

In silico Evaluation of Bioactive Compounds from Endophytic Fungus *Nigrospora oryzae* UILRZ1 as Potential Inhibitors of Targets in Resistant Bacteria

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ABSTRACT

The widespread use of antibiotics has led to an increase in hospital-acquired infections. The increasing prevalence of these infections has renewed interest in exploring bioactive compounds from sources such as endophytic fungi. Thus, this study aimed to use *in silico* methods to evaluate the antibacterial activity of γ -tocopherol, α -myrin, and 3 β -acetoxy-5-bisnorcholeic acid obtained from the endophytic *Nigrospora oryzae* UILRZ1 as potential inhibitors of clinically relevant resistant bacteria. In this study, we used three-dimensional structures of protein targets, including tyrosyl-tRNA synthetase, DNA gyrase, SHV-1 beta-lactamase, penicillin-binding proteins, topoisomerase, and dihydrofolate reductase. The UniProt database was used to retrieve the targets, and Glide's Protein Wizard was used to prepare them. γ -tocopherol, 3 β -acetoxy-5-bisnorcholeic acid, and α -myrin were obtained from the PubChem database as ligands. SiteMap was chosen for binding site prediction, and Schrödinger's Glide software was employed for molecular docking. Lipinski's Rule of Five was used to evaluate the pharmacokinetic and pharmacodynamic properties of the ligands. The results showed that the ligand-protein interactions were stable and specific. γ -tocopherol (−5.43 kcal/mol) exhibited the strongest affinity, making it a promising candidate for developing novel antibiotics effective against resistant pathogens. The findings highlight that the identified bioactive compounds can be obtained from *Nigrospora oryzae* and exhibit promising antibacterial activity, which warrants further exploration in future studies. The results also have implications for the discovery of novel derivatives from endophytic fungi for addressing antibiotic resistance.

1. Introduction

The development of effective therapies against antimicrobial resistance can be achieved through innovative approaches, such as *in silico* and computational methods to predict ligand orientation in receptor-binding pockets, thereby promoting sustainable antimicrobial use [1–3]. Molecular docking enables effective screening of microorganisms for potential metabolites and introduces a more rationalized drug discovery process. Many metabolites express their activity through interactions with receptor targets within microorganisms. Several biomolecular compounds have been isolated from endophytic fungi through molecular docking studies [2].

Endophytic fungi have been shown to produce metabolites that are structurally and functionally similar to those of plants [4,5]. They have

also been shown to protect plants against stress and pathogens due to the secondary metabolites they produce [6,7]. This characteristic attribute has led to an increased interest in research into the therapeutic potential of endophytic fungi [8]. Endophytic fungi are known to produce metabolites that contribute to protein synthesis, respiration, and enzyme functions [9]. In addition, they are a natural source of antibiotics that can be used as adjuvants to enhance antimicrobial activity [10,11]. Harnessing the diverse potential of endophytic fungi is essential to the development of novel antimicrobial agents [12–14]. They are a suitable alternative source of natural products that contribute to the conservation of medicinal plants and the environment [15,16]. *Nigrospora oryzae* is an ascomycete fungus classified under the genus *Nigrospora*. It is widely recognized as a plant pathogen with a varied host range; however, recent studies have noted its isolation from medicinal plants [26],

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Table 1
Bioactive compounds with their PubChem ID, Molecular formula, and Canonical Simplified Molecular-Input Line-Entry System (SMILES).

Compound	PubChem CID	Molecular Formula	Canonical SMILES
Alpha-Amyrin	225688	C ₃₀ H ₅₀ O	CC1CCC2(CCC3(C(=CCC4C3(CCC5C4(CCC(C5(C)C)O)C)C)C2C1O)C)C[C@H](CCC(=O)O)[C@H]1CC[C@H]2[C@H]1CC[C@H]3[C@H]2CC=C4[C@H]3CC[C@H](C4)OC(=O)C
3β-Acetoxy-5-Bisnorchenolic Acid	91742734	C ₂₄ H ₃₆ O ₄	CC1=C(C=C2CCC(OC2=C1C)(C)CCCC(C)CCCC(C)CCCC(C)C)O
γ-tocopherol	14986	C ₂₈ H ₄₈ O ₂	CC(C1CCC(C(O1)OC2C(C(C(C2O)OC3C(C(C(CO3)C)O)NC)O)N)N)NC
Gentamicin	3467	C ₂₁ H ₄₃ N ₅ O ₇	

Table 2
Synergistic Effects of the Fractions on Selected Pathogens.

Test organism	Fractions			
	A	B	C	D
<i>Escherichia coli</i>	—	—	—	—
<i>Staphylococcus aureus</i>	+	+	+	+
<i>Klebsiella pneumoniae</i>	—	—	—	—
<i>Acinetobacter baumannii</i>	—	—	—	+
<i>Pseudomonas aeruginosa</i>	+	+	+	—
<i>Enterococcus faecalis</i>	+	+	+	+

KEY:
A: Fractions 1 and 2.
B: Fractions 2 and 3.
C: Fractions 1 and 3.
D: Fractions 1, 2, and 3.
+: Inhibition
–: No inhibition

Table 3
Minimum Inhibitory Concentration of Combined Fractions of *Nigrospora oryzae* UILRZ1 Metabolite (μg/mL).

Test bacteria	Fractions			
	A	B	C	D
<i>Escherichia coli</i>	128	128	128	128
<i>Staphylococcus aureus</i>	64	64	64	128
<i>Klebsiella pneumoniae</i>	256	256	128	256
<i>Acinetobacter baumannii</i>	64	128	128	256
<i>Pseudomonas aeruginosa</i>	128	128	128	256
<i>Enterococcus faecalis</i>	64	64	128	64

KEY:
A: Fractions 1 and 2.
B: Fractions 2 and 3.
C: Fractions 1 and 3.
D: Fractions 1, 2, and 3.

as an endophytic fungus has been isolated from several medicinal plants, including *Helianthus annuus*, *Sclerocarya birrea*, and *Coccinia grandis*, among others [29–31]. *Nigrospora oryzae* produces secondary metabolites with antimicrobial and antioxidant activities, making it a suitable source of novel and common bioactive compounds for targeting resistant bacterial species [30].

Gamma-tocopherol is a naturally occurring isoform of vitamin E recognized for its potent antioxidant, anti-inflammatory, and cytoprotective properties. Recent studies have highlighted that, unlike alpha-tocopherol, γ-tocopherol exhibits superior scavenging activity against reactive nitrogen species (RNS), modulates inflammatory signaling

pathways, and plays a crucial role in cellular defense against oxidative damage. These biochemical features are particularly relevant in antimicrobial defense, as oxidative and nitrosative stresses strongly influence bacterial survival and pathogenicity [22]. The enhanced redox-regulating ability of γ-tocopherol therefore provides a strong mechanistic basis for its potential antibacterial activity. Recent membrane-interaction studies demonstrate that vitamin E isoforms significantly alter lipid bilayer dynamics, increasing susceptibility of microbial membranes to oxidative injury and functional collapse [23]. Furthermore, γ-tocopherol has been shown to regulate immune and inflammatory mediators, thereby indirectly enhancing host antimicrobial defenses. These properties strongly support its evaluation as a membrane-active antibacterial candidate.

The primary focus of *in silico* studies is drug discovery, with the synthesis of bioactive metabolites to counter rising infections caused by pathogenic microorganisms. *In vitro* and *in silico* studies are typically conducted to assess the action and safety of metabolites produced by microbes. The outcomes of these studies help save time, effort, and resources by predicting the efficacy of new bioactive compounds before synthesis. Lipophilicity refers to the affinity of molecules to a lipophilic environment [17]. This attribute is an important factor that determines the behavior of bioactive compounds. It also influences the ADMET properties (absorption, distribution, metabolism, excretion, and toxicity) of compounds [17,18]. The lipophilicity of molecules is measured as the partition or distribution coefficient of molecules within non-polar or fat-based environments [19,20]. Drug-likeness is evaluated with the use of Lipinski’s Rule of Five. It is used to identify the properties that influence the oral availability of drugs [21]. Compounds that violate these criteria tend to be poorly absorbed by the system [21]. Thus, a potential drug candidate should not violate more than one of these conditions for it to be considered orally active.

The global rise of antimicrobial resistance has intensified the search for novel, safe, and naturally derived antimicrobial agents. In this context, *in silico* molecular docking has emerged as a rapid, cost-effective, and reliable method for screening bioactive compounds against essential bacterial proteins, including those involved in cell wall synthesis, DNA replication, and metabolic regulation. Recent studies (2023) demonstrate that computational docking and ADMET profiling can effectively predict antibacterial potential and guide experimental validation of phytochemicals and nutraceutical compounds [24]. Applying *in silico* approaches to bioactive compounds, therefore, offers an efficient strategy to confirm their antibacterial activity, identify molecular targets, and optimize their therapeutic potential before laboratory investigations [24]. Consequently, exploring the antibacterial potential of bioactive compounds using *in silico* molecular docking is scientifically justified, as it integrates recent advances in computational biology with nutraceutical drug discovery [25]. This strategy provides mechanistic insight into ligand-target interactions, predicts binding affinity and stability, and supports the rational development of bioactive compounds as novel antimicrobial or adjunct therapeutic agents. While studies have identified compounds such as γ-tocopherol, α-amyirin, and 3β-acetoxy-5-bisnorchenolic acid from various natural studies, limited information is available on their occurrence in *Nigrospora oryzae*. In addition, only a few have explored a computational-based approach to testing these compounds against bacterial protein targets for their antibacterial activity. This study aims to address these limitations by using *in silico* methods to evaluate γ-tocopherol, α-amyirin, and 3β-acetoxy-5-bisnorchenolic acid obtained from the endophytic *Nigrospora oryzae* UILRZ1 as potential inhibitors of clinically isolated multidrug-resistant bacteria.

2. Materials and methods

2.1. Source of *Nigrospora oryzae* UILRZ1

Nigrospora oryzae UILRZ1 was obtained from a previous study in

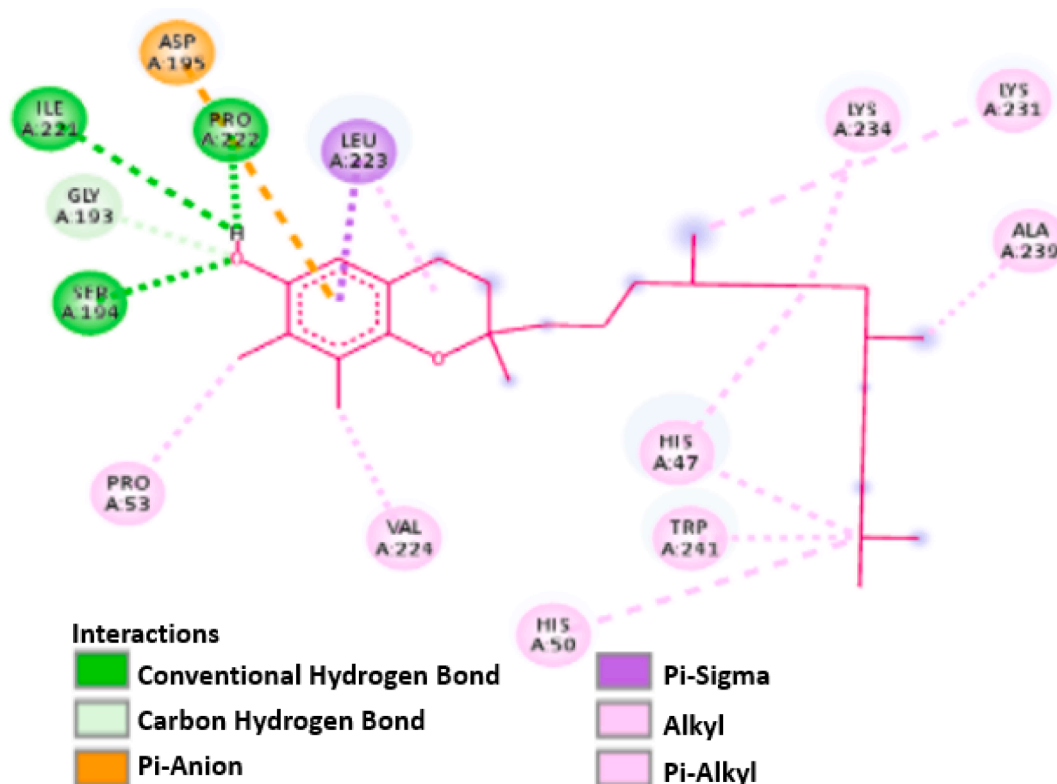


Fig. 1a. Post-docking analysis of Tyrosyl-tRNA synthetase (PDB ID: 1JLJ) binding to γ -tocopherol.

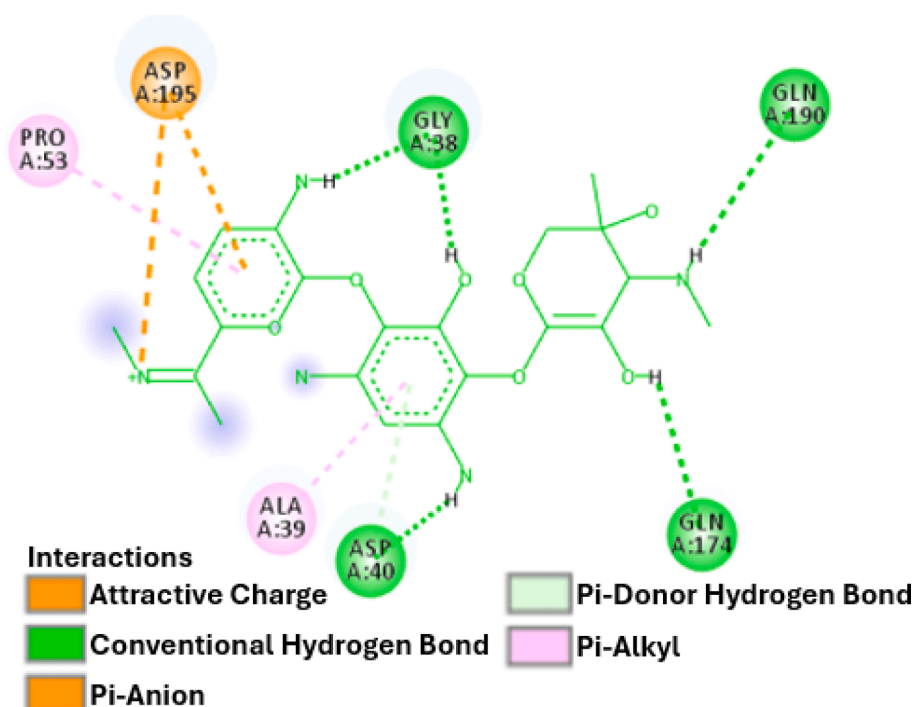


Fig. 1b. Interaction of Gentamicin within the Binding Pocket of 1JLJ.

which we evaluated the antibacterial potential of fractionated bioactive compounds from endophytic fungi [28]. Our findings showed that fractions from *Nigrospora oryzae* UILRZ1 had higher antibacterial activity and statistical effectiveness compared to other screened isolates [28]. Based on this result, *Nigrospora oryzae* UILRZ1 was selected for the present study.

2.2. Assessment of synergism of fractions of *Nigrospora oryzae* UILRZ1 metabolites

One of the most effective methods for increasing the antimicrobial activity of a substance is to combine it with one or more other antimicrobial agents [27]. In this study, direct TLC-bioautography and

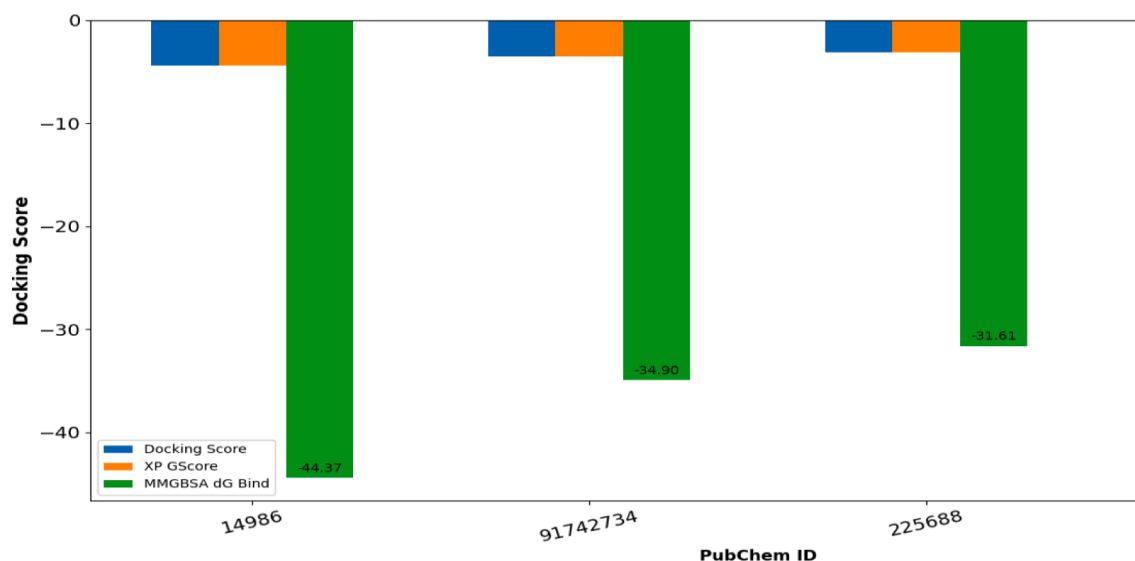


Fig. 1c. Post-docking analysis of Tyrosyl-tRNA synthetase (PDB ID: 1JJJ) binding to γ -tocopherol, indicating docking scores, Xp GScore, and MMGBSA.

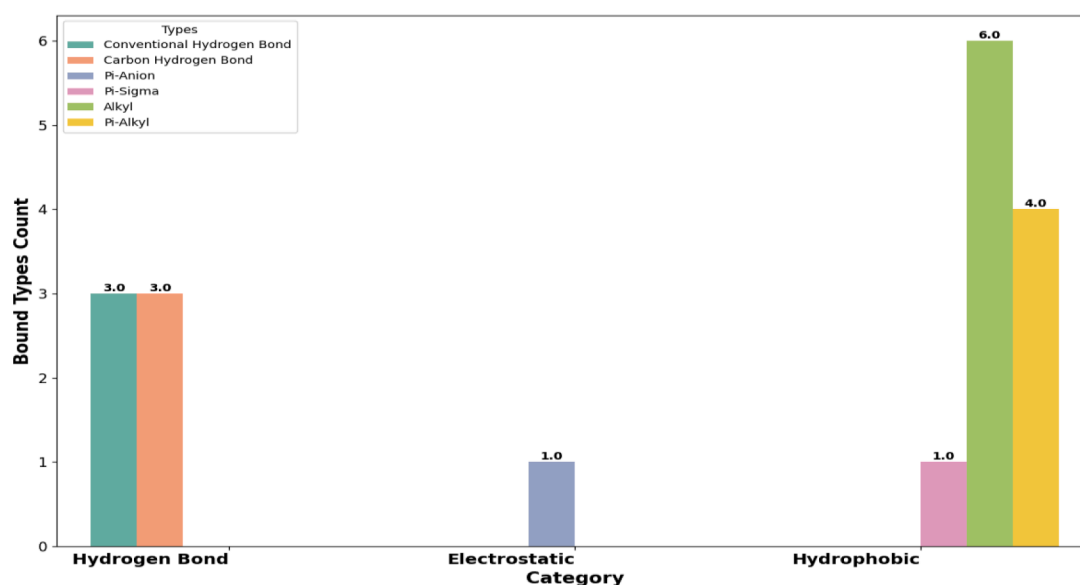


Fig. 1d. Bond categories and types involving Tyrosyl-tRNA synthetase (PDB ID: 1JJJ) binding to γ -tocopherol.

microbroth dilution methods were used to determine the antibacterial activity of the combined fractions prepared at a 1:1 ratio against the selected test organism, as described in the previous study [28].

2.3. Selection and preparation of ligands

Three bioactive compounds, γ -tocopherol, 3 β -acetoxy-5-bisnorcholelic acid, and α -amyrin were selected for the *in silico* analysis based on previous reports of their antimicrobial activity [28]. These compounds were derived from the *Nigrospora oryzae* UILRZ1 fractions. In addition to their antimicrobial activity, virtual screening was performed to assess whether the compounds (ligands) could bind to the microbial targets (receptors). Their structures were retrieved from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov>). Ligands were optimized using the LigPrep 2.4 module of Schrödinger's Maestro software. This process generated low-energy conformations with appropriate protonation states, optimized geometry, and assigned proper bond orders.

2.4. Retrieval and preparation of bacterial protein targets

The three-dimensional crystal structures of the target bacterial proteins — Tyrosyl-tRNA synthetase (1JJJ), DNA gyrase (1KZN), SHV-1 beta-lactamase (3MKF), Penicillin-binding protein (3UE3), Penicillin-binding protein 3 (PBP3) (3VSL), Topoisomerase IV (4EMV), and Dihydrofolate reductase (4M7U) were retrieved from the Protein Data Bank (PDB) [17]. These proteins were prepared using the Protein Preparation Wizard in Schrödinger's Maestro interface. For this preparation, non-essential water molecules and heteroatoms were removed, and missing hydrogen atoms were added. The side chains and loops were also corrected through loop refinement methods. Lastly, the OPLS-2005 force field was applied to reduce energy and ensure appropriate geometry and steric accuracy.

2.5. Binding site prediction, grid generation, and docking

The SiteMap [Schrödinger] tool was used to evaluate the size, volume and hydrophobic/hydrophilic balance of cavities to predict pockets

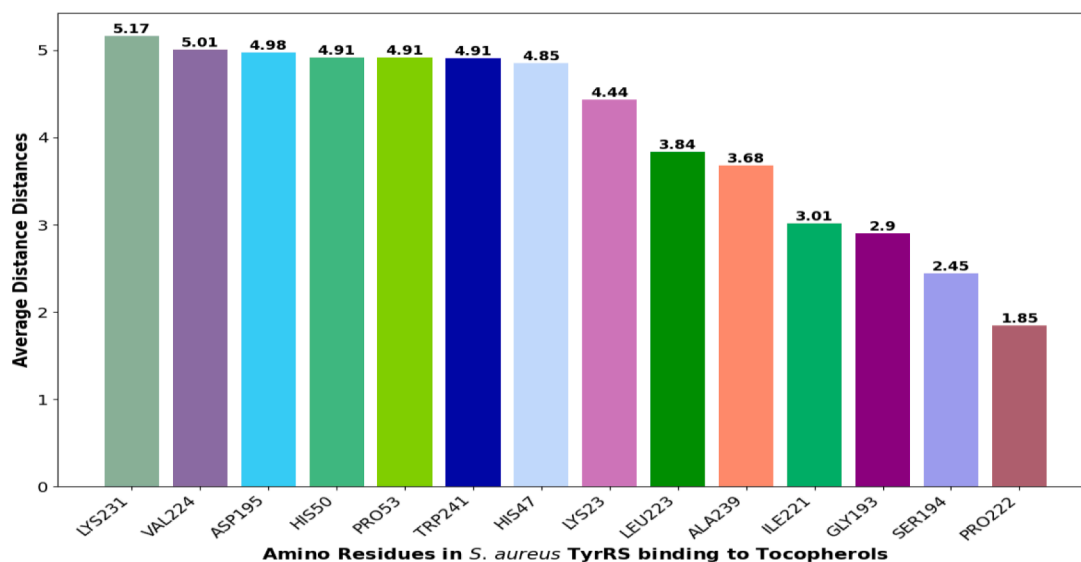


Fig. 1e. Amino acid residues at the binding site of Tyrosyl-tRNA synthetase (PDB ID: 1JLJ) involved in binding to γ -tocopherol.

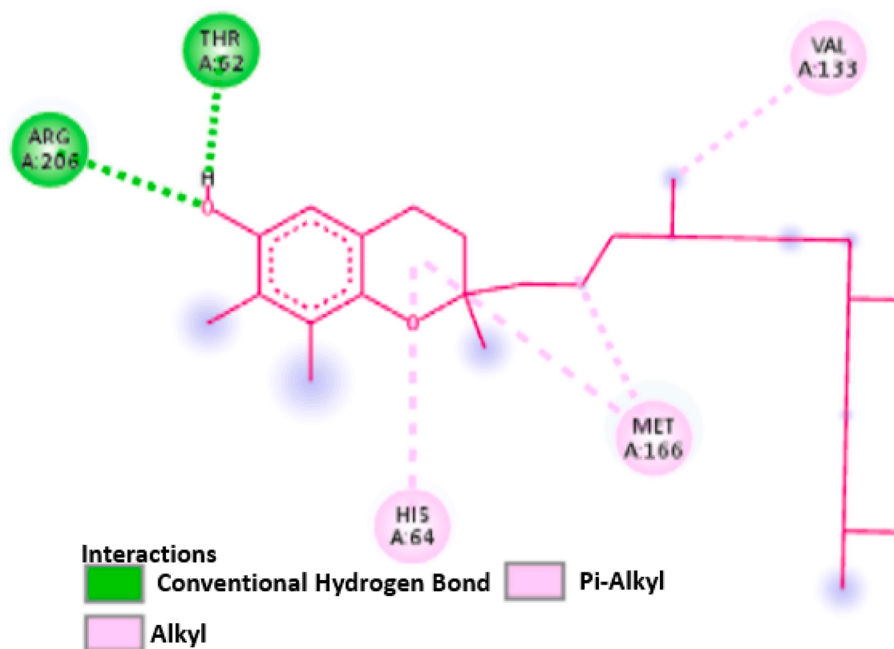


Fig. 2a. Post-docking analysis of DNA gyrase binding to γ -tocopherol.

that are druggable. It was employed to determine the potential binding sites of ligands in each protein. The site that had the highest SiteScore value was selected for the generation of docking grids. Docking grids were generated with the use of Receptor Grid Generation in Glide (Schrödinger). The receptor grid was centered at $x = 7.19$, $y = 14.21$, $z = -12.65$, with grid dimensions of $25 \times 25 \times 25 \text{ \AA}$, ensuring complete coverage of the predicted ligand-binding site and enabling adequate conformational sampling during docking. Prepared ligands were subsequently docked into the generated receptor grids using the Glide Standard Precision (SP) mode. The best docking poses were selected based on the GlideScore (predicted binding affinity) and favorable interactions with key active-site amino acid residues [5,10].

2.6. Analysis of docking results

Analysis of the docking results was done using PyMOL and LigPlot +

to visualize the hydrogen bonds, hydrophobic interactions, and π - π stacking. The amino acid residues forming key interactions with ligands were identified. Compounds with the most favorable docking scores were considered potential inhibitors of bacterial targets.

2.7. ADMET and drug-likeness analysis

The pharmacokinetic and pharmacodynamic properties of the ligands were assessed using Lipinski's Rule of Five [13,18,21]. Parameters such as molecular weight, Log P, hydrogen-bond donors (HBD), hydrogen-bond acceptors (HBA), and topological polar surface area (TPSA) were calculated using SwissADME and QikProp. Ligands satisfying Lipinski's criteria were considered orally bioavailable and potentially drug-like.

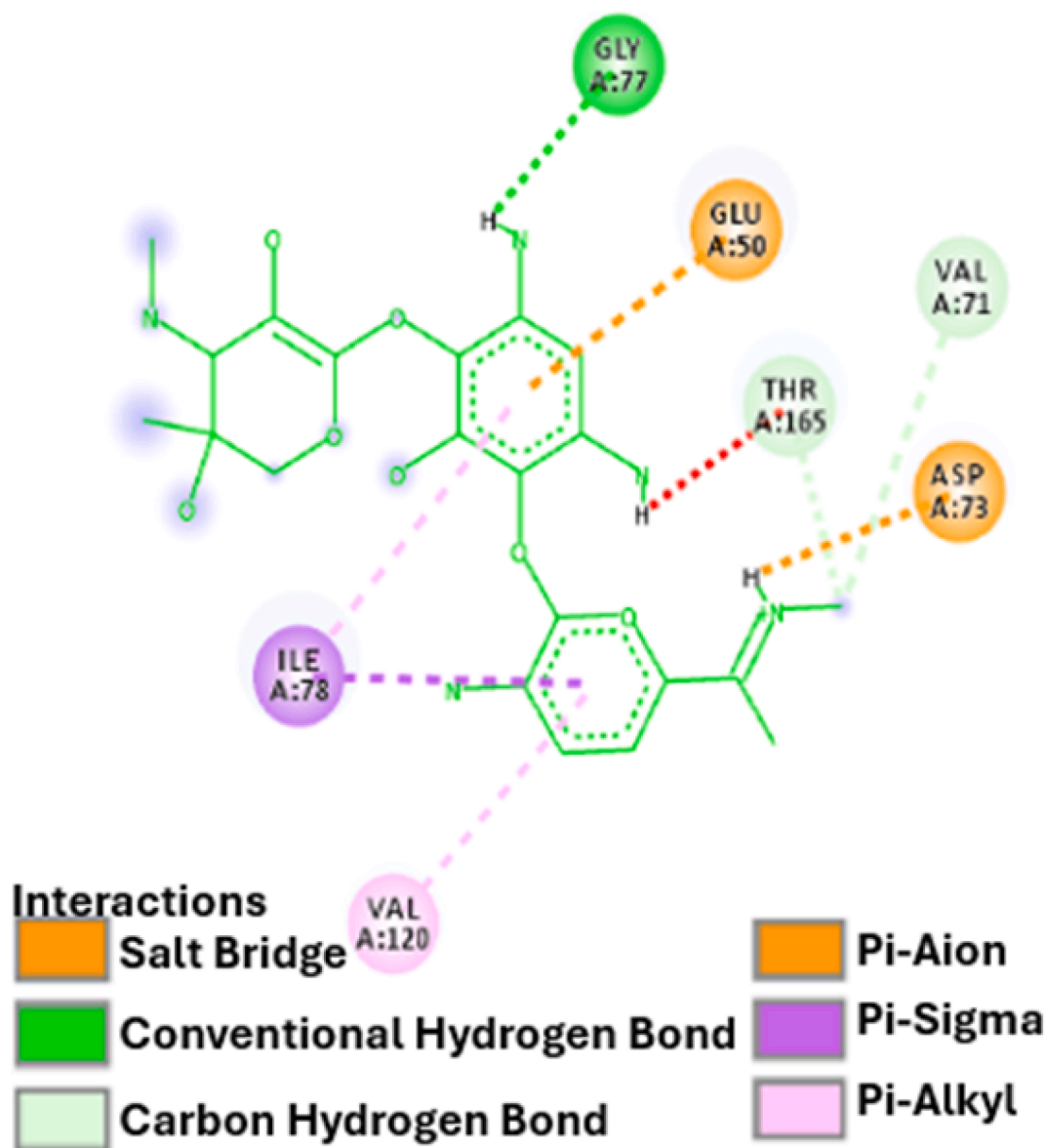


Fig. 2b. Interaction of Gentamicin within the Binding Pocket of DNA Gyrase.

2.8. Canonical simplified molecular-input line-entry system (SMILES)

Table 1 below shows a canonical simplified molecular-input line-entry system (SMILES) of the ligands prepared for docking. These are unique to each ligand and facilitate conversion into two- or three-dimensional crystallography models.

3. Results and discussion

3.1. Effect of the fractions in combinations on selected bacterial isolates

The four fractions were subjected to susceptibility testing in varying combinations. All the combinations inhibited *S. aureus* and *E. faecalis*. Combinations A, B, and C inhibited *P. aeruginosa* as shown in Table 2 below.

3.2. Interactions of γ -tocopherol within the binding pockets of Tyrosyl-tRNA synthetase

The interaction between Tyrosyl-tRNA synthetase and γ -tocopherol

involves multiple amino acid residues that form a range of bonding interactions critical for the stability of the complex. Notably, residues such as SER194 (1.89 Å), ILE221 (3.01 Å), and PRO222 (1.85 Å) participate in conventional hydrogen bonds, contributing to the specificity and strength of the interaction. These hydrogen bonds are crucial as they help to position γ -tocopherol within the active site of Tyrosyl-tRNA synthetase, thereby influencing its binding affinity and possibly its catalytic efficiency. In addition, GLY193 formed carbon-hydrogen bonds at distances of 2.86 Å and 2.95 Å, thereby enhancing stability. These bonds highlight the roles that interactions between side chains and the backbone play in maintaining the complex's structural integrity (Table 3).

The hydrophobic interactions between residues like LEU223 (2.64 Å), LYS234 (4.44 Å), and TRP241 (4.91 Å) that form alkyl and Pi-alkyl bonds contribute to structural stability. These interactions help to protect the γ -tocopherol by creating a hydrophobic environment. The γ -tocopherol is further anchored at the active site with the electrostatic Pi-Anion interaction involving ASP195 (4.98 Å). Overall, the highly specific binding of γ -tocopherol to Tyrosyl-tRNA synthetase is attributed to these interactions identified above. These associations have potential implications for the biological activity of γ -tocopherol and how it

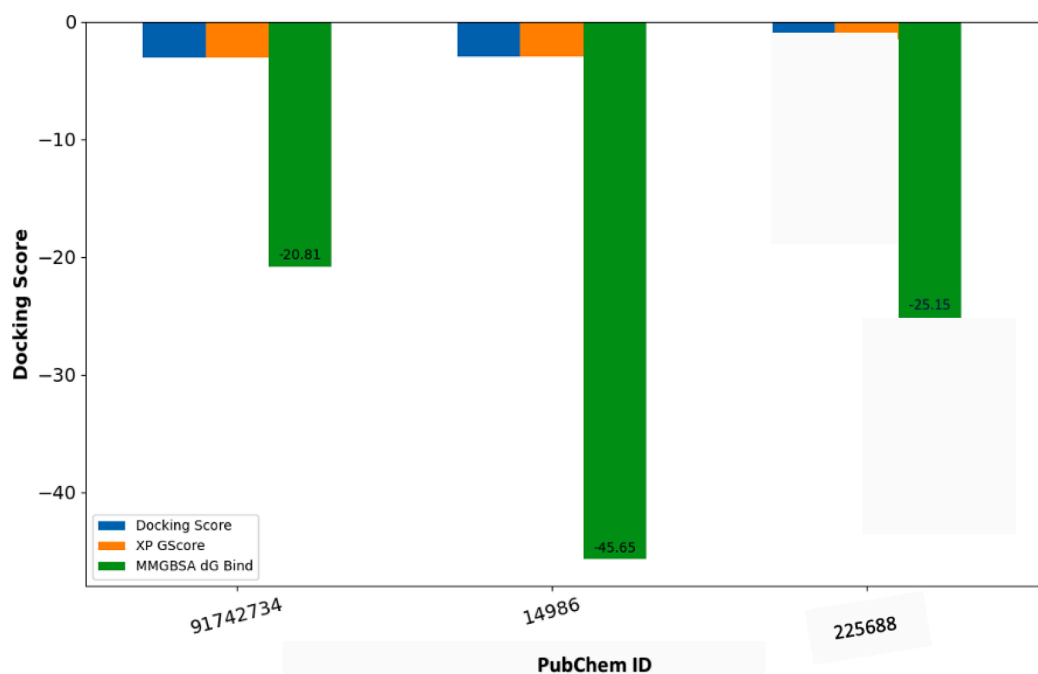


Fig. 2c. Post-docking analysis of DNA gyrase [PDB ID: 1KZN] binding to γ -tocopherol indicating docking scores, Xp GScore, and MMGBSA.

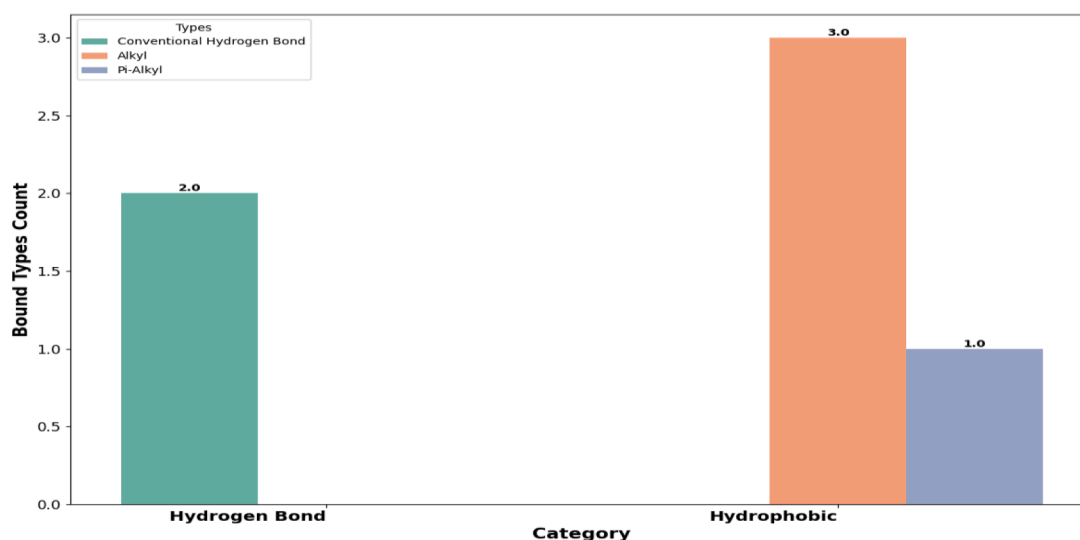


Fig. 2d. Bond categories and types involving DNA gyrase (PDB ID: 1KZN) binding to γ -tocopherol.

functions as a protein. This is indicated in Fig. 1a below.

The intermolecular interaction between the control ligand, gentamicin, and the amino acids of 1JIJ shows a total of seven amino acids exhibiting bonding with ten intermolecular interactions: six hydrogen bonds, two electrostatic, and one hydrophobic, indicating higher specificity rather than binding stability within the binding pocket as seen in Fig. 1b below. Compared with α -tocopherol, γ -tocopherol exhibited greater binding stability, resulting in a more thermodynamically stable complex.

3.3. Effect of the docking score on post-docking analysis of Tyrosyl-tRNA synthetase to γ -tocopherol

The post-docking analysis of Tyrosyl-tRNA synthetase showed that tocopherol (PubChem ID 14986) had the most favorable docking score and XP GScore (-4.39) alongside a highly negative MMGBSA ΔG Bind

(-44.37 kcal/mol), indicating both optimal fit within the active site and strong thermodynamic stability. 3 β -acetoxy-5-bisnorcholeic acid (PubChem ID 91742734) followed with moderate docking (-3.53) and MMGBSA ΔG Bind (-34.90 kcal/mol), while Alpha-Amyrin (PubChem ID 225688) exhibited lower binding affinity (docking -3.11 ; MMGBSA -31.61 kcal/mol). The consistent trend between docking scores and binding free energies highlights that more negative docking and MMGBSA values correlate with stronger predicted binding, emphasizing tocopherol as the most promising ligand for Tyrosyl-tRNA synthetase (Fig. 1c).

The bar graph in Fig. 1d illustrates the interaction of different bond types within the binding of Tyrosyl-tRNA synthetase to γ -tocopherol. These bonds include hydrogen bonds, electrostatic, and hydrophobic interactions. The hydrogen bonds include three cases: normal hydrogen bonding and carbon-hydrogen bonding. The electrostatic interactions comprise one case of Pi-Anion interaction, while the hydrophobic

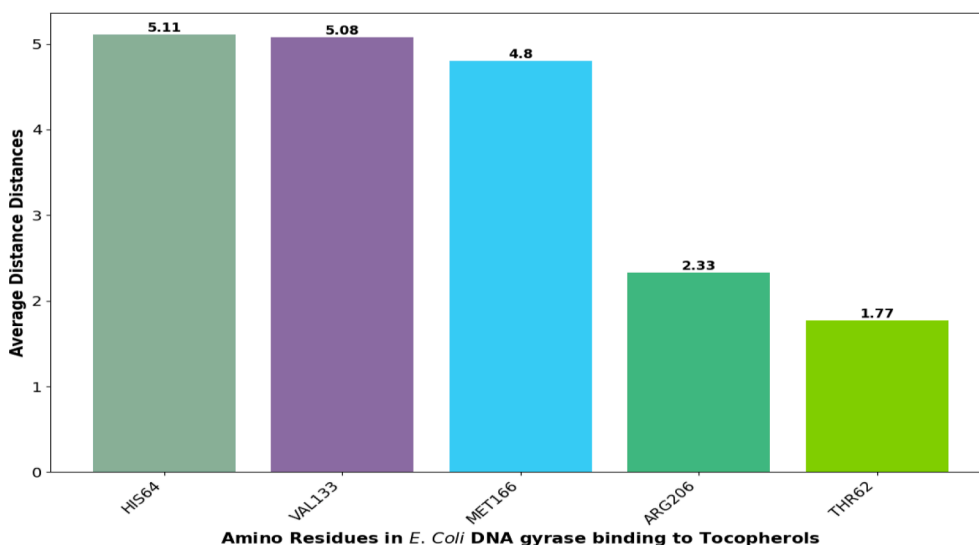


Fig. 2e. Amino acid residues at the binding site of DNA gyrase (PDB ID: 1KZN) involved in binding of γ -tocopherol.

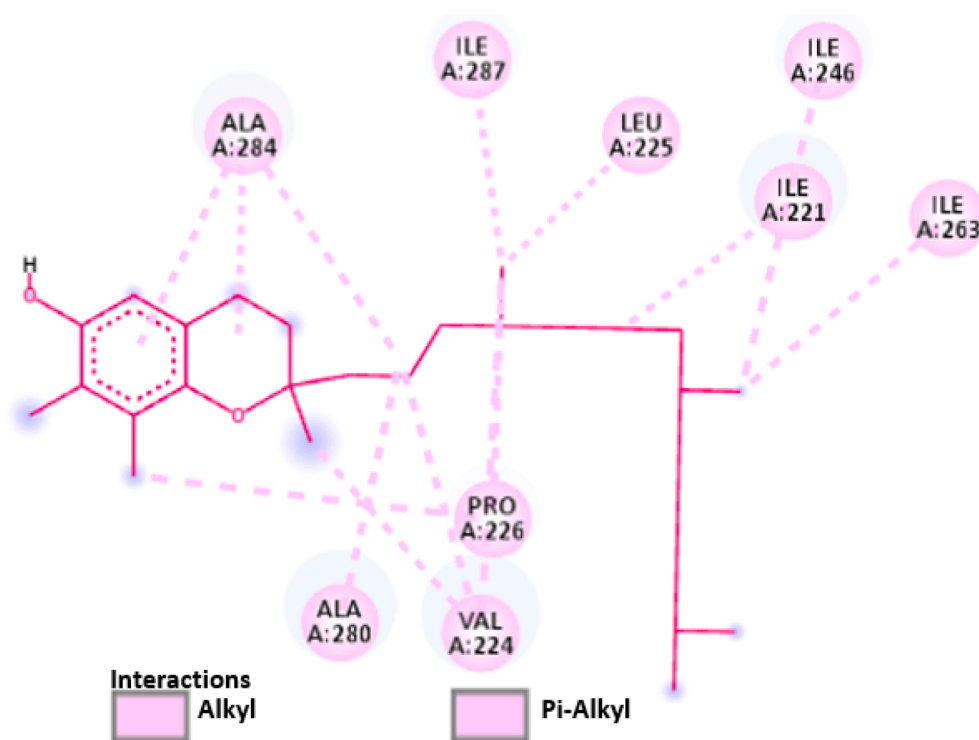


Fig. 3a. Post-docking analysis of SHV-1 beta-lactamase (PDB ID: 3MKF) binding to γ -tocopherol.

interactions comprise six Alkyl bonds, four Pi-Alkyl bonds, and one Pi-Sigma bond. The prevalence of hydrophobic interactions compared to the other bonds highlights its significance in the binding.

The average distances between specific *Staphylococcus aureus* amino acid residues in the DNA gyrase and Tyrosyl-tRNA synthetase (TyrRS) binding to tocopherol are presented in Fig. 1e below. The distances suggest how close the amino acids are to the tocopherol at the binding site. This influences the binding affinity and interaction strength within the complex. Distances that are shorter are considered to produce stronger and more direct interactions, which is necessary for a binding that is stable and specific. Greater distances play a role in the interaction's overall structural stability.

3.4. Interactions of γ -tocopherol and gentamicin within the binding pockets of DNA gyrase

Several amino acids contribute to the hydrogen and hydrophobic bonds present in the ligand formed by DNA gyrase and γ -tocopherol. The interactions from the hydrogen bonds are attributed to ARG206 (2.33 Å) and THR62 (1.77 Å). The bond distances at these residues are strong and specific, which allows them to contribute to stabilizing the ligand at the DNA gyrase active site. The hydrogen bonds help to anchor the ligand and ensure proper orientation and specificity of the interactions in the complex. The alkyl and pi-alkyl interactions from the hydrophobic bonds are attributed to MET166 (5.21 Å), MET166 (4.39 Å), VAL133 (5.08 Å) and HIS64 (5.11 Å) (Fig. 2a). These hydrophobic interactions help to create supportive hydrophobic regions in the ligand that enhance

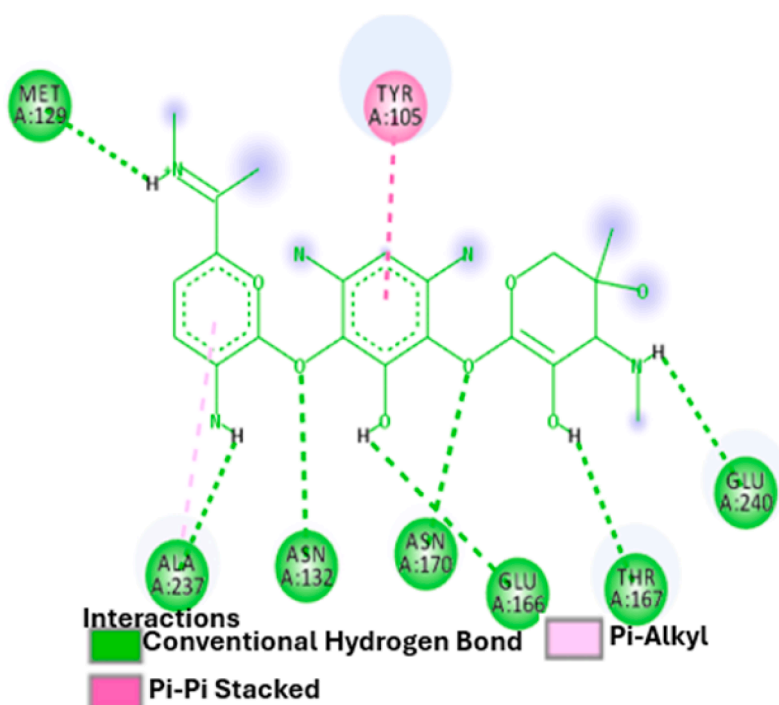


Fig. 3b. Interaction of Gentamicin within the Binding Pocket of 3MKF.

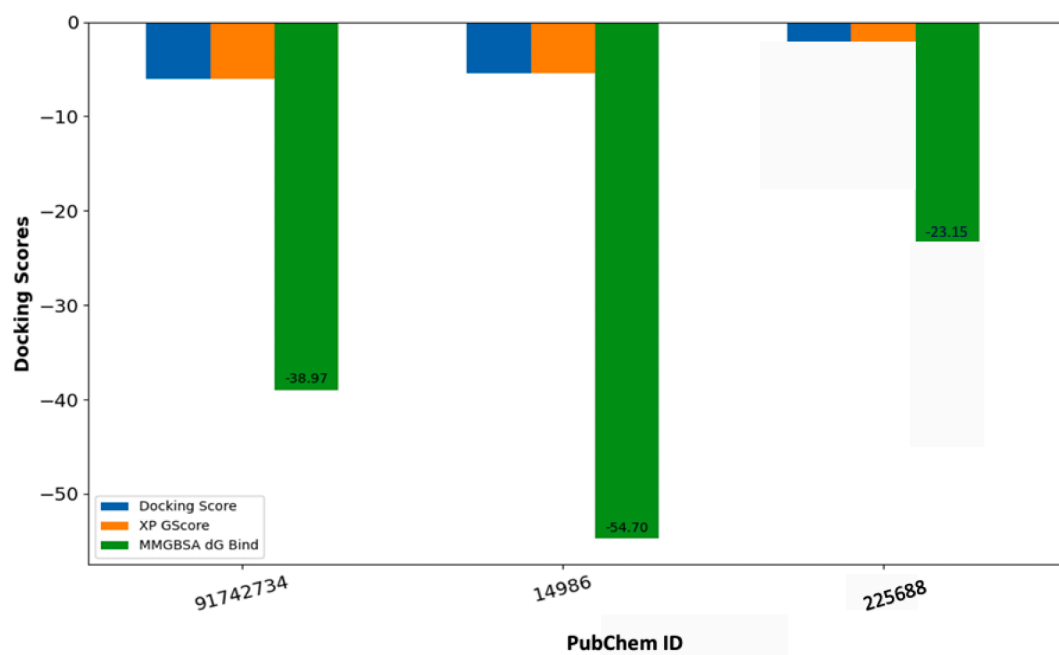


Fig. 3c. Post-docking analysis of SHV-1 beta-lactamase (PDB ID: 3MKF) binding to γ -tocopherol indicating docking scores, Xp GScore, and MMGBSA.

overall affinity for binding. The presence of a dual bond demonstrates how balanced the forces are that DNA gyrase uses to interact with γ -tocopherol. This suggests that γ -tocopherol may act as a potential inhibitor of DNA gyrase.

The crystallographic and chemical interactions of gentamicin with amino acid. The receptor-ligand complex exhibited seven different types of intermolecular interaction which confirms its instability as seen in Fig. 2b. While gentamicin demonstrated precise molecular recognition, γ -tocopherol appeared to achieve stronger binding stability, highlighting their potential as promising modulators of DNA gyrase activity

(Fig. 2c).

Fig. 2d below highlights the types and frequencies of bonds that are present in the interaction between DNA gyrase and γ -tocopherol. The bonds are “hydrogen bonds” for which conventional hydrogen bonds appear once and “hydrophobic” for which alkyl bond appears three times and pi-Alkyl bond is once. These bond types facilitate the stability and specificity of the interaction.

The average distances between specific *E. coli* amino acid residues in the DNA gyrase and γ -tocopherol is presented in Fig. 2e below. The interactions from the hydrogen bonds are attributed to HIS64, VAL133,

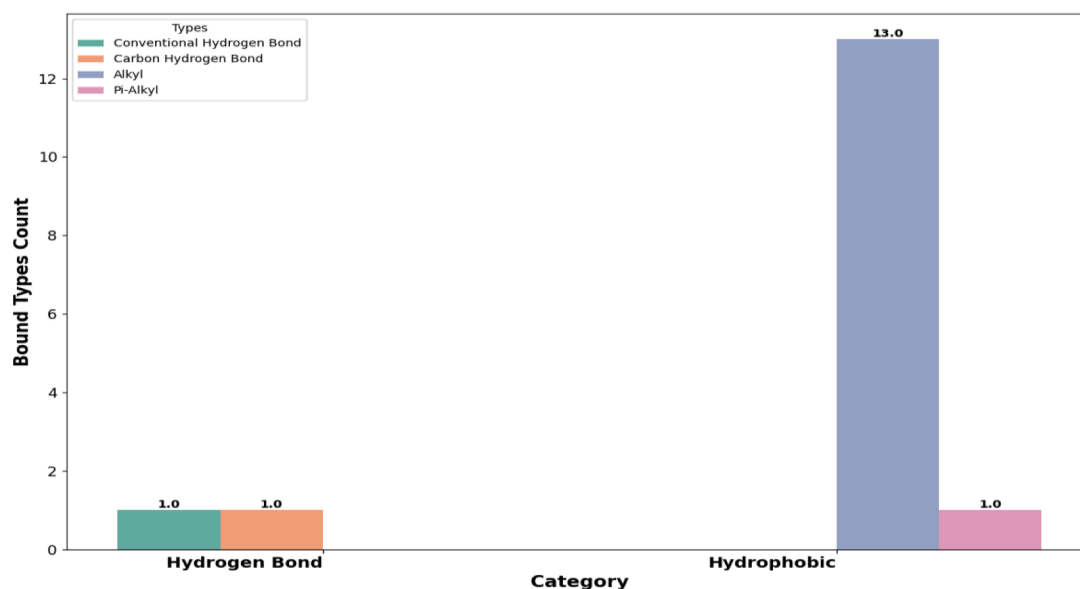


Fig. 3d. Bond categories and types involving SHV-1 beta-lactamase (PDB ID: 3MKF) binding to γ -tocopherol.

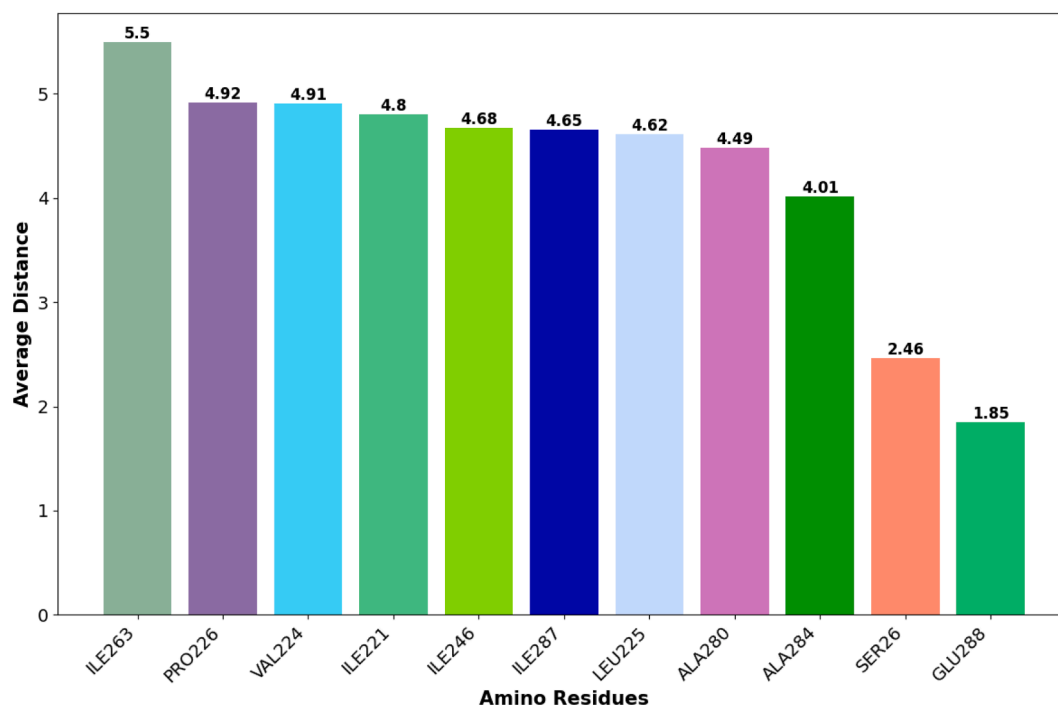


Fig. 3e. Amino acid residues at the binding site of SHV-1 beta-lactamase (PDB ID: 3MKF) binding to γ -tocopherol.

MET166, ARG206 and THR62. The distances include 5.11 Å for HIS64, 5.08 Å for VAL133, 4.80 Å for MET166, 2.33 Å for ARG206 and 1.77 Å for THR62. The distances suggest how close the amino acids are to the γ -tocopherol at the binding site. The closest is THR62 which suggests that it plays a major role in the stability of the interaction.

3.5. Interactions of γ -tocopherol and gentamicin within the binding pockets of SHV-1 beta-lactamase

Fig. 3a below shows the interaction between SHV-1 beta-lactamase and γ -tocopherol. The hydrogen bond interactions are attributed to GLU288 with a distance of 1.85 Å. This low distance suggests that the bond is strong, conventional and helps to maintain the specific geometry

of the active site of the enzyme. This close interaction likely contributes to the high specificity of γ -tocopherol for SHV-1 beta-lactamase. It is also possible because hydrogen bonds are crucial in molecular recognition and enzyme catalysis.

Crystallographic interaction of the control, gentamicin within the binding pocket of 3MKF. The ligand-receptor interaction shows hydrogen bonding in green colour dominating the interaction which signifies specificity of the interaction that took place in Fig. 3b below. Comparatively, γ -tocopherol exhibited targeted specificity with single site hydrogen while gentamicin exhibited broad specificity with multiple hydrogen bonds.

For the binding study involving γ -tocopherol and SHV-1 beta-lactamase (PDB ID: 3MKF), γ -tocopherol [PubChem ID 14986] shows the

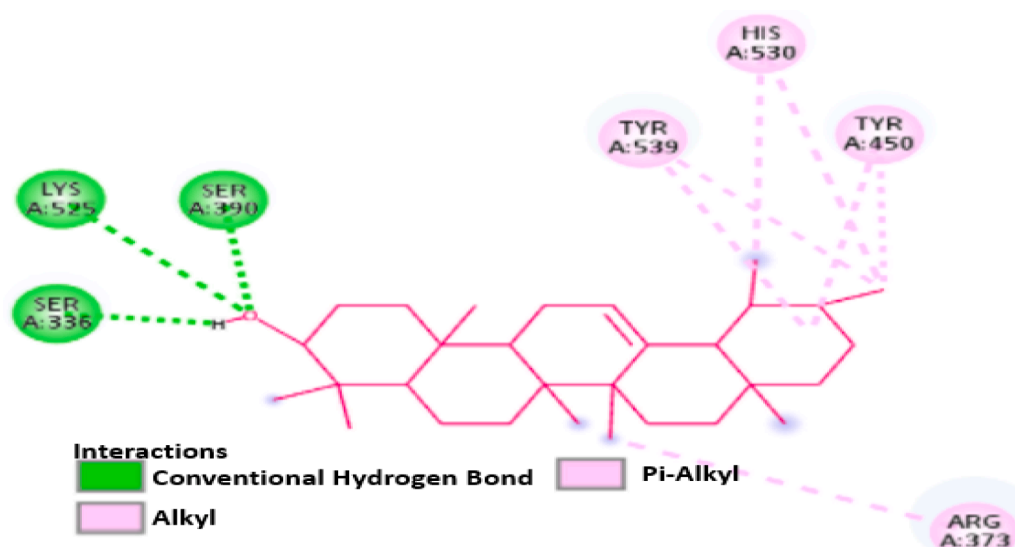


Fig. 4a. Post-docking analysis of Penicillin binding protein (3UE3) binding to 3β-acetoxy-5-bisnorcholenic acid.

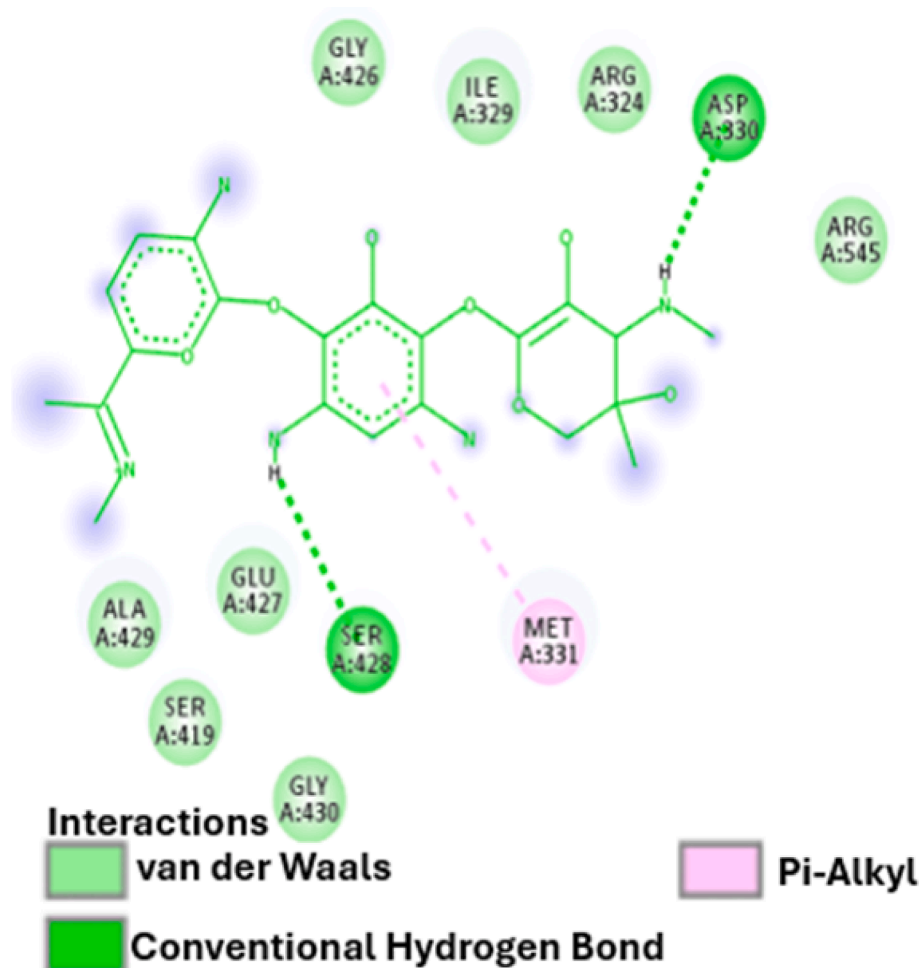


Fig. 4b. Interaction of Gentamicin within the Binding Pocket of 3UE3.

most favorable binding interaction with the lowest MMGBSA ΔG Bind score of -54.70 , indicating a strong binding free energy. It also has a docking score of -5.43 and an XP GScore of -5.90 . The compound with PubChem ID 91742734 has a slightly less favorable MMGBSA ΔG Bind

score of -38.97 , with docking and XP GScores of -6.03 and -6.04 , respectively. Lastly, the compound with PubChem ID 225688 shows the least favorable interaction, with an MMGBSA ΔG Bind score of -23.15 , a docking score of -2.20 , and an XP GScore of -2.50 (Fig. 3c).

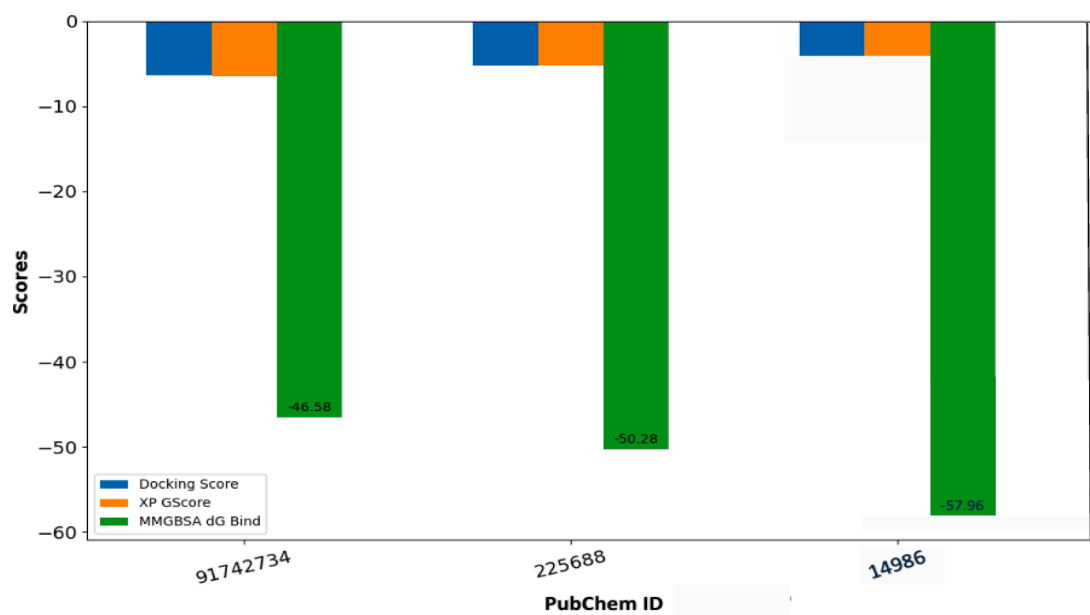


Fig. 4c. Post-docking analysis of Penicillin binding protein (3UE3) binding to 3 β -acetoxy-5-bisnorcholenic acid indicating docking scores, Xp GScore, and MMGBSA.

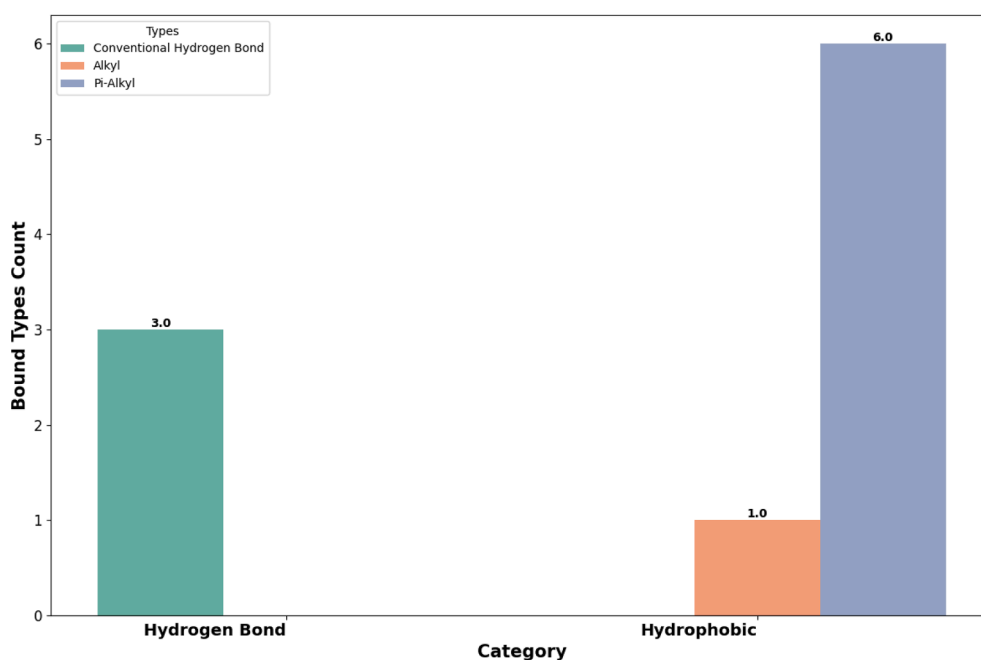


Fig. 4d. Bond categories and types involved in Penicillin binding protein (3UE3) binding to 3 β -acetoxy-5-bisnorcholenic acid.

The binding of SHV-1 beta-lactamase (PDB ID: 3MKF) to γ -tocopherol involves various bond types categorized primarily into hydrogen bonds and hydrophobic interactions. These comprise two conventional hydrogen bonds, one carbon-hydrogen bond and 13 non-polar alkyl interactions. The significance of the hydrophobic forces shows that they are important to the stability of γ -tocopherol to SHV-1 beta-lactamase. Fig. 3d below and highlights the combined effect of different bond types in the binding affinity.

Fig. 3e below shows the bond distances associated with the hydrophobic interactions of SHV-1 beta-lactamase and γ -tocopherol. The distances are 5.50 Å for ILE263 and 4.80 Å for ILE221. These distances reflect a weak interaction that is essential for the protein-ligand complex. Specific hydrophobic bonds are alkyl and pi-alkyl that help to maintain the non-polar regions of the enzyme and γ -tocopherol.

Additionally, ALA284 presents at several bond distances of 4.33 Å, 3.99 Å, and 3.71 Å. The repetition of these amino acids within the complex contribute to ensuring enzyme functionality during inhibition by the compound.

3.6. Interactions of 3 β -acetoxy-5-bisnorcholenic acid within the binding pockets of SHV-1 beta-lactamase

Penicillin-binding protein (3UE3) and 3 β -acetoxy-5-bisnorcholenic acid complexes show different bonds and distances. These bonds highlight that the molecular interactions in this complex are specific and stable. There is a presence of short and strong hydrogen bond distances of 1.72 Å for SER390 and 1.92 Å for SER336 (Fig. 4a). These bonds are conventional and help the binding of the compound to be specific at the

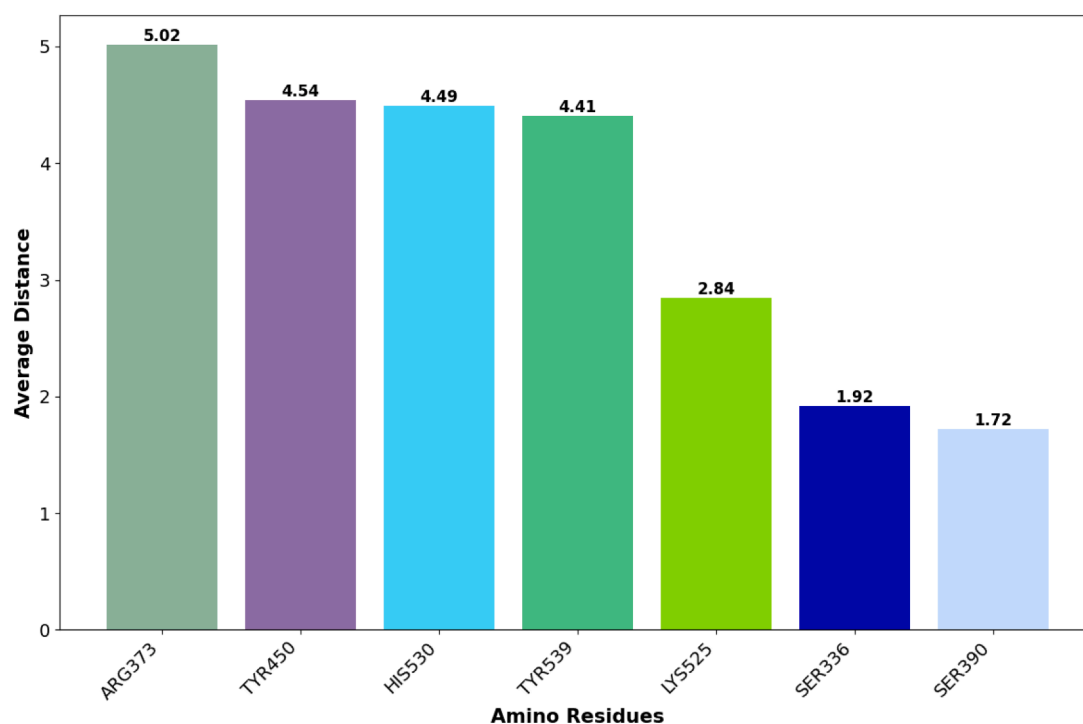


Fig. 4e. Penicillin binding protein (3UE3) binding to 3 β -acetoxy-5-bisnorcholenic acid, showing the amino acid residues and their bond distances.

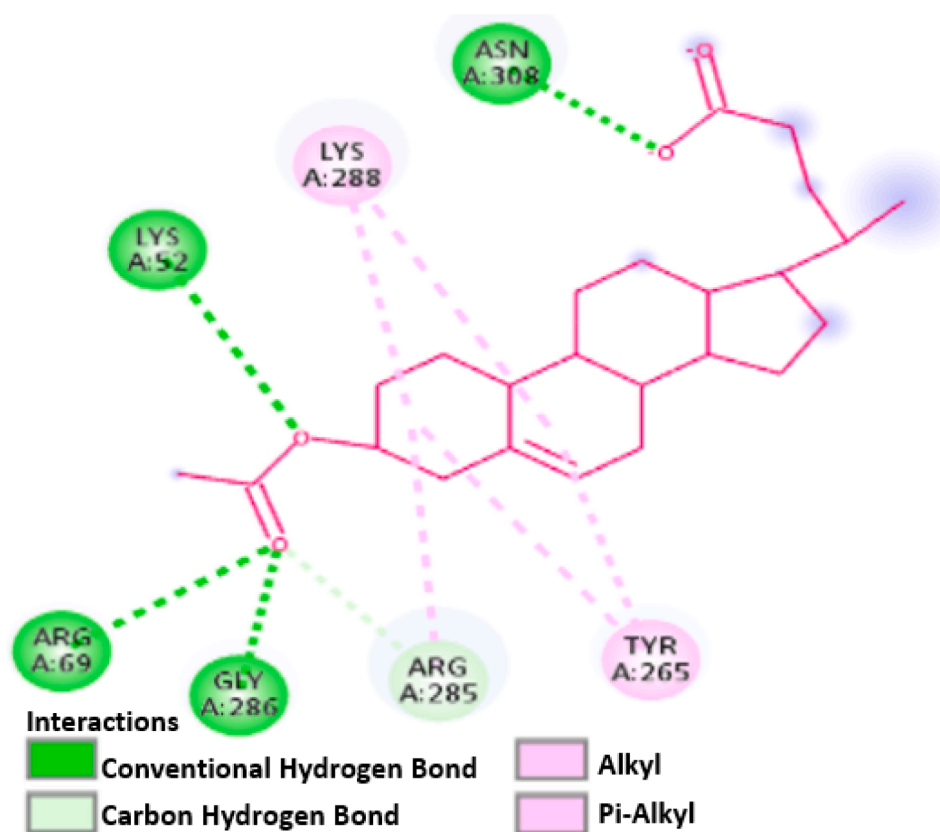


Fig. 5a. Post-docking analysis of Penicillin-binding protein 3 (PBP3) (3VSL) binding to 3 β -acetoxy-5-bisnorcholenic acid.

active site of the Penicillin-binding protein. In addition, the hydrogen bonds help to stabilise the structure of the protein-ligand complex. This then contributes to biological functions such as inhibiting the protein activity.

Crystallographic interaction of gentamicin with the amino acids of 3UE3 that also shows hydrogen bond intermolecular interaction of the ligand with SER428 and ASP330 while hydrophobic interaction occurred with MET331 of the receptor as seen in Fig. 4b below.

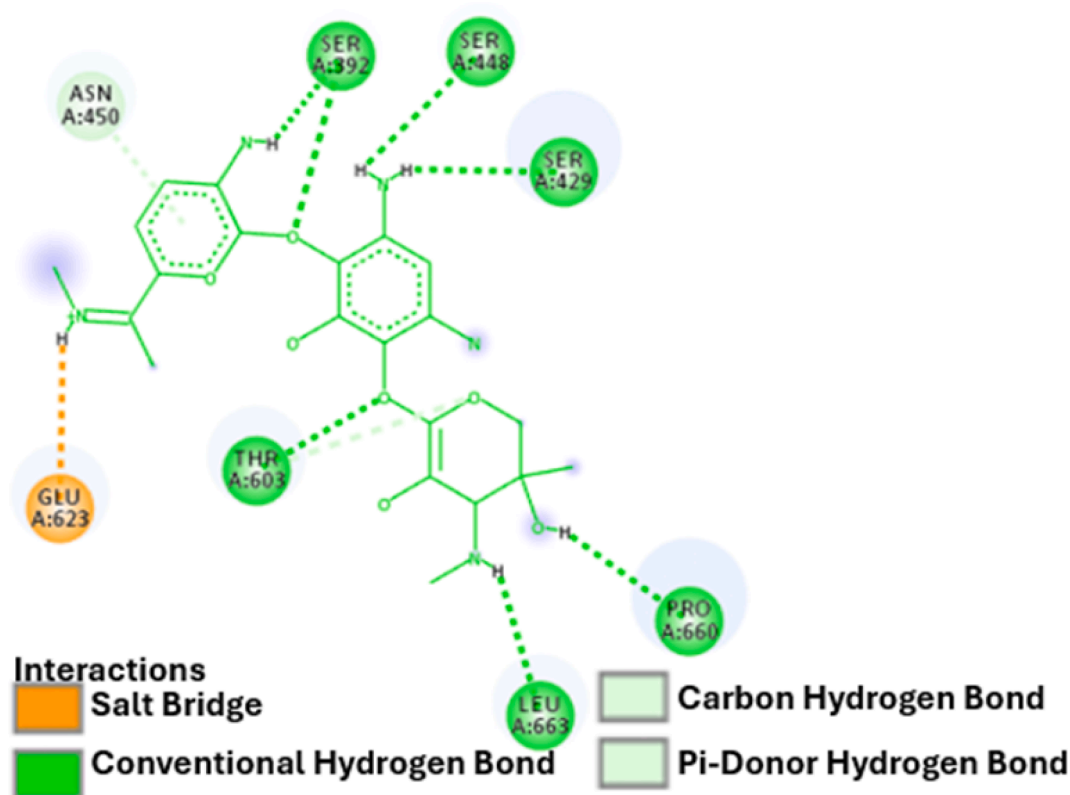


Fig. 5b. Interaction of Gentamicin within the Binding Pocket of 3VSL.

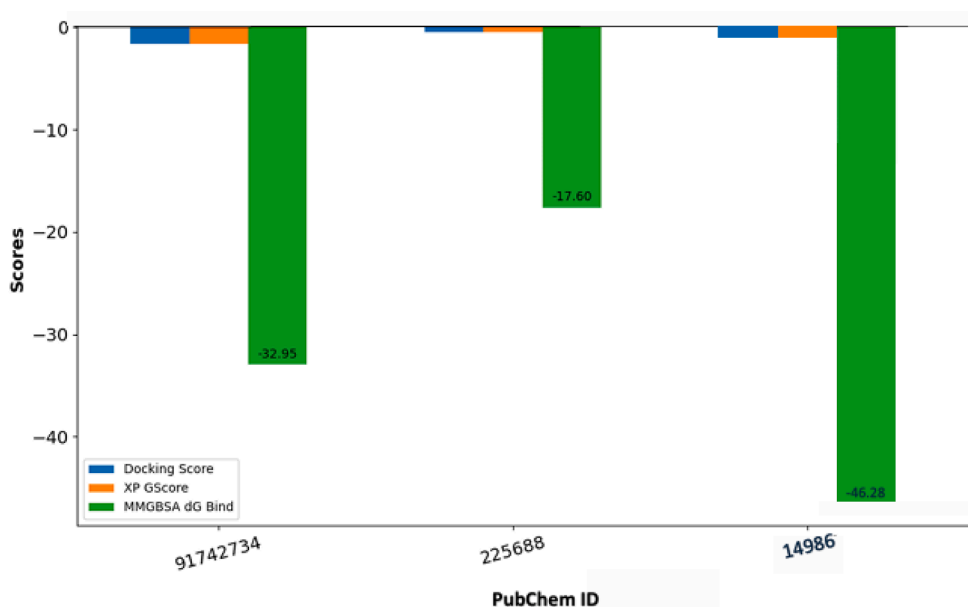


Fig. 5c. Post-docking analysis of Penicillin-binding protein 3 (PBP3) (3VSL) binding to 3 β -acetoxy-5-bisnorcholeonic acid, indicating the docking, Xp GScore, and MMGBSA scores.

The docking score of compound 91742734 was -6.44 , XP GScore of -6.45 and a ΔG Bind value of -46.58 . This result shows that the compound has a high potential for binding. This is in contrast to compound 225688 that has a lower docking score of -5.22 , XP GScore of -5.22 and ΔG Bind value of -57.96 . This suggests that the binding affinity of the compound is moderate. The last compound, 14986, has even lower values, indicating that its interactions are not suitable even if it binds

(Fig. 4c).

The hydrophobic interactions within the ligand are longer and weaker with bond distances of 4.97 Å and 4.12 Å for TYR450 as well as 4.39 Å and 4.60 Å for HIS530. These interactions are mostly alkyl and pi-alkyl bonds and ensure the stability of the compound within the protein hydrophobic pocket. TYR539 occurs repeatedly and at distances of 5.10 Å and 3.71 Å and ensures that the compound is effectively secured. This

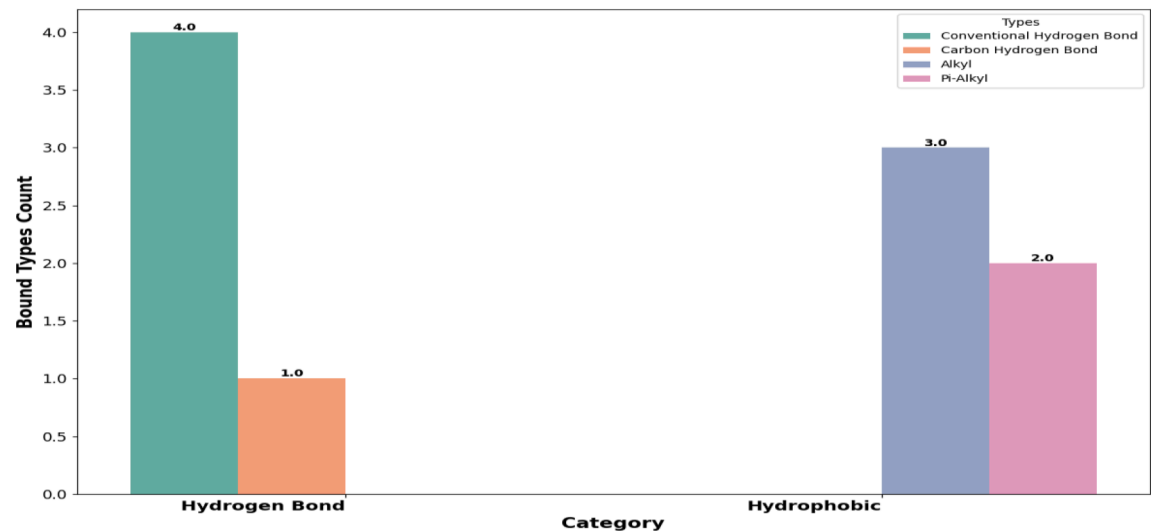


Fig. 5d. Bond categories and types involved Penicillin-binding protein 3 (PBP3) (3VSL) binding to 3β-acetoxy-5-bisnorcholonic acid.

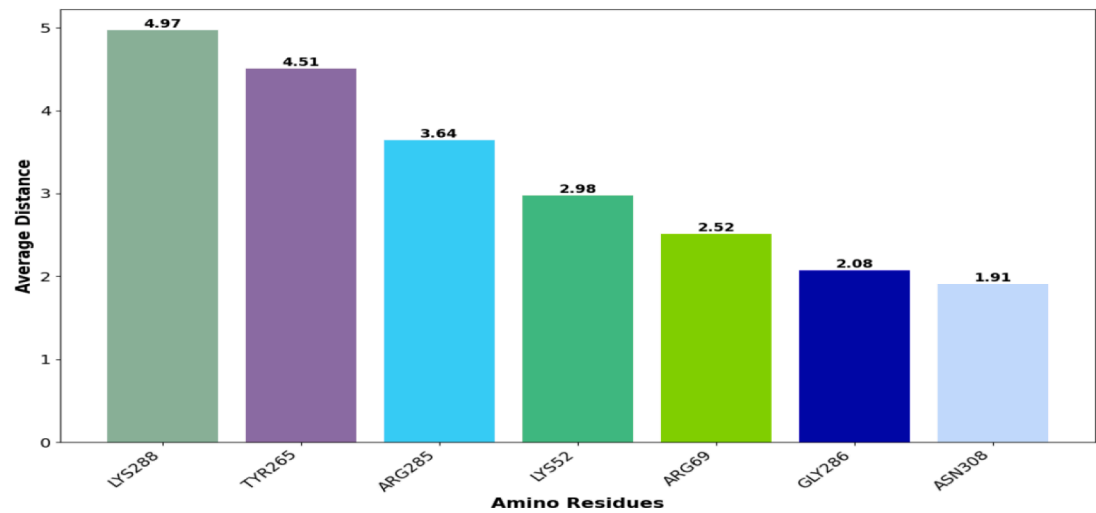


Fig. 5e. Amino acid residues at the binding site of Penicillin-binding protein 3 (PBP3) (3VSL) binding to 3β-acetoxy-5-bisnorcholonic acid.

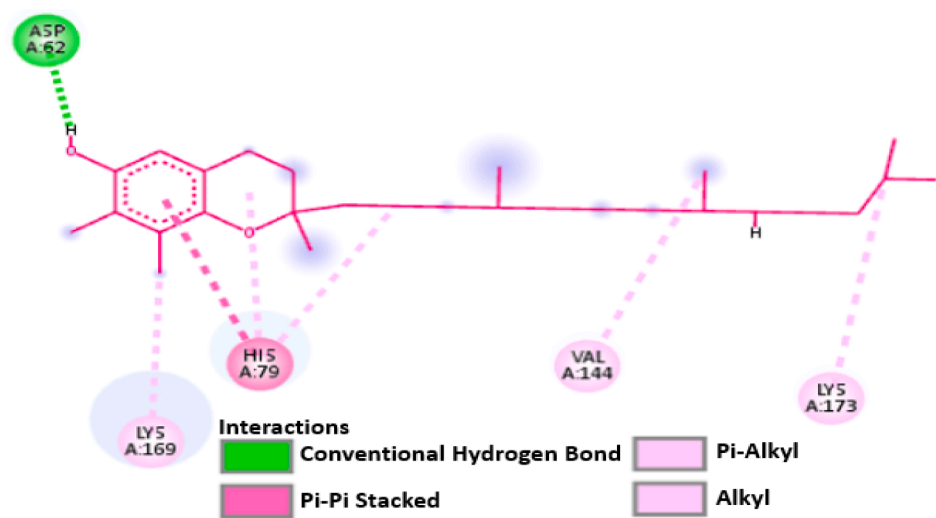


Fig. 6a. Post-docking analysis of Topoisomerase (PDB ID: 4EMV) binding to γ-tocopherol.

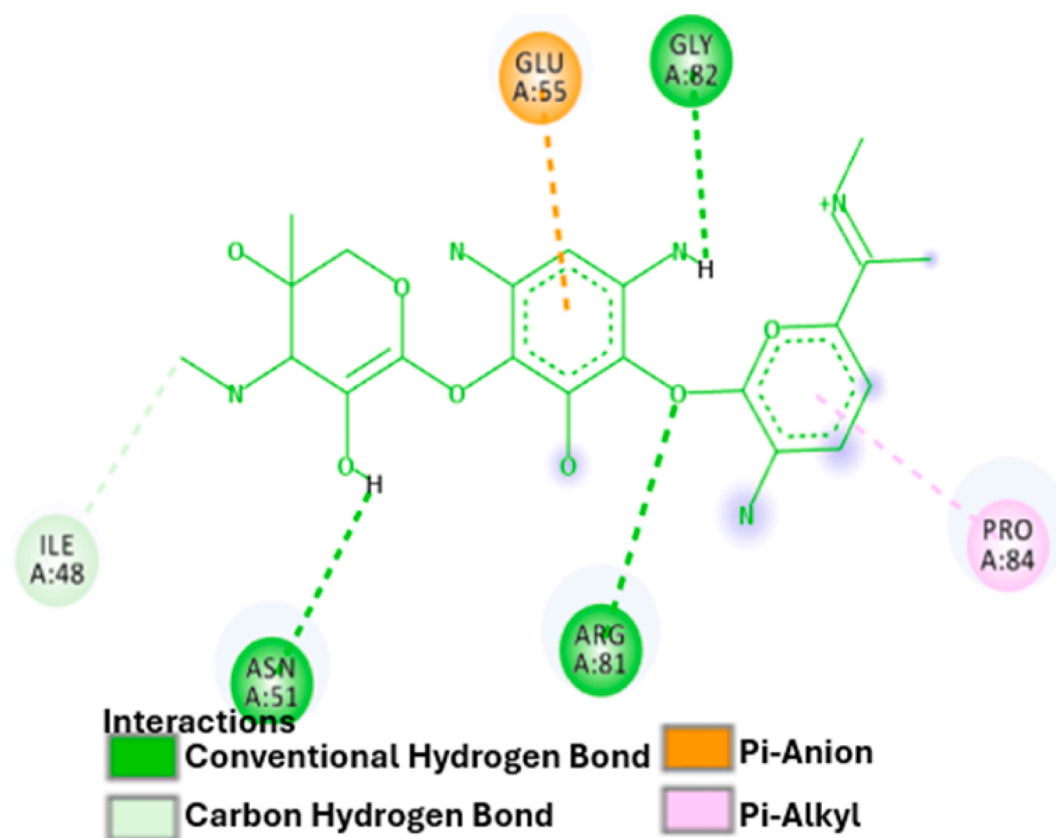


Fig. 6b. Interaction of Gentamicin within the Binding Pocket of 4EMV.

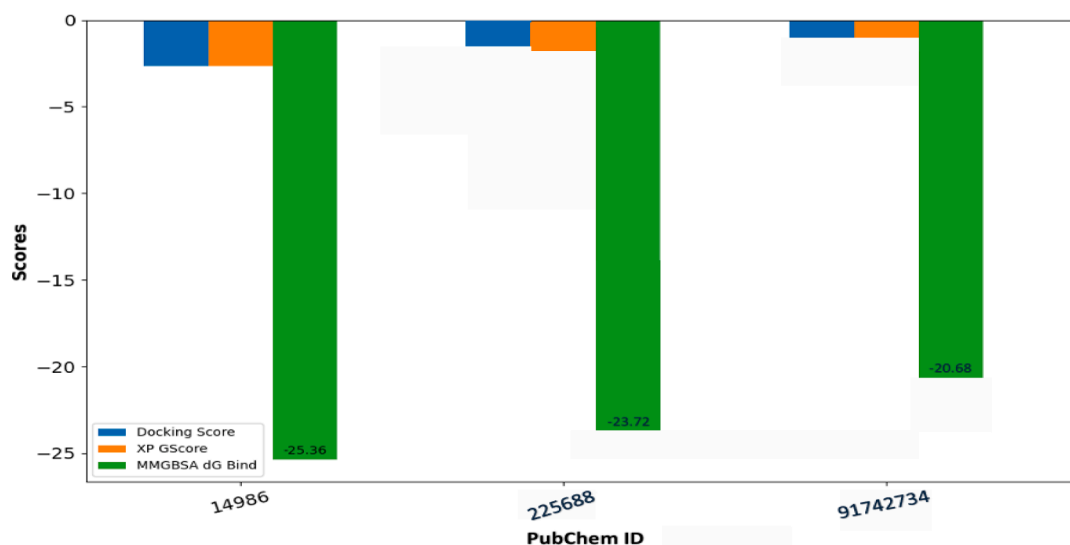


Fig. 6c. Post-docking analysis of Topoisomerase binding to γ -tocopherol indicating the docking, XpGscore, and MMGBSA scores.

also contributes to the activity of the compound in inhibiting the protein function (Fig. 4d below). The strong hydrogen bond and the hydrophobic bond creates a balance that ensures that the protein–ligand is specific and stable.

Penicillin Binding Protein binds to 3 β -acetoxy-5-bisnorcholonic acid with several amino acids (Fig. 4e below). The longest distance was by ARG373 at 5.02 Å making it an important contributor to stability. Other significant amino acids include TYR450 (4.54 Å) and HIS530 (4.49 Å) that facilitate strong binding of the compound and protein. These amino acids enhance the binding affinity of the complex.

The interaction between Penicillin-binding protein 3 (PBP3) and 3 β -acetoxy-5-bisnorcholonic acid shows that there is a balance of molecular interactions within the complex that make it specific and stable (Fig. 5a).

The crystallographic interaction of gentamicin with the amino acids of 3VSL receptors. Hydrogen bond intermolecular interaction was exhibited in all the receptor–ligand complexes, as in Fig. 5b below.

For penicillin-binding protein 3, 3 β -acetoxy-5-bisnorcholonic acid (PubChem ID 91742734) again has the most favorable docking score and XP GScore at -1.63 and -1.64 , respectively, and an MMGBSA dG Bind of -32.95 . Alpha-Amyrin (PubChem ID 225688) follows with a

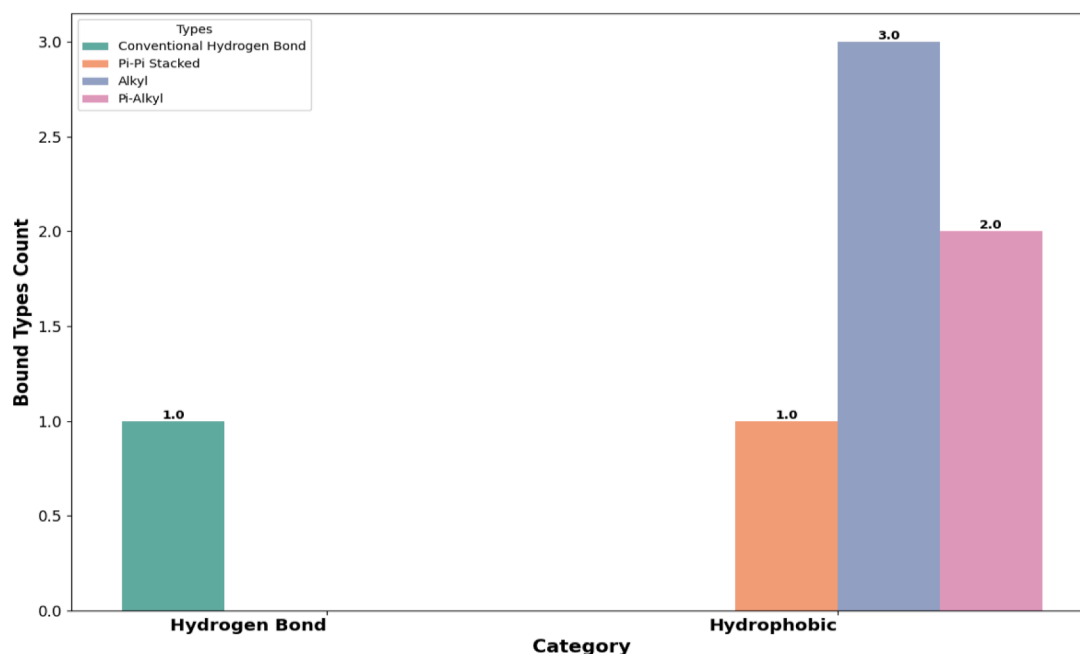


Fig. 6d. Bond categories and types involved in Topoisomerase binding to γ -tocopherol.

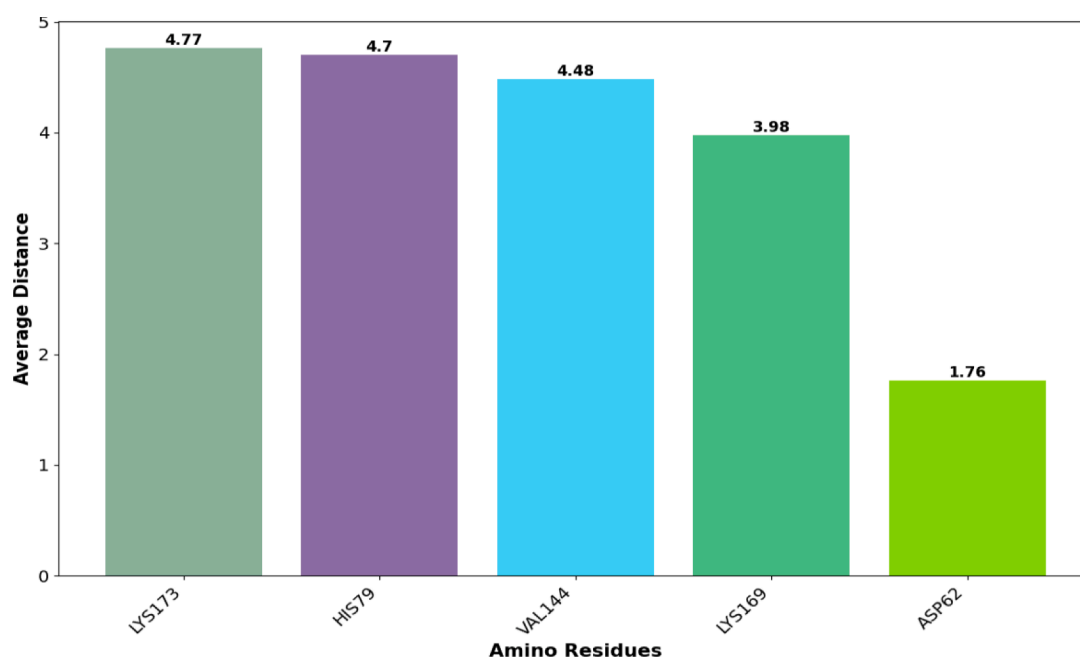


Fig. 6e. Amino acid residues at the binding site of Topoisomerase binding to γ -tocopherol.

docking score of -0.51 and an MMGBSA dG Bind of -17.60 . tocopherol (PubChem ID 14986) shows a positive docking score of 1.09 , which is unfavorable, but has a very favorable MMGBSA dG Bind of -46.28 , indicating strong binding free energy despite the docking score, as indicated in Fig. 5c below.

The bond distances associated with conventional hydrogen bonds, such as 2.98 Å for LYS52, 2.52 Å for ARG69, 2.07 Å for GLY286, and 1.91 Å for ASN308, indicate very close and strong interactions. These distances suggest that these residues are involved in highly specific hydrogen-bonding interactions with 3β -acetoxy-5-bisnorcholenic acid, which are crucial for the compound's precise recognition and binding. Tight hydrogen bonding is essential for stabilizing the ligand within the active site of PBP3, ensuring that the inhibitor effectively binds and

potentially disrupts the protein's function, as indicated in Fig. 5d below.

On the other hand, the longer bond distances associated with hydrophobic interactions, such as 4.42 Å for ARG285, 5.14 Å for LYS288, 4.80 Å for LYS288, 3.93 Å for TYR265, and 5.08 Å for TYR265, as indicated in Fig. 5e, highlight the role of these interactions in maintaining the overall stability of the protein-ligand complex. The alkyl and pi-alkyl interactions contribute to the hydrophobic core of the binding site, where the non-polar regions of 3β -acetoxy-5-bisnorcholenic acid are sequestered away from the aqueous environment. These interactions are weak but contribute to the complex's stability. They help the ligand to remain connected and prevent early dissociation. The strong hydrogen bond and weak hydrophobic interaction ensure a balance of specificity and stability in the complex. These interactions ensure that

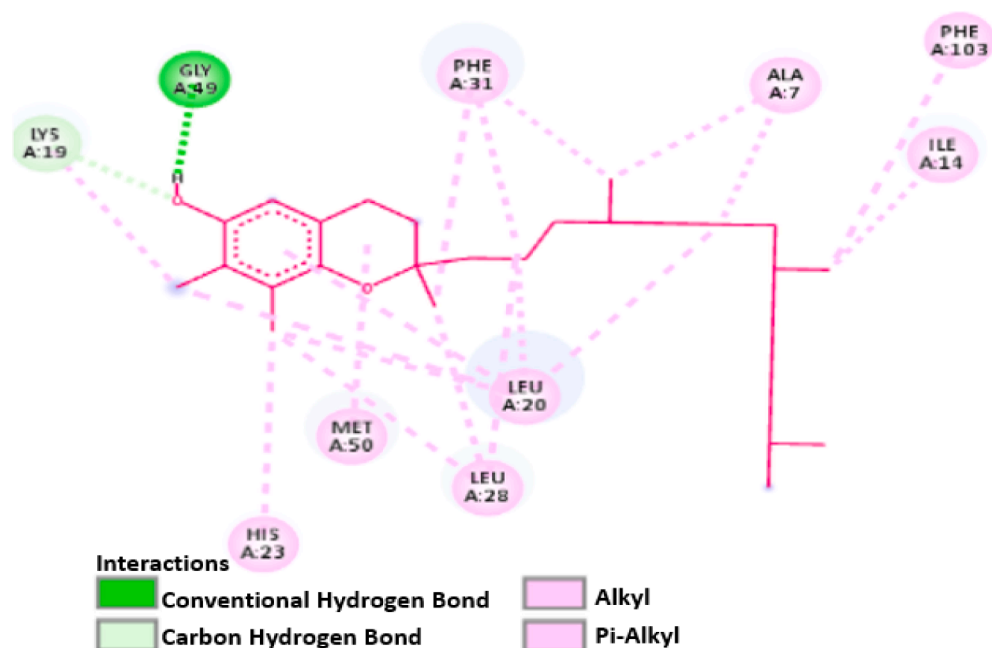


Fig. 7a. Post-docking analysis of Dihydrofolate reductase (PDB ID: 4M7U) binding to γ -tocopherol at the binding site.

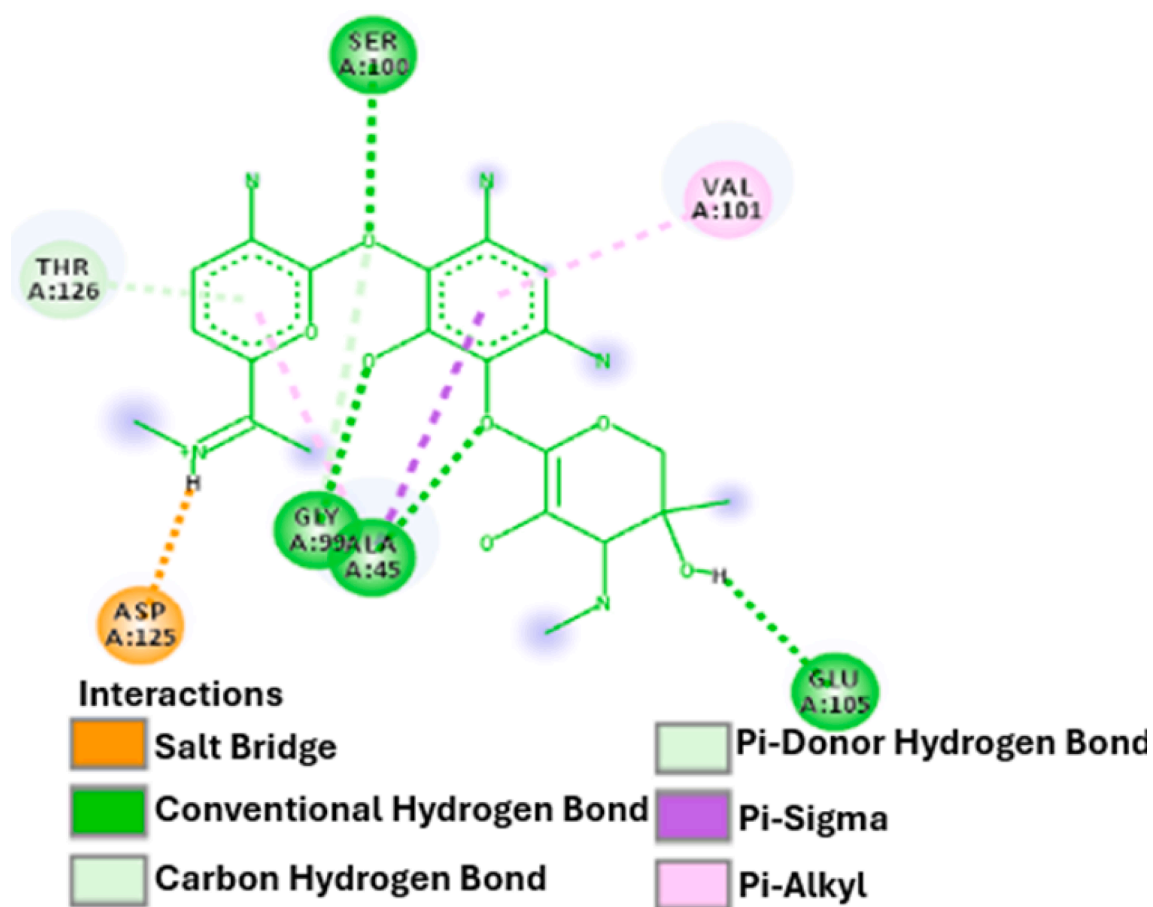


Fig. 7b. Interaction of Gentamicin within the Binding Pocket of 4M7U.

PBP3 is well inhibited by the compound.

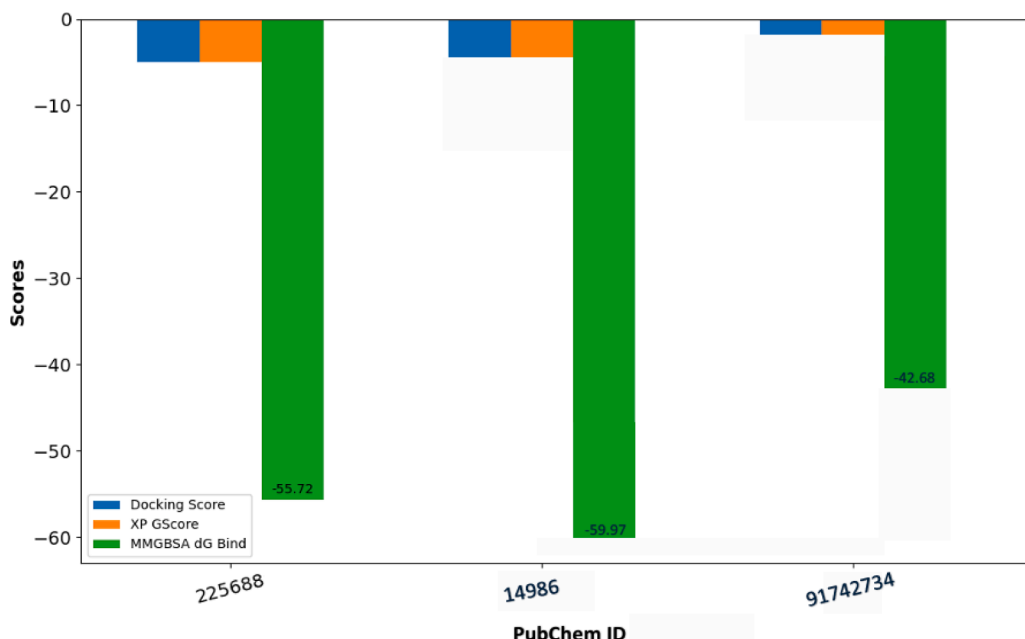


Fig. 7c. Post-docking analysis of Dihydrofolate reductase binding to γ -tocopherol.

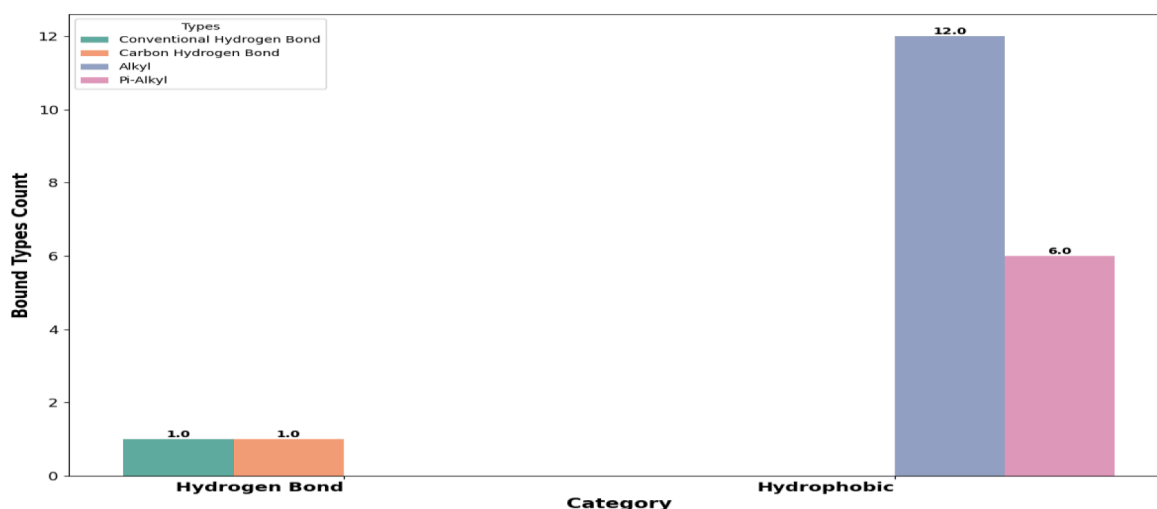


Fig. 7d. Bond categories and types involved in Dihydrofolate reductase binding to γ -tocopherol.

3.7. Interactions of γ -tocopherol within the binding pockets of topoisomerase

In the interaction between Topoisomerase (PDB ID: 4EMV) and γ -tocopherol, as indicated in Fig. 6a below, the bond distances and types (Figs. 6c and 6d) provide insight into how specificity and stability are achieved in this molecular complex.

The crystallographic intermolecular interaction of gentamicin within the binding pocket of 4EMV in Fig. 6b below shows four types of intermolecular interaction occurring involving six amino acids: GLY82, ASN51, ARG81, ILE48, GLU55, and PRO84. The hydrogen-bond intermolecular interaction at the shortest distance to GLY82 indicated stronger binding affinity.

3.8. Post-docking analysis of Topoisomerase binding to γ -tocopherol

In the analysis of Topoisomerase, γ -tocopherol (PubChem ID 14986) showed a docking score of -2.65 and an MMGBSA dG Bind of -25.36 .

Alpha-Amyrin (PubChem ID 225688) has a docking score of -1.54 and an MMGBSA dG Bind of -23.72 , while 3β -acetoxy-5-bisnorchenolic acid [PubChem ID 91742734] has a docking score of -1.03 and an MMGBSA dG Bind of -20.68 . Here, tocopherol stands out with the most favorable docking score and binding free energy as indicated in Fig. 6c below.

The bond distance of $1.76 \text{ \AA}$ associated with ASP62 indicates a strong conventional hydrogen bond. This tight binding is crucial for the specificity of γ -tocopherol within the Topoisomerase binding site, as hydrogen bonds are highly directional and contribute significantly to the precision of the interaction. The γ -tocopherol interacts with Topoisomerase due to the strong hydrogen bonds that ensures the ligand is stabilized. This hydrogen bond also potentially contributes to the activity of the enzyme (Fig. 6d).

The hydrophobic interactions between Topoisomerase and γ -tocopherol exhibit long bond distances, as shown in Fig. 6d. These distances include $4.26 \text{ \AA}$ for HIS79 (pi-pi stacked), $4.48 \text{ \AA}$ for VAL144, $4.77 \text{ \AA}$ for LYS173 (alkyl), and $3.98 \text{ \AA}$ for LYS169 (alkyl) (Fig. 6e). These distances are essential to complex stability. Similarly, the weaker pi-pi

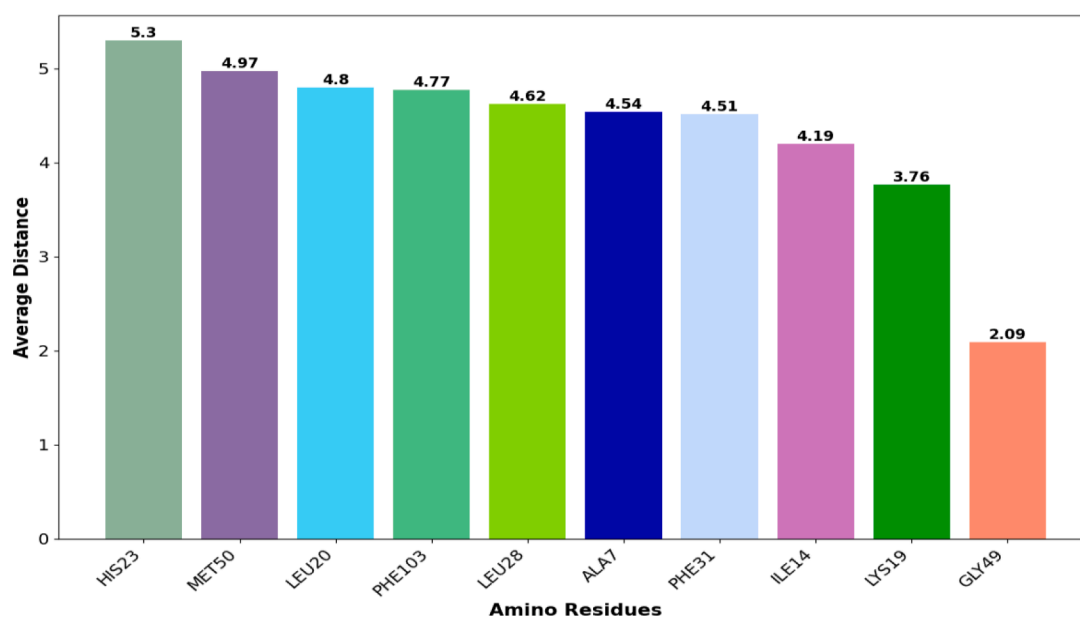


Fig. 7e. Post-docking analysis of Dihydrofolate reductase binding to γ -tocopherol.

Table 4

Chemical Properties of Ligands for Drug Likeness.

	Alpha-Amyrin	3 β -Acetoxy-5-Bisnorcholenic acid	γ -tocopherol	Gentamicin
Molecular Weight (g/mol)	426.72	388.54	416.68	477.60
XlogP	5.08	9.01	10.33	−4.10
Hydrogen bond donor	1	1	1	8
Hydrogen bond acceptor	4	1	2	12
Lipinski Rule Violation	1	1	1	2
Rotatable bond count	6	0	12	7
Topological polar surface area (TPSA)	63.60	20.23	29.46	199.73
Bioavailability	0.85	0.55	0.55	0.17

stacked and pi-alkyl bonds help γ -tocopherol bind to the hydrophobic Topoisomerase binding site. The interactions within this ligand help prevent it from being exposed to nonpolar aqueous regions. The hydrophobic contacts facilitate the effective binding of γ -tocopherol, thereby ensuring affinity and stability in the complex (Fig. 6e).

Table 5

Prediction of ADMET properties from pkCSM.

ADMET properties	Alpha-Amyrin	3 β -Acetoxy-5-Bisnorcholenic Acid	γ -tocopherol	Gentamicin
Intestinal Absorption(%)	0.915	0.667	0.772	0.029
Caco-2 (log Papp in 10 ^{−6} cm/s)	−4.726	−5.056	−4.733	−6.119
Volume Distribution(log L/kg)	0.306	−0.699	−0.1	−0.94
CYP3A4Substrate	Yes	No	No	No
CYP2D6 Substrate &Inhibitor	No	No	No	No
Total Clearance (log mL/min/kg)	1.286	1.26	1.518	0.247
AMES Toxicity	Non-mutagenicity	Non-mutagenicity	Non-mutagenicity	Non-mutagenicity

3.9. Interactions of γ -tocopherol within the binding pockets of dihydrofolate reductase

Fig. 7a shows the interaction between Dihydrofolate reductase (DHFR) and γ -tocopherol. This interaction indicates how diverse types of bonds at different distances ensure the protein–ligand complexes are specific and stable.

The figure below is a 2D crystallographic interaction of gentamicin within the binding pocket of 4M7U. Varying types of hydrogen bond intermolecular interaction were involved with seven amino acids, which was an indication that the ligand was of more specificity than stability (Fig. 7b).

Compound 14,986 was shown to be the most promising candidate for binding to DHFR based on MMGBSA ΔG bind values. Although it has a low docking score of -4.33 , its ΔG Bind of -59.97 indicates a stronger affinity. This contrasts with compound 225,688 which has a high docking score of -5.04 and a ΔG Bind of -55.72 as shown on Fig. 7c. This analysis shows that MMGBSA is an important tool for determining binding strength.

Strong hydrogen bonds characterize the interaction between the DHFR γ -tocopherol ligands. The conventional hydrogen bond at 2.09 Å for GLY49 indicates that the bond is close and specific. This bond anchors γ -tocopherol within the active site of DHFR and maintains its attachment. Similarly, LYS19 at a distance of 2.91 Å fosters the stability of the ligand (Fig. 7d). These bonds help to ensure that the enzyme has the appropriate orientation necessary for potential activity or inhibition.

The hydrophobic interactions between DHFR and γ -tocopherol

ligands are more (16 out of 20 bonds) with longer distances (Fig. 7e). The hydrophobic bond is mainly an alkyl bond at distances of 4.63 Å and 4.45 Å with ALA7 and 4.97 Å with MET50. Pi-alkyl interactions are also present at distances of 5.30 Å for HIS23 and 4.23 Å for PHE31. These interactions help ensure that γ -tocopherol is bound to the hydrophobic region of DHFR and that ligand stability is maintained within the binding pocket. The molecular and biochemical activity of the complex can be attributed to the strong hydrogen bonds and high hydrophobic interactions. These bonds combine to make the complex both specific and stable.

3.10. Lipinski rule of five, permeability and bioavailability

The Lipinski rule of five was used to assess the permeability and solubility of the identified ligands, as shown in Table 4. The XlogP was violated by all the ligands except the standard antibiotic, which had a value of -4.10 , below 5 XlogP. Meanwhile, standard antibiotics violated 2 other rules: the hydrogen-bond donor was more than 5, and the hydrogen-bond acceptor was more than 10. The TPSA of control failed the acceptable limit (140 Å^2). Also, all other investigated compounds passed oral bioavailability screening with a rotatable bond count of less than 10, except for γ -tocopherol. The bioavailability value is not meant to be more than 0.55, which is passed by all except alpha amyrin.

Table 5 below shows the profiling properties that predicted the drug likeness of the ligands after clinical administration. The cytochrome enzyme, CYP3A4, substrates present in the liver were only accessible by alpha amyrin for metabolism, which means alpha amyrin is the only ligand that the liver can easily metabolize. All ligands, including the standard antibiotics, were non-mutagenic. This study observed that the ligands failed only one rule, whereas the standard antibiotics (control) failed two rules of the Lipinski rule of five. It can be deduced that the identified ligands are of higher drug likeness.

4. Conclusion

From this study, it can be deduced that the molecular simulation analysis revealed the inhibitory potential of the evaluated endophytic fungal metabolites against key bacterial protein targets. The results demonstrated that these compounds, particularly γ -tocopherol, exhibited stable, specific interactions with critical enzymes, including Tyrosyl-tRNA synthetase, DNA gyrase, SHV-1 beta-lactamase, penicillin-binding proteins, topoisomerase, and dihydrofolate reductase.

These interactions suggest the likelihood of forming novel inhibitory complexes that could disrupt essential bacterial processes, thereby suppressing the activity of ESKAPE pathogens known for their multidrug resistance. Future research could explore the isolation and antimicrobial activity of the bioactive γ -tocopherol molecule from other natural products to establish its efficacy against multidrug-resistant pathogens.

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CRediT authorship contribution statement

Rahmat Folashade Zakariyah: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Risikat Nike Ahmed:** Supervision, Conceptualization. **Kamoldeen Abiodun Ajijolakewu:** Visualization, Supervision, Project administration, Data curation. **Iyiola Olatunji Oladunjoye:** Writing – review & editing, Visualization, Software, Resources, Methodology, Investigation. **Elohor Precious Samuel:** Writing – review & editing, Validation, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- [1] Alnweiri KHM. Endophytic fungi: a new strategy to conserve medicinal plants in Jordan. *Int Sci J Microbiol Biol* 2020;5(2):20–8. <https://doi.org/10.21608/mb.2021.135182>.
- [2] Ambrose GO, Afees OJ, Faoziyat SA, Oluwaseun AS, Terkuma C, Alexander KA, et al. QSAR Study and Molecular Docking of 2-Phenylaminoimidazo[4,5-H]isoquinolin-9-ones as potent inhibitors of p56lck tyrosine kinase (LCK) in breast cancer therapy. *J Biomed Pharm Sci* 2018;1:108.
- [3] Ndagi U, Falaki AA, Abdullahi M, Lawal MM, Soliman ME. Antibiotics resistance: Bioinformatics-based understanding as a functional strategy for drug design. *RSC Adv* 2020;10:18451–68.
- [4] Fadji AE, Babalola OO. Elucidating mechanisms of endophytes used in plant protection and other bioactivities with multifunctional prospects. *Front Bioeng Biotechnol* 2020;8:467. <https://doi.org/10.3389/fbioe.2020.00467>.
- [5] Friesner RA, Banks JL, Murphy RB, Halgren TA, Klicic JJ, Mainz DT, et al. Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. *J Med Chem* 2004;47(7):1739–49. <https://doi.org/10.1021/jm0306430>.
- [6] Geisinger E, Mortman NJ, Dai Y, Cokol M, Syal S, Farinha A, et al. Antibiotic susceptibility signatures identify potential antimicrobial targets in *Acinetobacter baumannii*. *Nat Commun* 2020;11:4522. <https://doi.org/10.1038/s41467-020-18301-2>.
- [7] Newman DJ, Cragg GM. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *J Nat Prod* 2020;83:770–803. <https://doi.org/10.1021/acs.jnatprod.9b01285>.
- [8] Moussa AY. Endophytes: a uniquely tailored source of potential antibiotic adjuvants. *Arch Microbiol* 2024;206:207. <https://doi.org/10.1007/s00203-024-03891-y>.
- [9] Ignjatovic J, Dajic N, Krmar J, Protic A, Strukelj B, Otasevic B. Molecular docking study on biomolecules isolated from endophytic fungi. *J Serb Chem Soc* 2021;86(00):2. <https://doi.org/10.2298/JSC200815002I>.
- [10] Halgren TA, Murphy RB, Friesner RA, Beard HS, Frye LL, Pollard WT, et al. Glide: a new approach for rapid, accurate docking and scoring. 2. Enrichment factors in database screening. *J Med Chem* 2004;47(7):1750–9. <https://doi.org/10.1021/jm030644s>.
- [11] Kirk PM, Cannon PF, Minter DW, Stalpers JA. *Dictionary of the fungi*. 10th ed. Wallingford: CABI; 2008.
- [12] Lade H, Kim JS. Bacterial targets of antibiotics in methicillin-resistant *Staphylococcus aureus*. *Antibiotics* 2021;10(4):398. <https://doi.org/10.3390/antibiotics10040398>.
- [13] Khan IH, Patel NB, Patel VM. Synthesis, in silico molecular docking and pharmacokinetic studies, in vitro antimicrobial and antimicrobial studies of new imidazolones clubbed with thiazolidinedione. *Curr Comput-Aided Drug Des* 2018;14:269–83. <https://doi.org/10.2174/1573409914666180516113552>.
- [14] Khare E, Mishra J, Arora NK. Multifaceted interactions between endophytes and plant: developments and prospects. *Front Microbiol* 2018;9:2732. <https://doi.org/10.3389/fmicb.2018.02732>.
- [15] Kumara PM, Shweta S, Vasanthakumari MM, Sachin N, Manjunatha BL, Jadhav SS, et al. Endophytes and plant secondary metabolite synthesis: Molecular and evolutionary perspective. In: Verma VC, Gange AC, editors. *Adv Endophytic Res* Singapore: Springer; 2014. p. 145–62. doi:10.1007/978-81-322-1575-2_9.
- [16] Yu M, Chen JC, Qu JZ, Liu F, Zhou M, Ma YM, et al. Exposure to endophytic fungi quantitatively and compositionally alters anthocyanins in grape cells. *Plant Physiol Biochem* 2020;149:144–52. <https://doi.org/10.1016/j.plaphy.2020.02.006>.
- [17] Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, et al. The protein data bank. *Nucleic Acids Res* 2000;28(1):235–42. <https://doi.org/10.1093/nar/28.1.235>.
- [18] Arannilewa AJ, Alakase OS, Adeleye AO, Malachi OI, Obaido IM, Ekun OE, et al. Molecular docking analysis of Cianidanol from *Ginkgo biloba* with HER2+ breast cancer target. *Bioinformation* 2018;14(9):482–7.
- [19] Nandy S, Mukherjee A, Pandey DK, Dey A. *Bacopa monnieri*: the neuroprotective elixir from the East—phytochemistry, pharmacology, and biotechnological improvement. In: Singh J, Meshram V, Gupta M, editors. *Bioactive Natural Products in Drug Discovery*. Singapore: Springer; 2020. p. 97–126. https://doi.org/10.1007/978-981-15-1394-7_2.
- [20] Waring MJ. Lipophilicity in drug discovery. *Expert Opin Drug Discov* 2010;5(3):235–48.
- [21] Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* 1997;23:3–25.
- [22] Jiang Q, Im S, Wagner JG, Hernandez ML, Peden DB. Gamma-tocopherol, a major form of vitamin E in diets: Insights into antioxidant and anti-inflammatory effects, mechanisms, and roles in disease management. *Free Radic Biol Med* 2022;178:347–59. <https://doi.org/10.1016/j.freeradbiomed.2021.12.012>.
- [23] Missio D, Leivas FG, Cibin F, Emanuelli T, Somacal S, Buss V, et al. Vitamin E reduces reactive oxygen species production in the dominant follicle during

- negative energy balance in cattle. *Reprod Domest Anim* 2023;58:1662–71. <https://doi.org/10.1111/rda.14481>.
- [24] Assefa et al., 2023. <https://doi.org/10.1021/acsomega.3c08866?urlappend=%3Fref%3DPDF&jav=VoR&rel=cite-as>.
- [25] Manimegalai P, Selvam K, Prakash P, Kirubakaran D, Shivakumar MS, SenthilNathan S. In-vitro antibacterial, antioxidant and anti-inflammatory and In-silico ADMET, molecular docking study on *Hardwickia binata* phytocompunds with potential inhibitor of skin cancer protein. *In Silico Pharmacol* 2023;11(1):25. <https://doi.org/10.1007/s40203-023-00163-3>.
- [26] Thanabalasingam D, Kumar NS, Jayasinghe L, Fujimoto Y. Endophytic Fungus *nigrospora oryzae* from a medicinal plant *coccinia grandis*, a high yielding new source of phenazine-l-carboxamide. *Nat Product Comm* 2015;10:1934578X1501001. <https://doi.org/10.1177/1934578x1501001008>.
- [27] Roshanak S, Shahidi F, Yazdi FT, Javadmanesh A, Moaffagh J. Evaluation of antimicrobial activity of buforin I and nisin and synergistic effect of the combination of them as a novel antimicrobial preservative. *J Food Prot*, 2020;83(11):2018–2025. Doi:10.4315/jfp-20-127.
- [28] Zakariyah RF, Ahmed RN, Ajijolakewu KA, Hamid AA. Enhanced antibacterial potential of fractionated bioactive compounds isolated from endophytic *Nigrospora oryzae* UILRZ1. *J Trop Biodivers Biotechnol* 2025;10(1):jtbb12019. <https://doi.org/10.22146/jtbb.v10i1>.
- [29] Safi A, Mehrabi-Koushki M, Arzanlou M. Endophytic species of *Nigrospora* from grasses and shrubs of Shadegan International Wetland, with new species and records from Iran. *Antonie Van Leeuwenhoek* 2024;117. <https://doi.org/10.1007/s10482-024-01976-8>.
- [30] Supaphon P, Preedanon S. Evaluation of in vitro alpha-glucosidase inhibitory, antimicrobial, and cytotoxic activities of secondary metabolites from the endophytic fungus, *Nigrospora sphaerica*, isolated from *Helianthus annuus*. *Ann Microbiol* 2019;69:1397–406. <https://doi.org/10.1007/s13213-019-01523-1>.
- [31] Fanele A, Ndlovu SI. Endophytic fungal species *Nigrospora oryzae* and *Alternaria alternata* exhibit antimicrobial activity against gram-positive and gram-negative multi-drug resistant clinical bacterial isolates. *BMC Complementary Med Ther* 2023;23:323. <https://doi.org/10.1186/s12906-023-04157-8>.