligand, (Bicine) in Chimera Version 1.14 (http://www.cgl.ucsf.edu/chimera/). The protein was saved in protein database (pdb) format. The 2OV5 contains three similar chains (A, B, C) and the chain A was used for protein preparation.

Ligand Selection and Preparation

Following an extensive literature search, the 3D structures of phytocompounds with reported antibacterial activity from various plants were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/) in structural data file (sdf) format. These phytocompounds, Biocine which was co-crystalized with the receptor and Imipenem as a positive control were used as ligands for molecular docking. Energy minimizations of the ligands was carried out using Chimera Version 1.14.

Molecular Docking and Validation

The Docking process was conducted in two (2) stages: Virtual Screening and Site-Specific docking.

Virtual Screening

As described by Rahayu et al. (2024), PyRx software was used for blind docking virtual screening with all the ligands against the KPC-2 receptor (PDB ID- 2OV5) to check for binding affinity. Bicine and Imipenem were used for docking validation and positive control (reference) respectively. The results were obtained in terms of docking scores measured in Kcal/mol. Ligands with greater or equal binding affinity based on docking scores to Bicine and reference antibiotic (Imipenem) were proceeded through UCSF Chimera for site-specific docking.

Site-Specific Docking

The active binding sites of the receptor (2OV5) and active binding residues were obtained from the PDBSum (https://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl). Using Biovia Discovery Studio 2020 the XYZ attributes (55.98, -22.92, -2.19) retrieved were used for the grid box dimensions for the active site-specific docking. Molecular docking of the ligands with higher docking score after virtual screening were performed using AutoDock Vina in UCSF Chimera 1.14 as described by Butt et al. (2020).

Molecular Docking validation

The validation technique was conducted to verify the precision of the molecular docking process using PyMol. Bicine was re-docked to the protein's binding site pocket. The validation process utilized the root mean square deviation (RMSD) as the parameter, measured in angstroms (Å) (Jannah and Wahid, 2023).

ADMET Property Prediction

The Compounds with greater binding affinity (best poses) after site-specific docking were advanced to ADMET property prediction. The Absorption, Distribution, Metabolism and Excretion and Toxicity properties of each of the hit compounds were evaluated using the SwissADME (http://swissadme.ch/) and admetSARS (http://lmmd.ecust.edu.cn/admetsar2/) websites. Other drug-like properties including synthetic accessibility, and solubility of the hit compounds were also tested using these tools. This was achieved by uploading the canonical smiles of each hit compound on SwissADME. To determine the toxicity profile, the canonical smiles of the hit compounds were uploaded on admetSARS and toxicity predictions like hepatic toxicity and nephron toxicity were fully elucidated (Saliu *et al*., 2021).

Elucidation of 2D-structure of Best Poses and Validation with PDBSum

The docking results from the site-specific docking using UCSF chimera were analyzed to show hydrogen and non-hydrogen bond interactions of the best pose ligands with the active binding amino acid residues in the active site of the KPC-2 receptor using Proteins Plus webserver (https://proteins.plus/) for 2D and PyMol Software which is an open source for modern advance in molecular visualization and animation https://www.schrodinger.com/platform/products/pymol/. The binding residues were then checked against the deposited active binding residues in the PDBSum database. If similar, it shows that the binding can produce good inhibitory activity against the KPC-2.

Molecular Dynamic (MD) Simulation

The system was configured using the CHARMM36 forcefield (Best *et al*., 2012) in conjunction with the CHARMM-GUI1-3 online interface (Lee *et al*., 2016). Every single simulation was conducted using the NAMD 2.13 tool (Phillips *et al*., 2005). Using the TIP3P explicit solvation model, the periodic boundary conditions for the system were modified to dimensions of 99.46, 100.128, and 99.97 in x, y, and z, respectively. The Top docking results (binding affinity) were evaluated using a set of parameters generated by the CHARMM general force field after the system was neutralized by Na+ ions. A 2-fs integration time step and several techniques, such as minimization, equilibration, and production, were employed in all MD simulations for 150 ns time.

3. Result and Discussion

Receptor and Ligands Search

The 3D structure of 2OV5 receptor retrieved from the PDB, the prepared chain A of 2OV5 receptor, the co-crystalized ligand (Bicine), and the Imipenem (used for positive control) were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/)and are presented in Figure 1. The with the receptor and the. KPC-2 Carbapenemase (2OV5) has been a druggable target of choice in many studies in the search of carbapenemase inhibitors (Galdadas *et al.*, 2018; Oselusi *et al*., 2023). KPC-2 presents an overall significant clinical challenge as it readily

hydrolyzes available β-lactam inhibitors (Papp-Wallace *et al.*, 2010). Hence, KPC-2 (PBD.ID:2OV5) was selected as the drug target receptor for this study.

Phytocompounds from medicinal plants with antimicrobial activity have been deposited at a PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Through the use of PubChem, 105 3D structures of phytocompounds were retrieved for virtual screening. Natural phytocompounds have always been a rich source of medicinal compounds for many diseases. Certain medicinal plants have been shown to have antibacterial activity and some of these have been used traditionally for the management of microbial infections (Rahimi *et al*., 2022).

Molecular Docking Analysis

Virtual Screening

After the virtual screening of the 105 phytocompounds, Bicine (BCN) and Imipenem against the receptor (2OV5), ligands with greater binding affinity in terms of lower docking scores than the Bicine and Imipenem are presented in Table 1. A total of 23 phytocompounds and Imipenem had lower docking scores (better activity) than the co-crystalized ligand (Bicine) with the receptor while 18 ligands had better inhibitory activity than the reference antibiotic (Imipenem). The lower the docking score the better the inhibition of the receptor (Ercan and Cınar, 2021).

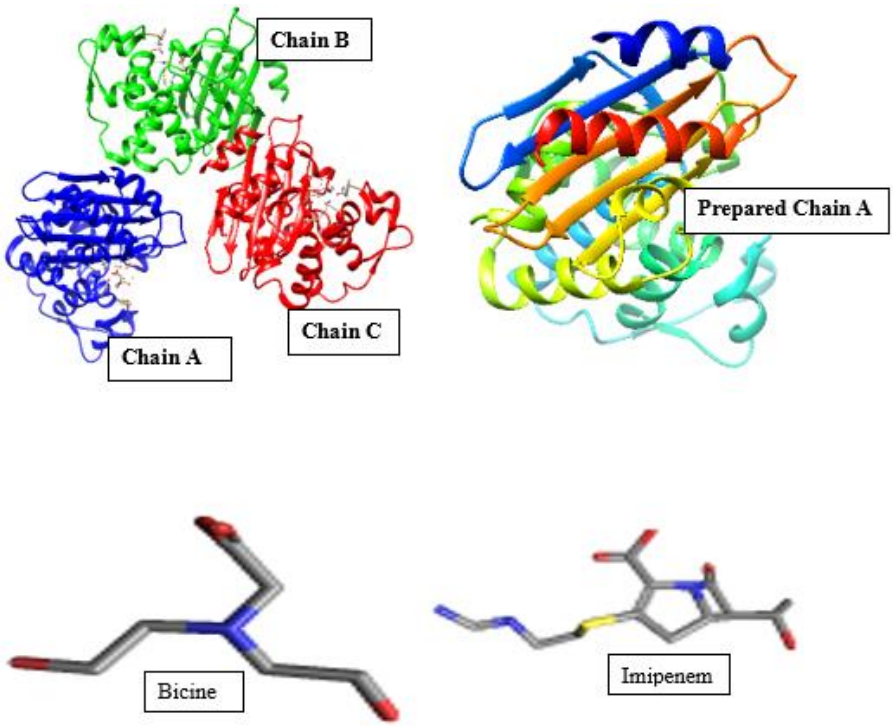


Figure 1: 3D KPC-2 receptor (2OV5) Retrieved from Protein Databank, the Prepared Chain A, Bicine and Imipenem.

Site-Specific Docking

Eighteen ligands that presented better activity than the Imipenem after virtual screening were again docked individually using UCSF Chimera 1.14 software. The docking scores were comparable to that of the virtual screening with Hypericin having the lowest docking score (best activity) followed by Withaferin A, Eriodictyol-7-O-glucoside and Glabridin. The values of the scores were also similar to that of virtual screening as presented in Table 2 while the 2D structures of the four promising phytocompounds are presented in Figure 2.

Docking Results Analysis

The best four (4) phytocompounds however interacted with the KPC-2 active site differently. The active residues of the KPC-2 given by the PDBSum (https://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl) are: SER130, THR235 and THR237 for the H-bond interaction. While a weaker van da Waal's interaction is with SER70, SER105, THR216, ARG220, LYS234 and GY236 amino acid residues. Hypericin (Figure 3a & b) had H-bonds with the residues that are not in the active site of the KPC-2, therefore may not be clinically effective. However, Withaferin A (Figure 4 a & b), Eriodictyol-7-O-glucoside (Figure 5 a & b) and Glabridin (Figure 6 a & b) interacted with the proper various active amino acid residues through both H-bonding and weak van da Waal's interaction. Consequently, these three phytocompounds will be effective as non-β lactam inhibitors of KPC-2.

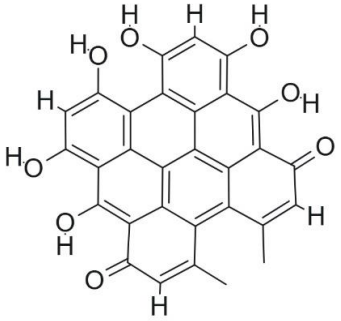
Since the clinical use of Imipenem is limited due to its low GI Absorption and hepatotoxicity, Glabridin that poses higher binding affinity, good GI absorption and no hepatotoxicity (Table 4) could serve as a better alternative antimicrobial against KPC-2. Moreso, Withaferin A has been reported to exhibit highly significant /potentiating effect with imipenem tested against A. baumannii clinical strain in metallo-βLactamase (Vasudevan et al. 2022). Also, Oselusi et al. (2023) reported that a phytocompound, Riboflavin, from Ehretia species could play a major role in the inhibition of carbapenemase producing bacteria.

Screening for ADME Properties

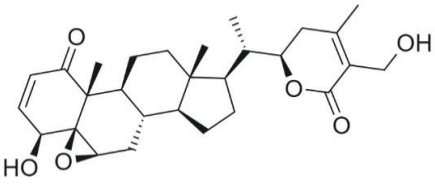
The ligands with better activity than the Imipenem were further screened for their Absorption, Distribution, Metabolism, Elimination, and Toxicity (ADMET) profiles using SwissADME and admetSARS. Table 3 shows the ADMET properties of the most promising four (4) phytocompounds in terms of binding affinity.

Table 1: List of 24 phytocompounds and Imipenem having better docking score than the co-crystalized ligand (Bicine)

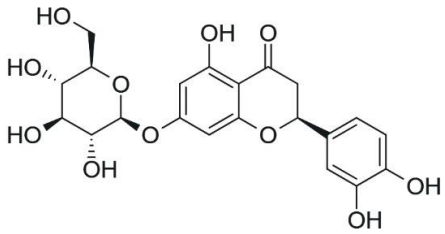
S/No	Name	Plant Source	M.Wt. (g/mol)	Binding Affinity KCal/Mol
1	Hypericin	Hypericum perforatum	504.4	-10.6
2	Withaferin A	Withania somniferum	470.6	-9.5
3	Eriodictyol-7-O-glucoside	Julgans regia	450.4	-8.8
4	Glabridin	Glycirrhiza glabra	324.4	-8.5
5	Balsaminol A	Momordica balsamina	474.7	-8.4
6	Ginsenosides	Panax notoginseng	444.7	-8.3
7	Quercetin	Julgans regia	302.23	-8.3
8	Glansreginin A	Julgans regia	593.6	-8.2
9	Carnosol	Rosmarinus officinalis	330.4	-8.2
10	Berberine	Berberis vulgaris	336.4	-8.1
11	Balsaminol B	Momordica balsamina	488.7	-8
12	Epigallocatechin	Camellia sinensis	306.27	-8
13	Catechin	Camellia sinensis	290.27	-8
14	Glabrol	Glycirrhiza glabra	392.5	-7.9
15	Chlorhexidine	Eucalyptus globulus	505.4	-7.8
16	Liquiritigenin	Glycirrhiza glabra	256.25	-7.5
17	Totarol	Podocarpus totara	286.5	-7.5
18	Piperine	Piper nigrum	285.34	-7.4
19	Imipenem	-	299.35	-7
20	Hyperforin	Hypericum perforatum	536.8	-6.5
21	Bromelain	Ananas comosus	577.97	-6.5
22	Capsaicin	Capsicum annuum	305.4	-6.3
23	Zingerone	Zingiber officinale	194.23	-6.1
24	Catechol	Piper betel	110.11	-5.3
25	Bicine	-	163.17	-5.1



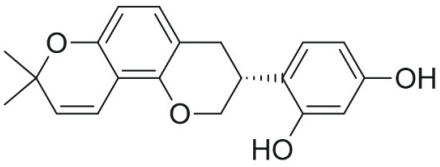
a. Hypericin from Hypericum perforatum plant



b. Withaferin A from *Withania somniferum*



c. Eriodictyol-7-O-glucoside from *Julgans regia* plant



d. Glabridin from *Glycirrhiza glabra* plant

Figure 2: Four promising phytocompounds: Hypericin (a), Withaferin A (b), Eriodictyol-7-O-glucoside (c) and Glabridin (d) from different plants

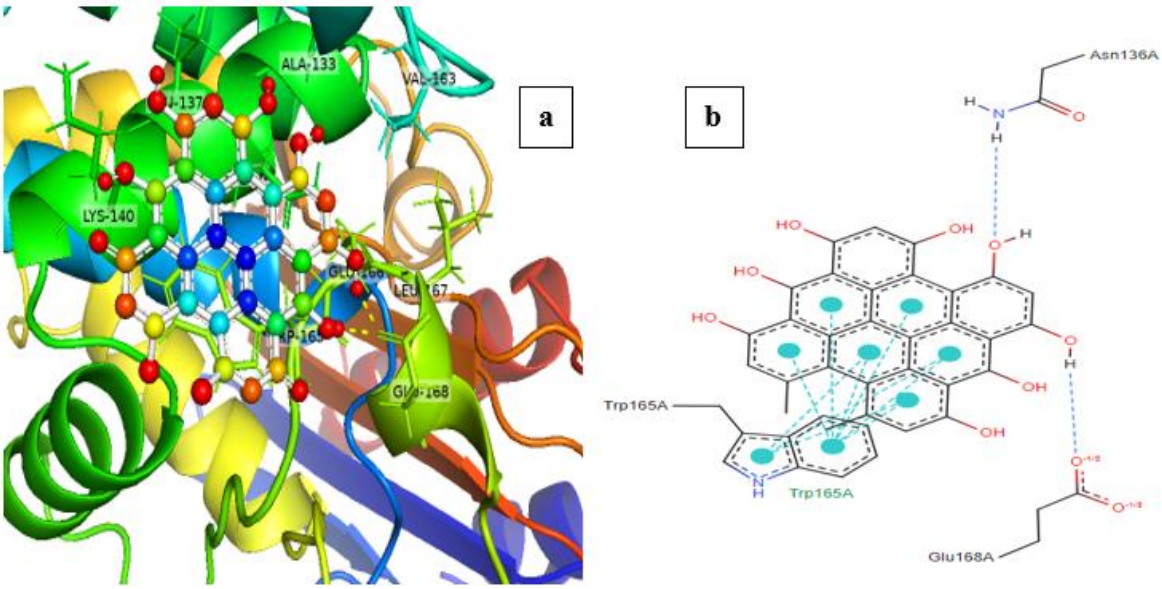


Figure 3: 3D (a) and 2D (b) diagrams showing interactions between 2OV5 and Hypericin

Table 2: Site specific docking using Chimera 1.14 of the phytocompounds having better inhibition activity that the Imipenem.

S/No.	Phytocompounds	Docking Score (Kcal/Mol)
1	Hypericin	-10.20
2	Withaferin A	-9.60
3	Eriodictyol-7-O-glucoside	-8.80
4	Glabridin	-8.60
5	Balsaminol A	-8.40
6	Ginsenosides	-8.20
7	Quercetin	-8.10
9	Carnosol	-8.10
10	Berberine	-8.10
11	Epigallocatechin	-8.00
12	Catechin	-7.90
13	Glabrol	-7.90
8	Glansreginin A	-7.70
16	Totarol	-7.70
15	Liquiritigenin	-7.60
14	Chlorhexidine	-7.50
17	Piperine	-7.30
18	Imipenem	-7.00

Table 3: ADMET Profiles of the top four phytocompounds

Molecule	Consensus Log P	Lipinski (violations)	Synthetic Accessibility	Blood Brain Barrier	Hepatotoxicity	Nephrotoxicity
Hypericin	4.26	2	3.89	-	+	-
Withaferin A	-	-	-	+	+	+
Eriodictyol-7-O-glucoside	-0.32	2	5.05	-	-	+
Glabridin	3.45	0	4.04	-	-	-
Imipenem	-0.04	0	4.49	-	+	-

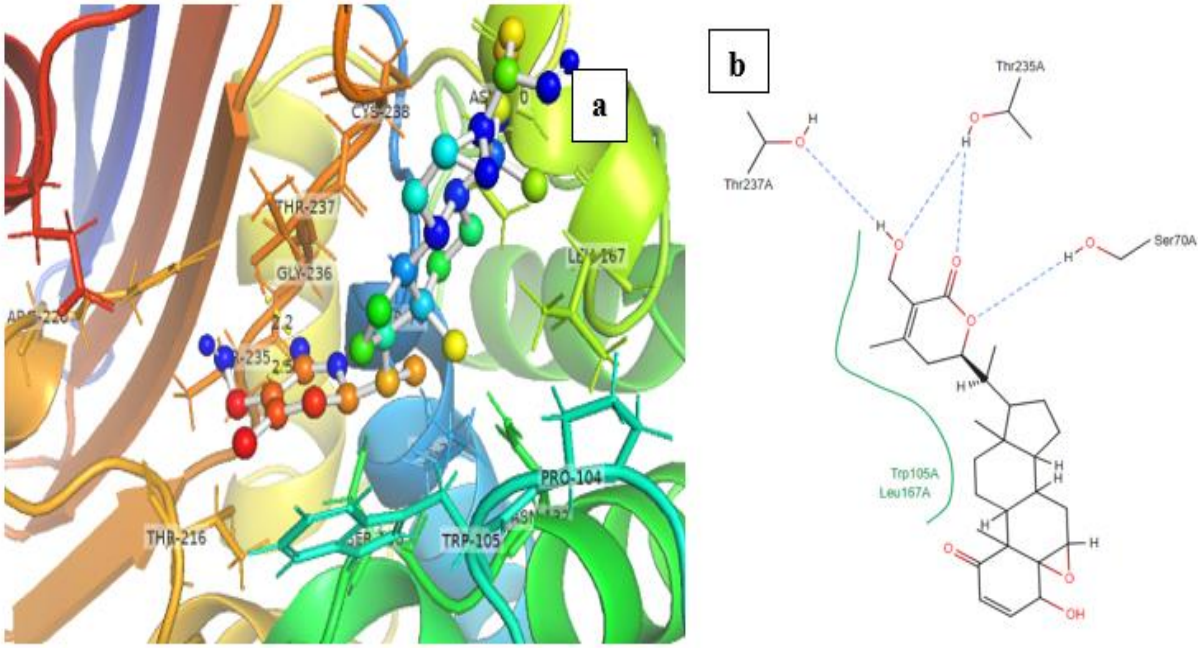


Figure 4: 3D (a) and 2D (b) diagrams showing interactions between 2OV5 and Withaferin A

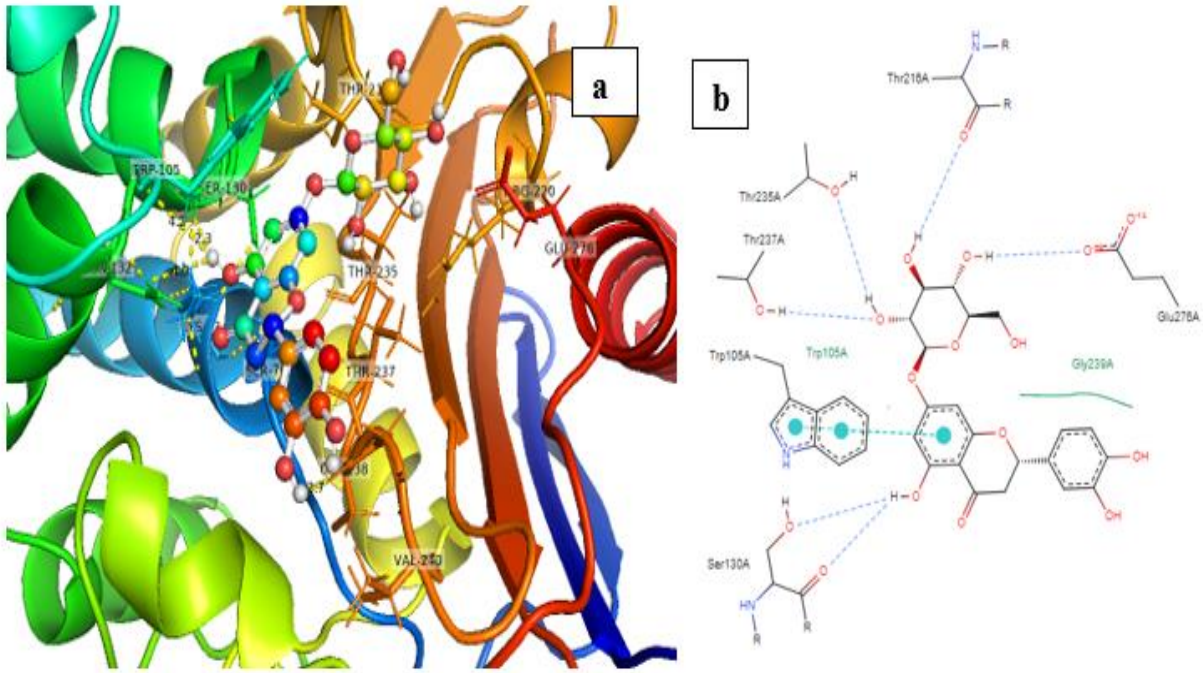


Figure 5: 3D (a) and 2D (b) diagrams showing interactions between 2OV5 and Eriodictyol-7-O-glucoside

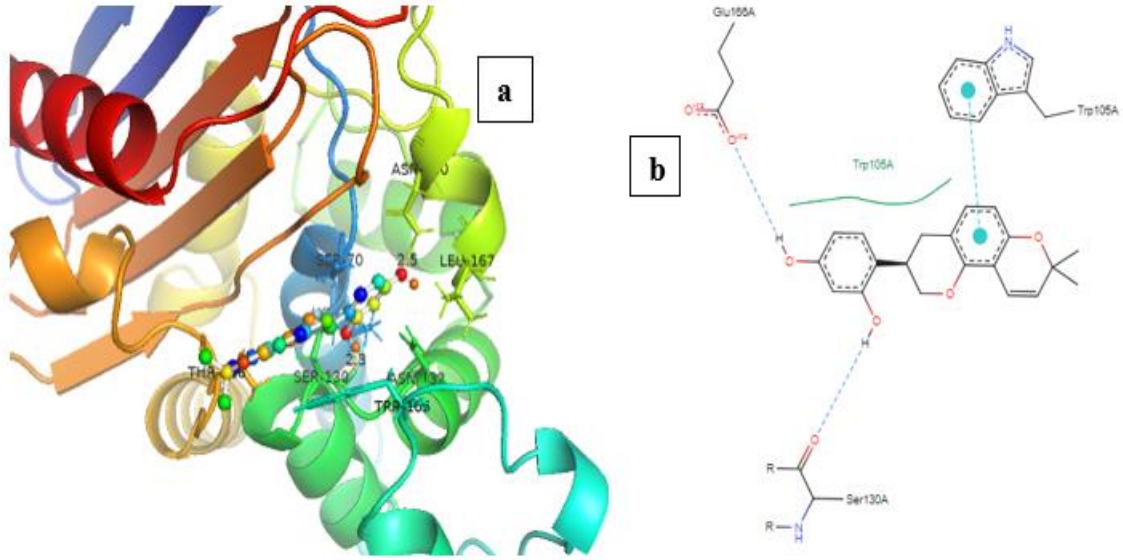


Figure 6: 3D (a) and 2D (b) diagrams showing interactions between 2OV5 and Glabridin

Hypericin and Glabridin are highly lipid soluble and would have good absorption when administered orally. None of the Lipinski's rules of a good orally administered drugs are violated by Glabridin and Imipenem. Also, there is easy synthesis of Hypericin and Glabridin. Importantly Glabridin is not hepatotoxic and not nephrotoxic. Having shown very good features of a drug candidate in terms of pharmacodynamic and pharmacokinetic, Glabridin was selected to the next and final stage of the screening, the Molecular Dynamic (MD) Simulation.

Molecular Dynamic (MD) Simulation

After the 150 ns MD Simulation of the Glabridin-2OV5 KPC-2 receptor complex, the root-mean-square deviation (RMSD of the protein, ligand and the complex were each within 2 Å (Figure 7a) and has a very low binding free energy of -69.57 kJ/mol which implies the stability of the conformations of 2OV5 and that no major conformational changes were observed. The low bond energy often supports the stability of the complex between the ligand and protein (Jannah and Wahid, 2023). There are three hydrogen bonds interactions (Figure 7b) during the simulation which corroborated the results of the molecular docking. The interactions between the amino acid residues of the receptor (2OV5) and the ligand (Glabridin) are also responsible for the complex's stable behaviour, so the ligand occupied a favorable point in the active site of the 2OV5.

The discovery of Glabridin and its characterization as inhibitors against KPC-2 carbapenemases from the numerous ligands showed that drug discovery is a very rigorous process with low chances of success but the use and application of computer-aided drug design and bioinformatics tools play an important role in minimizing cost and time. The bioactive potentials of phytochemicals against AMR pathogens have also been reported by Suganya et al. (2022). While further in-vitro studies, and optimization are necessary to validate the applicability of Glabidrin, it is sufficient to deduce that the compound display favorable properties in the inhibition of KPC-2 Carbapenemases in management of antimicrobial resistance.

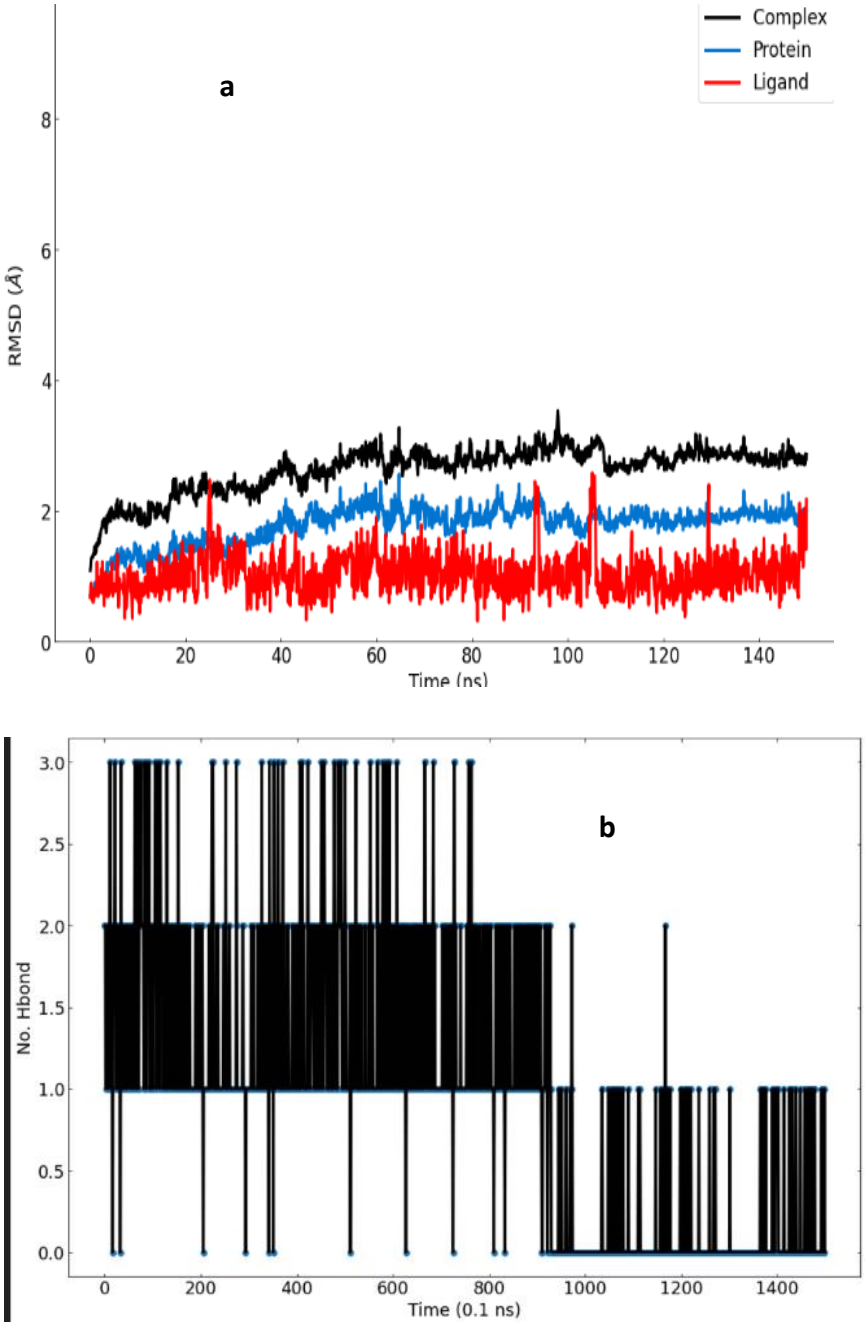


Figure 7: RMSD of the 2OV5_Glabridin Complex (a) and the H-bond interaction between the Glabridin and the amino acid Residues of the 2OV5 (b)

4. Conclusion

The study was able to successfully utilize in-silico techniques including molecular docking, Screening and MD simulation in discovering and designing potential lead compounds with proven activity and favourable physicochemical properties against carbapenamase-induced antimicrobial resistance in Klebsiella pneumoniae. Glabidrin, which is a natural phytocompound is a particularly good fit for inhibiting KPC-2 Carpenemase receptor responsible for antimicrobial resistance.

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