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Original Article

Molecular Docking of Some Phytochemicals Against DNA Gyrase of Gram-Negative and Gram-positive Bacteria

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Abstract: Molecular docking is a computational technique that predicts the binding orientation and affinity of a small molecule (ligand) to a target macromolecule (receptor). It involves the calculation of the intermolecular interactions between the ligand and receptor to estimate the binding energy and to identify the most stable and favorable binding conformation. Molecular docking can be used for drug discovery and design, as it can help identify potential drug candidates that bind to a specific target with high affinity and selectivity. It is also widely usedin structural biology, as it can provide insights into the structural basis of ligand- receptor interactions. The objective of this study was to investigate the potential activity of various phytochemicals against DNA gyrase of *Escherichia coli* and *Staphylococcus aureus*, using molecular docking technique. The study involved 43 phytochemical compounds, along with Ciprofloxacin and Nalidixic acid. The 3D structures of these compounds were obtained from PubChem database and prepared using PyRx-Vina Program 0.8 and OpenBabel software version 3.1.1. DNA gyrase of E. coli (ID 7P2M) and S. aureus (ID 3U2D) were obtained from the RCSB PDB database and refined through energy minimization using the PyRx-Vina Program 0.8. According to the binding affinity values, the results indicated that Diospyrinwas the most active compound against DNA gyrase of *E. coli* (-8.7) and *S. aureus* (-9.1), while Imidazoline was the least active against DNA gyrase of *E. coli* (-3) and Allyl methyl disulfide was the least active against DNA gyrase of *S. aureus* (-3) when compared with Ciprofloxacin and Nalidixic acid. The study also revealed that the number of amino acids that interacted with each compound varied. The findings suggest that phytochemical compounds could beused as potential antibacterial agents, and further in-vitro and in-vivo studies are recommended using the most active compounds.

Keywords: Phytochemicals, DNA Gyrase, Molecular Docking

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Introduction:

Molecular docking is a computational technique that aims to determine the arrangement of compounds resulting from the interaction of two or more separate molecules. The main goal of docking research is to predict the specific three-dimensional structures that may arise from these interactions. It's important to note that docking only produces appropriate binding structures and doesn't have any other effects on the molecules (Raval & Ganatra, 2022). Docking is a commonly used approach to predict how small molecules that are used for medicinal purposes will interact with specific proteins. This method helps researchers to anticipate the strength of the interaction between the small molecule and the protein target, as well as the small molecule's ability to produce a therapeutic effect (Raval & Ganatra, 2022). The molecular machines of the cell, i.e., proteins, are essential to many cellular processes such as signal transduction and cell regulation. Proteins seldom act alone in the cell, but they function through interacting with other small or macromolecules. Therefore, understanding protein interactions at the atomic level is critical to understanding biological processes (Maden *et al*., 2022). Phytochemicals, which are compounds produced by plants and derived from the Greek word "phyto" meaning "plant", have been utilized as traditional medicine due to the presence of biologically active compounds with immense therapeutic properties. The use of plants for medicinal purposes has a long history and continues to play a significant role in preventing and treating varioushuman diseases. Research has demonstrated that plants are a rich source of phytochemical ingredients and have great potential for medicinal value (Chanda & Ramachandra, 2019). Bacterial DNA gyrase, a type II topoisomerase, is a crucial target for the development of new antibacterials due to its ability to introduce negative supercoils to DNA substrates. This enzyme is a primary target of quinolones, which are broad-spectrum antibacterial agents that are commonly used as a first-line treatment for various infections. However, the effectiveness of currently used quinolones is decreasing due to the development of drug resistance. One of the most clinically relevant forms of resistance occurs through mutations in the enzyme targets. Other mechanisms that contribute to quinolone resistance include chromosomal mutations and/or the uptake of plasmid-genes, which can alter the concentration of quinolones within the cell and affect their interactions with the target or metabolism of the drug (Spencer & Panda, 2023). *Staphylococcus aureus* and *Escherichia coli* are common causes of both superficial and systemic infections. In Western industrialized countries, they are among the leading causes of bacteremia and sepsis, with proportions ranging from 16.3% to 21.6% for S. aureus and 5.6% to 24.2% for *E. coli*. These infections are associated with significant morbidity and mortality. Due to the high risk of secondary foci of infection and particularly high mortality rates, prolonged antibiotic therapy is recommended for *S. aureus*-associated bacteremia, regardless of whether the strain is methicillin-susceptible or methicillin-resistant. In addition to systemic infections, both species can cause superficial infections such as wound infections or urinary tract infections (Frickmann *et al*., 2019). The present study aimed to in-silico investigation of the potential activity of some phytochemicals in comparison with some quinolones toward DNA gyrase of *E. coli* and *S. aureus Staphylococcus aureus* has two genes, gyrA and gyrB, which encode its gyrase enzyme. The purification and partial characterization of *S. aureus* gyrase have been completed. However, the purification of the topoisomerase IV enzyme, which is encoded by grlA and grlB, has not been done yet. Genetic evidence has shown that topoisomerase IV is the main target of fluoroquinolones in *S. aureus* and not gyrase, which is different from what is observed in *E. coli* and *Neisseria gonorrhoeae* (Kato *et al*., 2022).

Materials and Methods:

3.1. Design of study

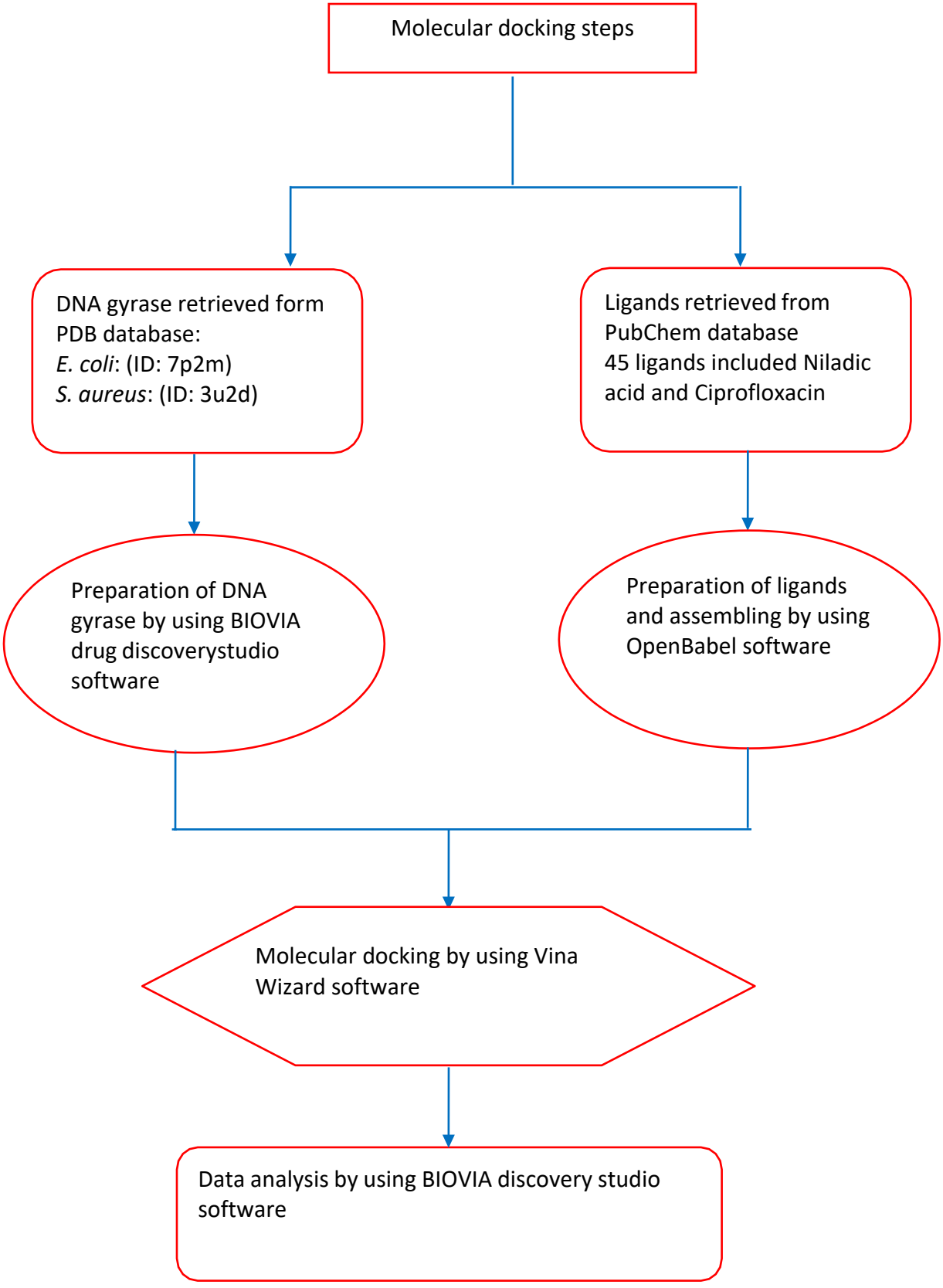
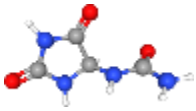
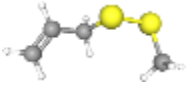
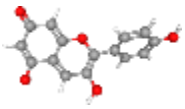
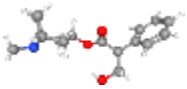
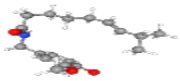
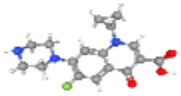
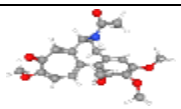
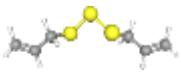
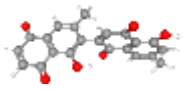


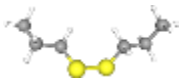
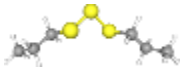
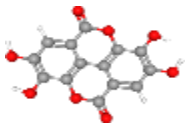
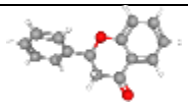
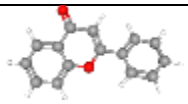
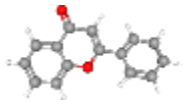
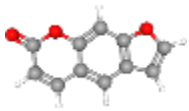
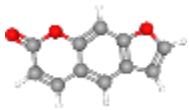
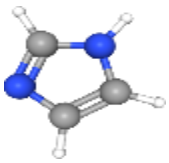
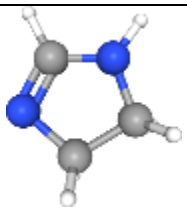
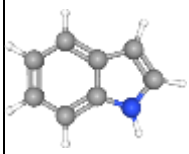
Figure 3-1: Design of study

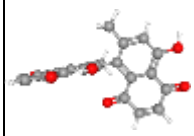
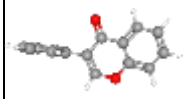
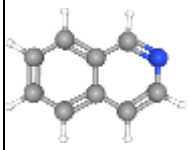
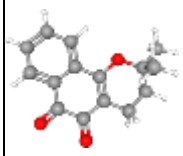
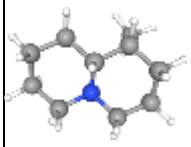
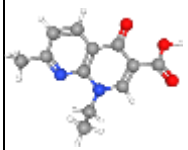
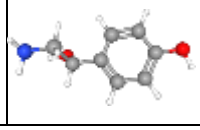
3.2. Preparation of ligands molecules

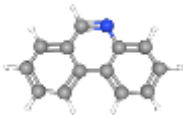
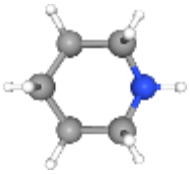
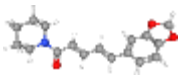
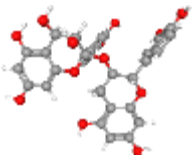
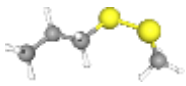
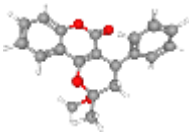
A total of 45 ligands molecules were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). These molecules included 43 phytochemicals compounds as well as Niladic acid and Ciprofloxacin. All ligands were used in 3D structure as shown in table 3-1. Then ligands were prepared using PyRx-Vina Program 0.8 and assembled by using OpenBabel software version 3.1.1. (Santoso, 2019) and then labeled from (L1 to L45).

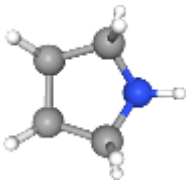
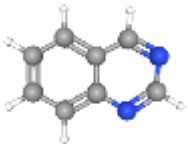
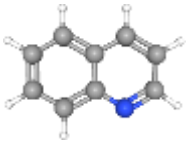
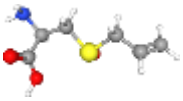
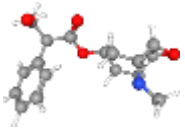
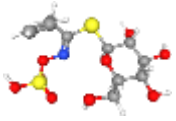
Table 3-1: Names and the 3D structures of the selected ligands

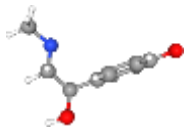
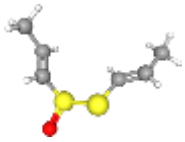
No.	Name of ligands	3D structure
1	Allantoin	
2	Allyl methyl disulfide	
3	Anthocyanidin	
4	Atropine	
5	Capsaicin	
6	Ciprofloxacin	
7	Colchicine	
8	Diallyl disulfide	
9	Diallyl trisulfide	
10	Diospyrin	

11	Dipropyl disulfide	
12	Dipropyl trisulfide	
13	Ellagic acid	
14	Flavanone	
15	Flavone	
16	Flavonol	
17	Furocoumarin	
18	Gluconapin	
19	Glyoxaline	
20	Imidazoline	
21	Indole	

22	Isodiospyrin	
23	Isoflavone	
24	Isoquinoline	
25	Lapachone	
26	Lupinane	
27	Nalidixic acid	
28	Octopamine	

29	Phenanthridine	
30	Piperidine	
31	Piperine	
32	Proanthocyanidins	
33	Propyl methyl disulfide	
34	Pyranocoumarin	

35	Pyrrolidine	
36	Quinazoline	
37	Quinoline	
38	S-allyl-L-cysteine sulfoxide	
39	Scopolamine	
40	Sinigrin	

41	Synephrine	
42	Thiosulfinate	
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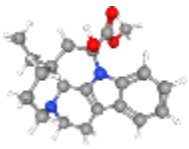
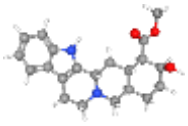
44	Vincamine	
45	Yohimbine	

3.3. Preparation of DNA gyrase

The goal was to examine how DNA gyrase of E. coli and S. aureus bind to their ligands and identify the active site. In order to do this, the study analyzed the binding site of the PO4301 ligand in the crystal structure with PDB ID 7P2M for E. coli, and PDB ID 3U2D for S. aureus, using BIOVIA discovery studio V21.1.0.20298. Before the molecular docking analysis, the target proteins were cleaned up by removing alternate conformations, adjusting the terminal residues, and correcting the bond orders. Then, the proteins were prepared for docking analysis by removing water molecules while keeping the target enzymes and their co-crystal ligands intact. The protein structures were further refined through energy minimization using the PyRx-Vina Program 0.8 (Santoso, 2019).

3.4. Molecular docking

All docking and scoring calculation were achieved using PyRx-Vina Program 0.8 with vina space serach (Center: X: 19.981, Y: 11.5986 and Z: 11.69.3) (Dimensions Å: X: 26.6637, Y: 29.4532 and Z: 27.8510) for DNA gyrase of E. coli, while vina space search for DNA gyrase of S. aureus was (Center: X: 3.46.47, Y: 4.4237 and Z: 25.8739) (Dimensions Å: X: 28.43.44, Y: 30.35.25 and Z: 27.8739) . The crystal structure of E. coli DNA gyrase (PDB entry: 7P2M) at a resolution of 1.16 Å and the crystal structure of S. aureus DNA gyrase (PDB entry: 3U2D) at a resolution of 1.85 Å

were obtained from the Protein Data Bank. A resolution between 1.5 and 2.5 Å is considered as a good quality for docking studies (Didierjean & Tête-Favier, 2016). It is known that the best score of Root Mean Square Deviation (RMSD) values should be near to 2 Å with an energy score less or equal to 7 Kcal/mol (Ramalho et al., 2009). These two values are often used as measure the validity of the result of molecular docking.

The protein-ligand interactions were analysis and visulized by using BIOVIA discovery studio V21.1.0.20298.

4. Results and discussion

4.1. Molecular docking of E. coli DNA gyrase

The molecular docking results of 45 ligands against DNA gyrase of E. coli have yielded valuable insights (appendix 1). Among them, Diospyrin, Yohimbine, and Ellagic acid demonstrated the highest level of activity with binding affinities of -8.7, -8.6, and -8.5, respectively. Following closely behind were Isodiospyrin (-8.4), Flavone (-8.3), and Pyranocoumarin (-8.2). Additionally, Isoflavone, Flavonol, Flavanone, and Anthocyanidin demonstrated relatively high levels of activity with binding affinities of (-8). These results were highly significant and can provide a basis for further exploration in this area of research when compared with results of Ciprofloxacin and Nalidixic acid with binding affinity (-7.2).The least active ligand was Imidazoline, displaying a binding infinity of (-3) as shown in Table (4-1).

The RMSD/UB (Root Mean Square Deviation/Upper Bound) and RMSD/LB (Root Mean Square Deviation/Lower Bound) value of almost all active compounds was (0) which indicated the accuracy and reliability of molecular docking results as appeared in table (4-1). When the RMSD value is 0.0 in molecular docking, it indicates that the predicted binding pose of the ligand is identical to the experimental or crystallographic structure of the protein-ligand complex. This means that the predicted structure is an exact match to the experimental structure, suggesting that the docking method has accurately predicted the binding mode of the ligand (Mandal & Munshi, 2021).

RMSD value of 0.0 is an ideal scenario, but it is very rare to achieve it in practice. This is because molecular docking methods are inherently approximations, and there are many factors that can contribute to errors and inaccuracies in the predicted binding pose. In addition, experimental structures can also have limitations and uncertainties, such as the resolution of the crystallographic data (Meng et al., 2011).

However, even if a RMSD value of 0.0 is not achieved, a low RMSD value indicates that the docking method has accurately predicted the binding mode of the ligand, and that the predicted structure can be a useful starting point for further refinement and optimization (Meng et al., 2011).

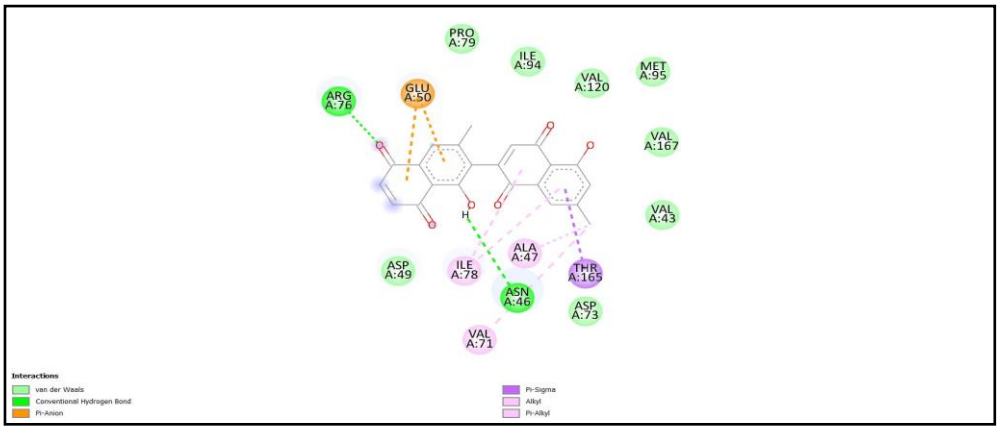
Table (4-1): The molecular docking results of the most active compounds against DNA gyrase of E. coli among 45 compounds in comparison with Ciprofloxacin and Nalidixic acid

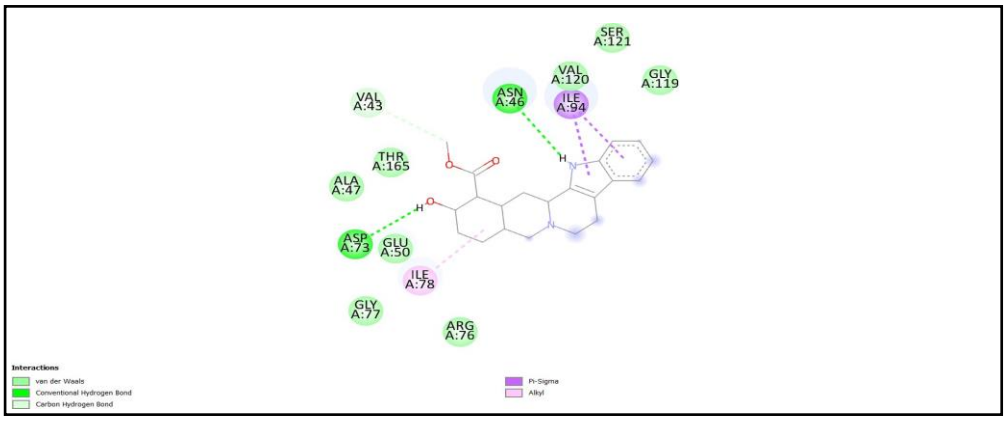
Ligand	Binding infinity	rmsd/ub	rmsd/lb
Diospyrin	-8.7	0	0
Yohimbine	-8.6	0	0
Ellagic acid	-8.5	0	0
Isodiospyrin	-8.4	0	0
Flavone	-8.3	0	0
Pyranocoumarin	-8.2	0	0
Isoflavone	-8	0	0
Flavonol	-8	0	0
Flavanone	-8	0	0
Anthocyanidin	-8	0	0
Ciprofloxacin	-7.2	0	0
Nalidixic acid	-7.2	0	0
Imidazoline	-3	0	0

Diospyrin is a natural product (Naphthoquinones) isolated from the leaves of the Indian Diospyros species of trees. It is a quinonoid pigment and has been shown to have a variety of biological activities, including anti-inflammatory, antitumor, and antiviral properties. Studies have shown that diospyrin has potent anticancer activity against various types of cancer cells, including breast, lung, and prostate cancer cells. It appears to work by inducing apoptosis (programmed cell death) in cancer cells and inhibiting their growth and proliferation. Diospyrin has also shown promise as a potential treatment for viral infections, including hepatitis B and C, HIV, and dengue fever. Although diospyrin is a promising natural product with potential therapeutic applications, more research is needed to fully understand its mechanisms of action and potential side effects before it can be used clinically (Pullella et al., 2020). There are many studies showed the activity of Naphthoquinones against DNA gyrase of E. coli (Hueso-Falcón et al., 2017; Karkare et al., 2013; Mohamady et al., 2020; Rajeswari, 2018).

Diospyrin, Yohimbine, Ellagic acid, Isodiospyrin, Flavone, Pyranocoumarin, Isoflavone, Flavonol, Flavanone, Anthocyanidin, Ciprofloxacin, Nalidixic acid, and Imidazoline were analyzed for their interaction with the active site of DNA gyrase. The amino acids involved in the interaction for each ligand were determined and presented in Figure 4-1 to Figure 4-13. The active site of DNA gyrase comprised specific amino acids, and the type and number of amino acids involved in the interaction varied among the ligands. For example, Diospyrin interacted with seven amino acids, whereas Flavonol interacted with four amino acids. The identification of the key amino acids involved in the interaction provides insights into the ligand-receptor interaction and can aid in the design and optimization of drugs.

Figure (4-1): Diospyrin interaction with DNA gyrase of E. coli





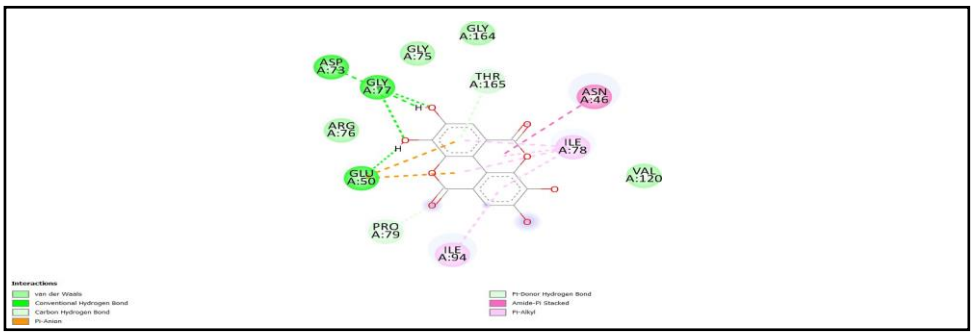


Figure (4-2): Yohimbine interaction with DNA gyrase of E. coli

Figure (4-3): Ellagic acid interaction with DNA gyrase of E. coli

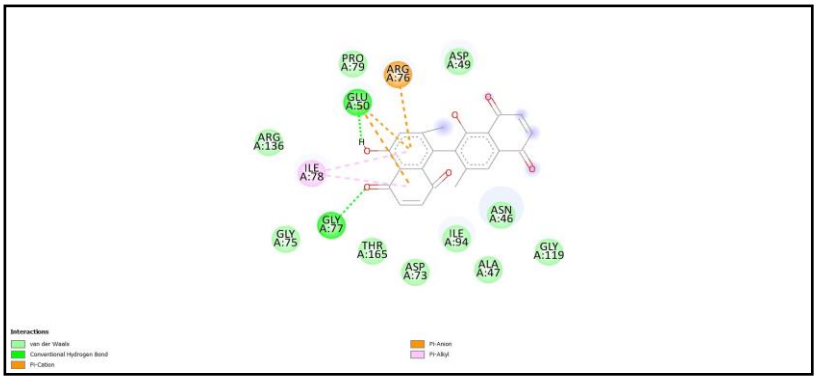
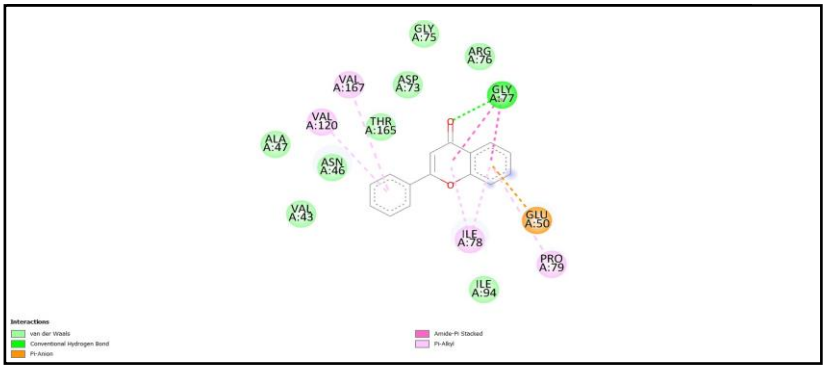


Figure (4-4): Isodiospyrin interaction with DNA gyrase of E. coli



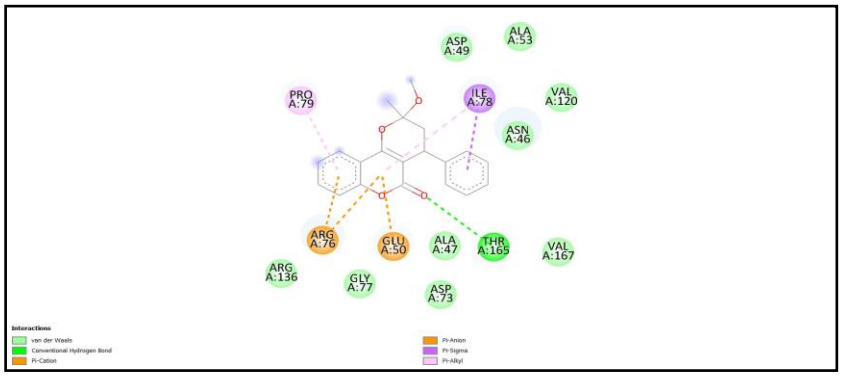


Figure (4-5): Flavone interaction with DNA gyrase of E. coli

Figure (4-6): Pyranocoumarin interaction with DNA gyrase of E. coli

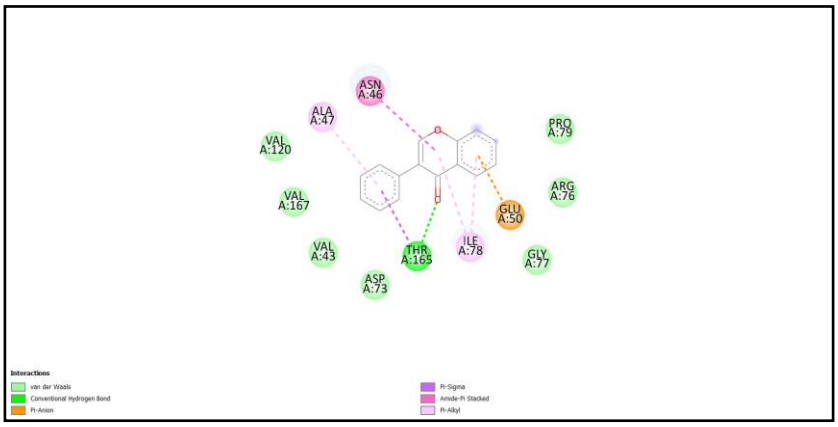


Figure (4-7): Isoflavone interaction with DNA gyrase of E. coli

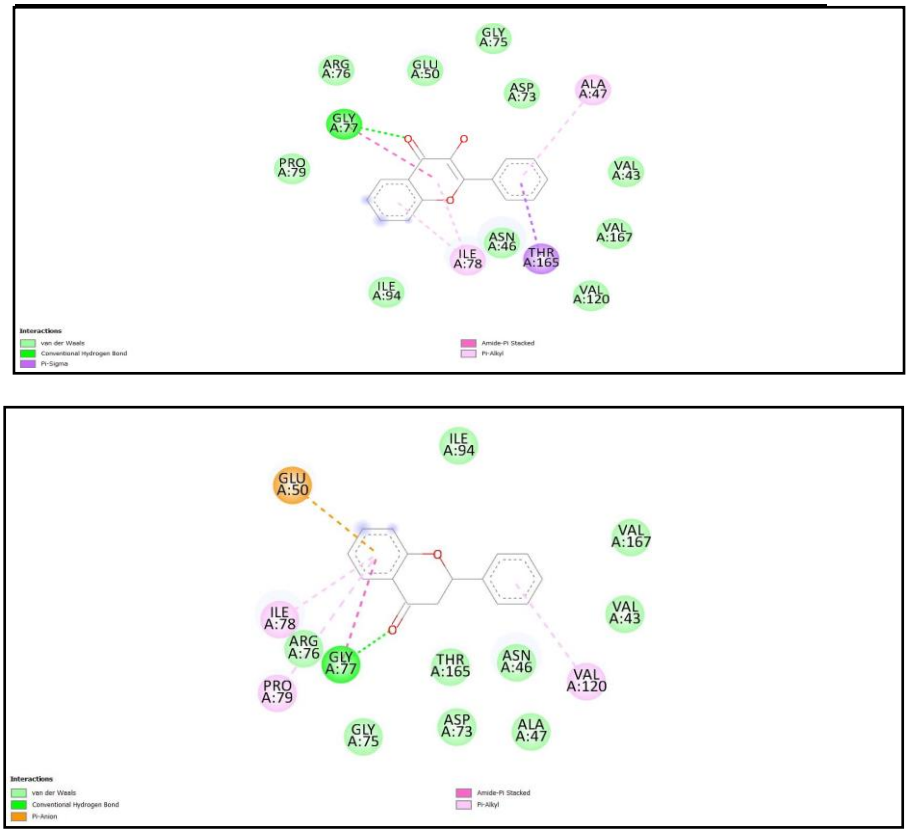


Figure (4-8): Flavonol interaction with DNA gyrase of E. coli

Figure (4-9): Flavanone interaction with DNA gyrase of E. coli

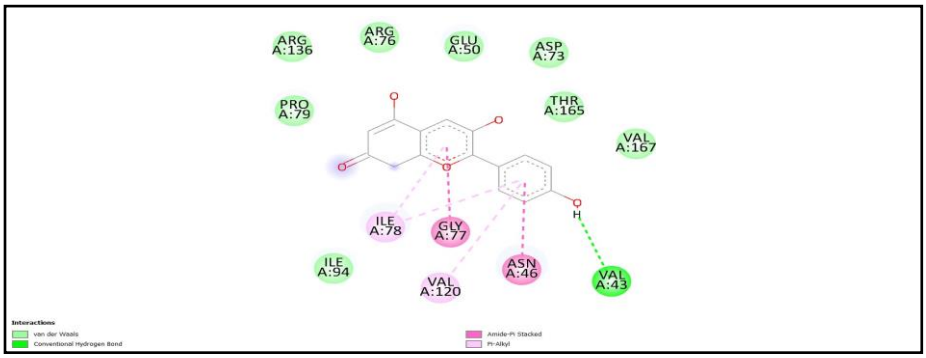


Figure (4-10): Anthocyanidin interaction with DNA gyrase of E. coli

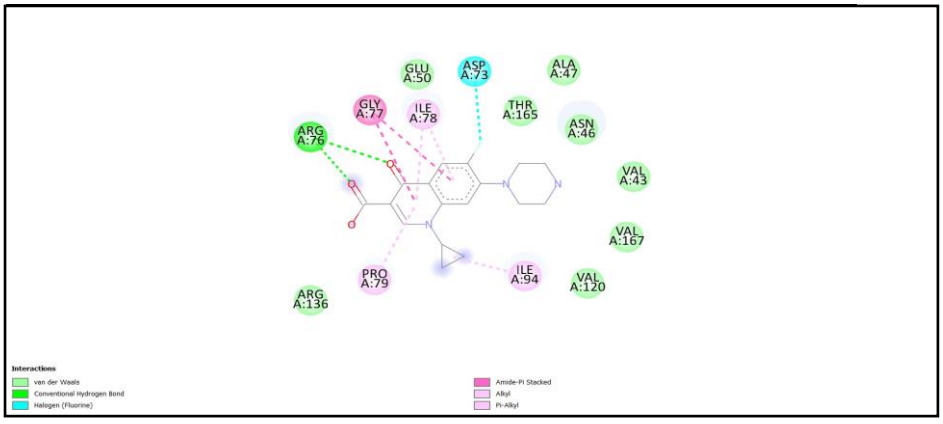
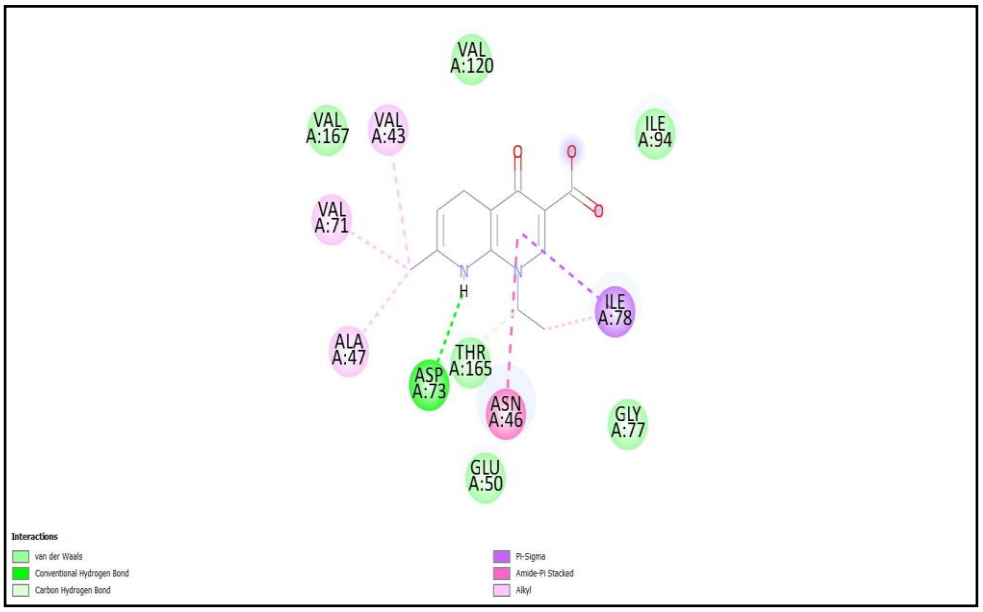


Figure (4-11): Ciprofloxacin interaction with DNA gyrase of E. coli



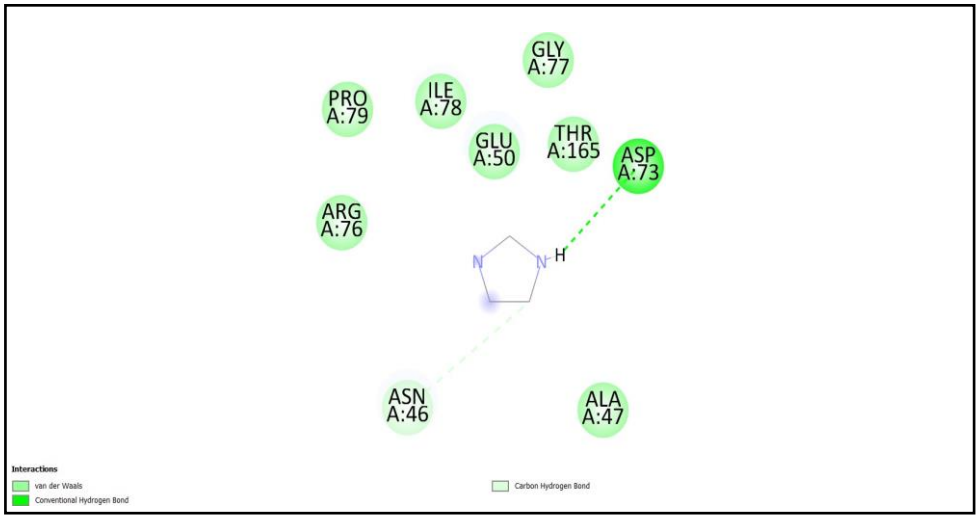


Figure (4-12): Nalidixic acid interaction with DNA gyrase of E. coli

Figure (4-13): Imidazoline interaction with DNA gyrase of E. coli

4.2. Molecular docking of S. aureus DNA gyrase

The results of molecular docking studies conducted on 45 ligands against S. aureus DNA gyrase have provided valuable insights (appendix 2). Among these ligands, Diospyrin, Isodiospyrin, and Pyranocoumarin demonstrated the highest level of activity, with binding affinities of -9.1, -8.9, and -8.6, respectively. Ellagic acid (-8.4), Yohimbine (-8.2), and Flavone (-8) followed closely behind. In contrast, Proanthocyanidins (-7.9), Flavonol, Flavanone, and Anthocyanidin exhibited relatively lower levels of activity, with binding affinities of -7.8. These results were significant and can serve as a basis for further research in this field, especially when compared with the results of Ciprofloxacin and Nalidixic acid, which had binding affinities of -7.4 and -7.1, respectively. Allyl methyl disulfide was found to be the least active ligand, with a binding affinity of -3, as shown in Table (4-2).

There were several studies which investigated the molecular dynamic of Naphthoquinones compounds against DNA gyrase of S. aureus and all these studies showed that the naphthoquinones interacted with several key residues in the active site of the enzyme, and that their binding affinities correlated with their antibacterial activities (Wu et al., 2015; Singh et al. 2019; Su et al., 2021).

Table (4-2): The molecular docking results of the most active compounds against DNA gyrase of S. aureus among 45 compounds in comparison with Ciprofloxacin and Nalidixic acid

Ligand	Binding infinity	rmsd/ub	rmsd/lb
Diospyrin	-9.1	0	0
Isodiospyrin	-8.9	0	0
Pyranocoumarin	-8.6	0	0
Ellagic acid	-8.4	0	0
Yohimbine	-8.2	0	0
Flavone	-8	0	0
Proanthocyanidins	-7.9	0	0
Flavonol	-7.8	0	0
Flavanone	-7.8	0	0
Anthocyanidin	-7.8	0	0
Ciprofloxacin	-7.4	0	0
Nalidixic acid	-7.1	0	0
Allyl methyl disulfide	-3	0	0

There several non-covalent interactions between the receptor and ligand are important in determining the binding affinity in molecular docking. These include:

1. Hydrogen bonds: Hydrogen bonds are formed between a hydrogen atom of the ligand and an electronegative atom of the receptor, such as oxygen or nitrogen. Hydrogen bonding is important for stabilizing the complex and can contribute significantly to the binding affinity.

2. Van der Waals interactions: Van der Waals interactions are weak, attractive forces between atoms or molecules that are in close proximity. These interactions play an important role in molecular recognition and can contribute significantly to the binding affinity.

3. Electrostatic interactions: Electrostatic interactions occur between charged groups on the ligand and receptor. These interactions can be attractive or repulsive, depending on the charge of the groups involved. Electrostatic interactions can contribute significantly to the binding affinity.

4. Pi-pi stacking: Pi-pi stacking occurs when the aromatic rings of the ligand and receptor are stacked on top of each other. This interaction is important for stabilizing the complex and can contribute significantly to the binding affinity.

5. Hydrophobic interactions: Hydrophobic interactions occur between nonpolar groups on the ligand and receptor. These interactions are important for stabilizing the complex and can contribute significantly to the binding affinity.

The strength and complementarity of these non-covalent interactions determine the overall binding affinity of the ligand for the receptor. Accurately predicting and optimizing these interactions is critical for the success of molecular docking studies (Maden et al., 2022).

In this study, the interaction of various compounds including Diospyrin, Isodiospyrin, Pyranocoumarin, Ellagic acid, Yohimbine, Flavone, Flavanone, Flavonol, Anthocyanidin, Ciprofloxacin, Nalidixic acid, and Allyl methyl disulfide

with the active site of DNA gyrase was analyzed. The specific amino acids involved in the interaction for each compound were determined and presented in Figure 4-14 to Figure 4-25. The active site of DNA gyrase was found to consist of particular amino acids, and the type and number of amino acids involved in the interaction varied among the compounds. For instance, Diospyrin interacted with seven amino acids, while Allyl methyl disulfide interacted with only one amino acid. Identification of the crucial amino acids that participate in the interaction provides valuable insights into the interaction between ligand and receptor, which can aid in the development and optimization of drugs

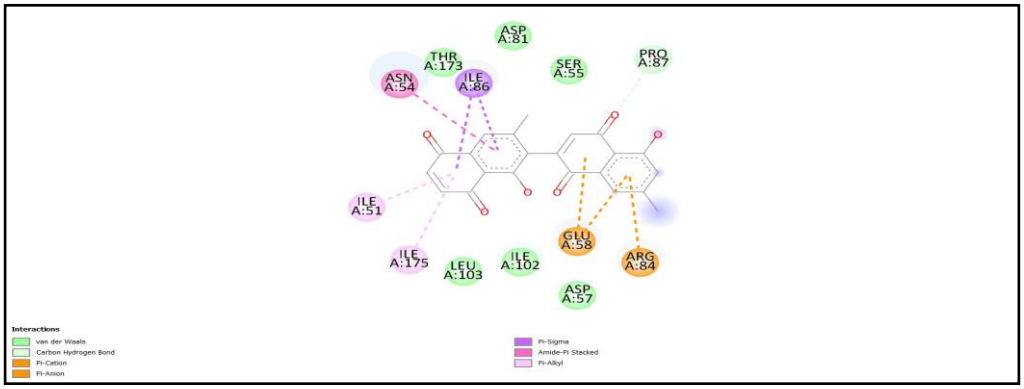


Figure (4-14): Diospyrin interaction with DNA gyrase of S. aureus

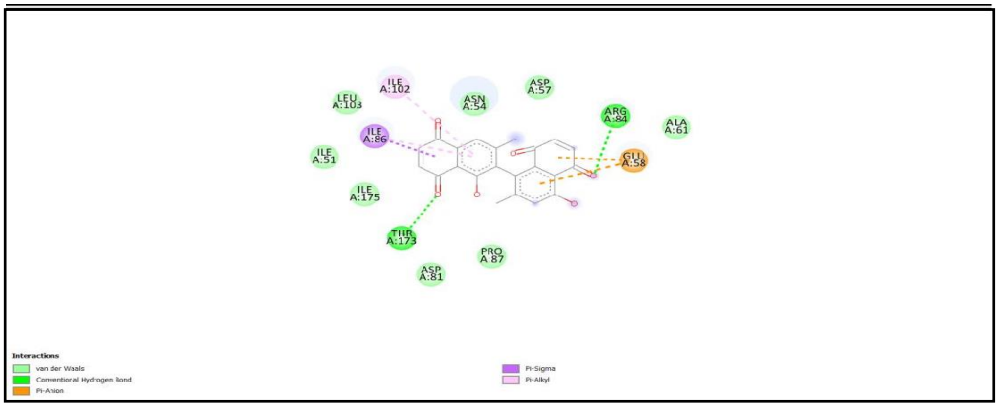


Figure (4-15): Isodiospyrin interaction with DNA gyrase of S. aureus

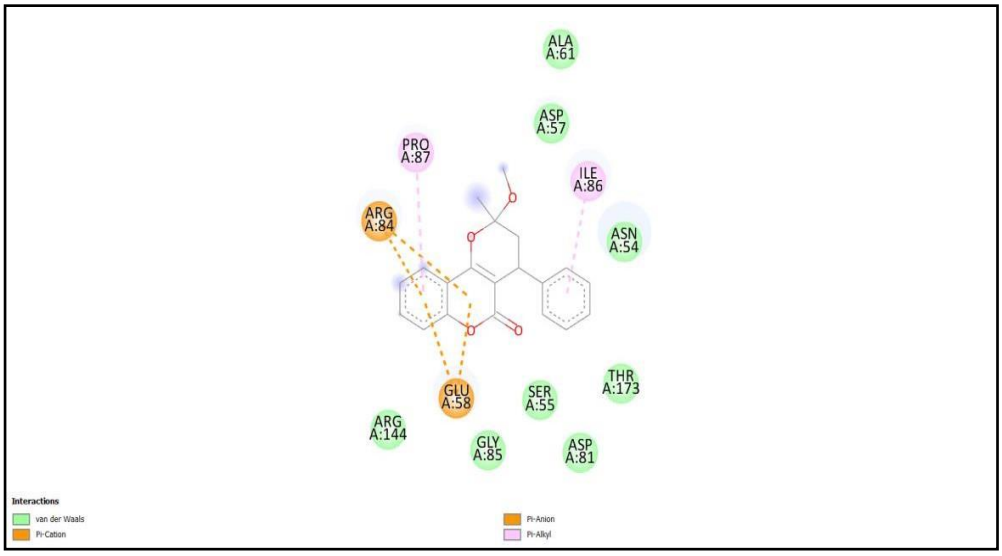


Figure (4-16): Pyranocoumarin interaction with DNA gyrase of S. aureus

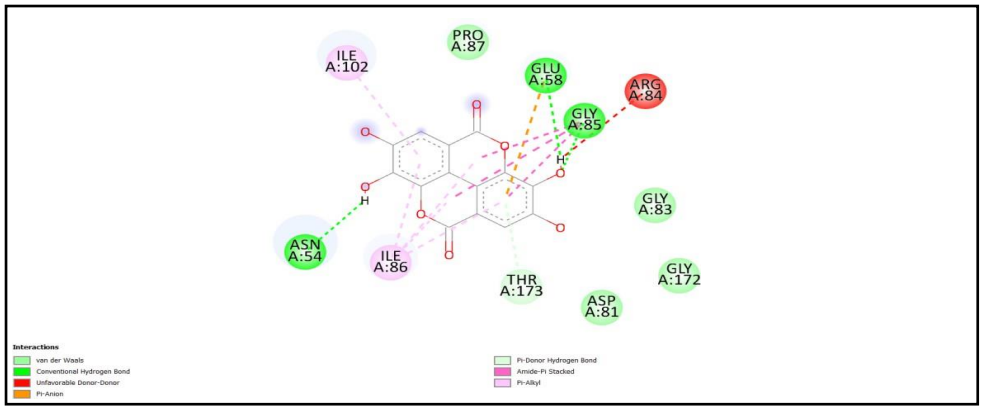


Figure (4-17): Ellagic acid interaction with DNA gyrase of S. aureus

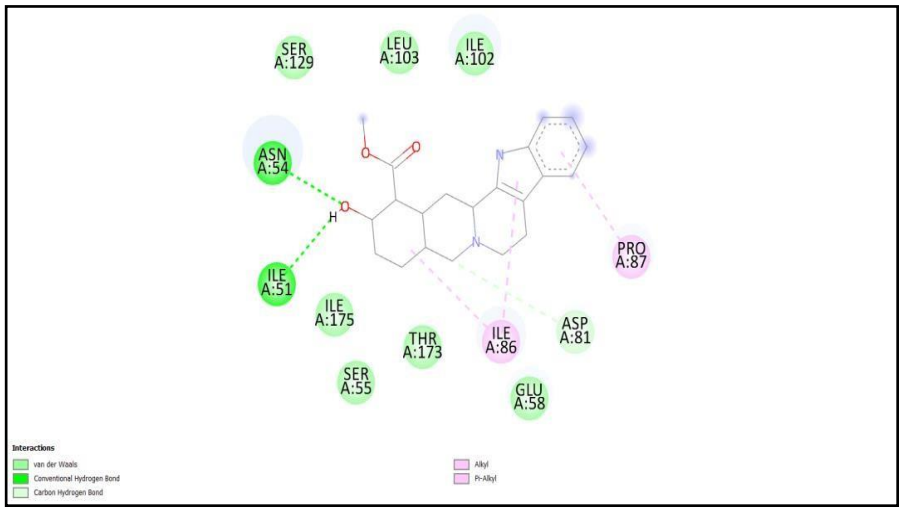


Figure (4-18): Yohimbine interaction with DNA gyrase of S. aureus

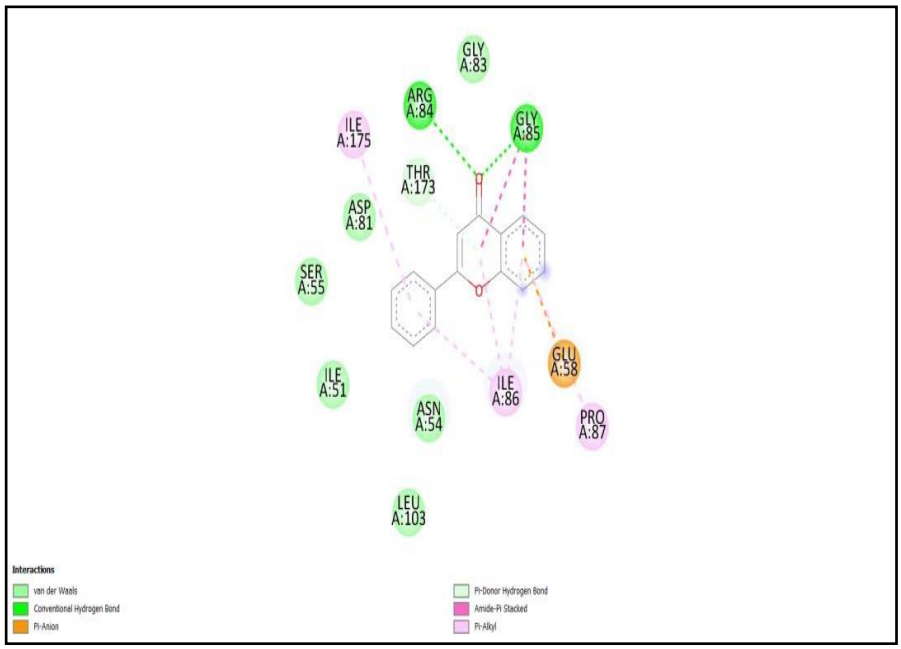


Figure (4-19): Flavone interaction with DNA gyrase of S. aureus

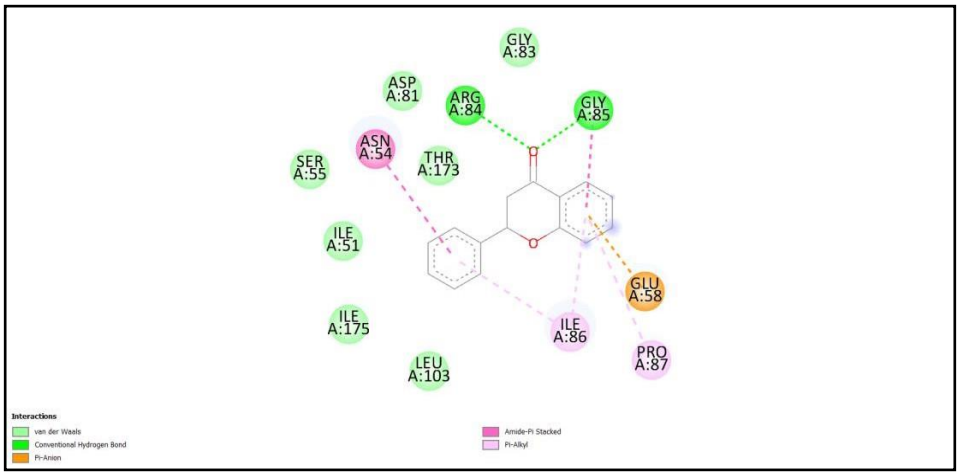


Figure (4-20): Flavanone interaction with DNA gyrase of S. aureus

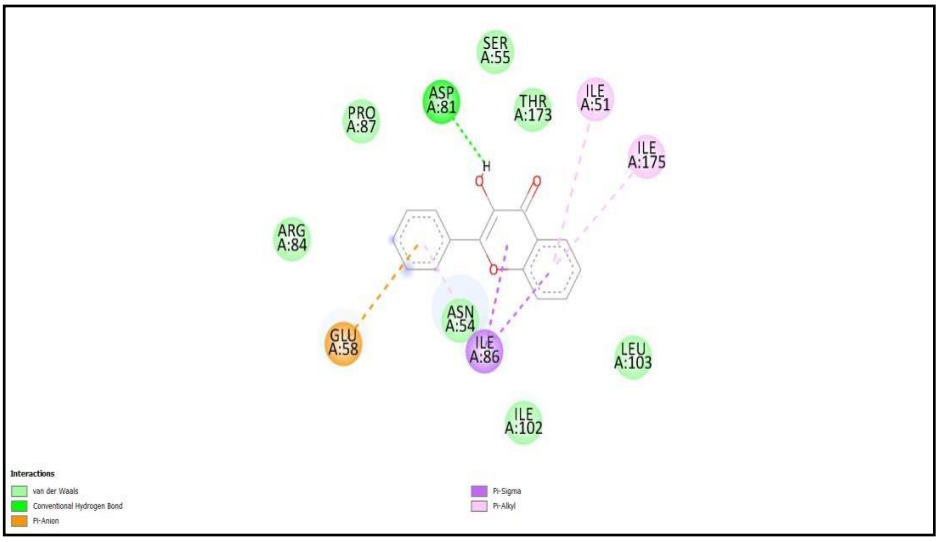


Figure (4-21): Flavonol interaction with DNA gyrase of S. aureus

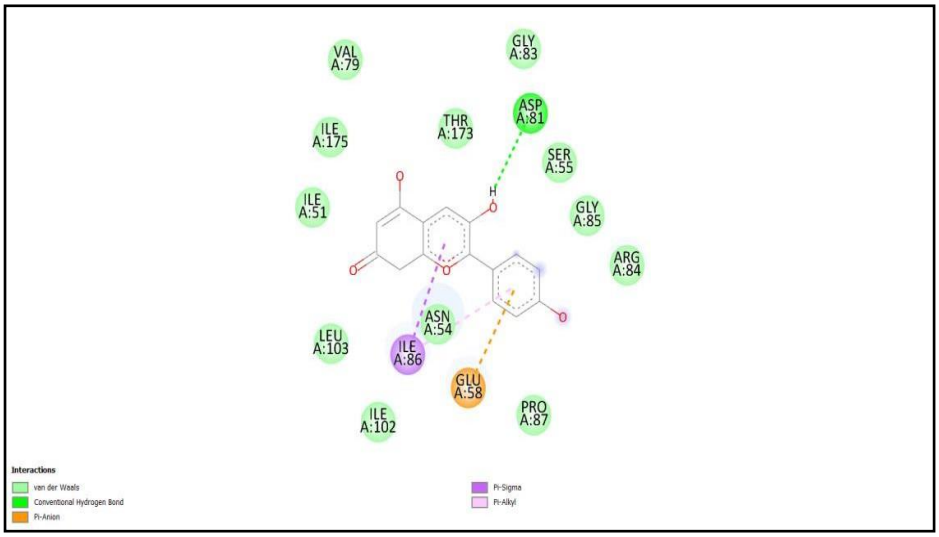


Figure (4-22): Anthocyanidin interaction with DNA gyrase of S. aureus

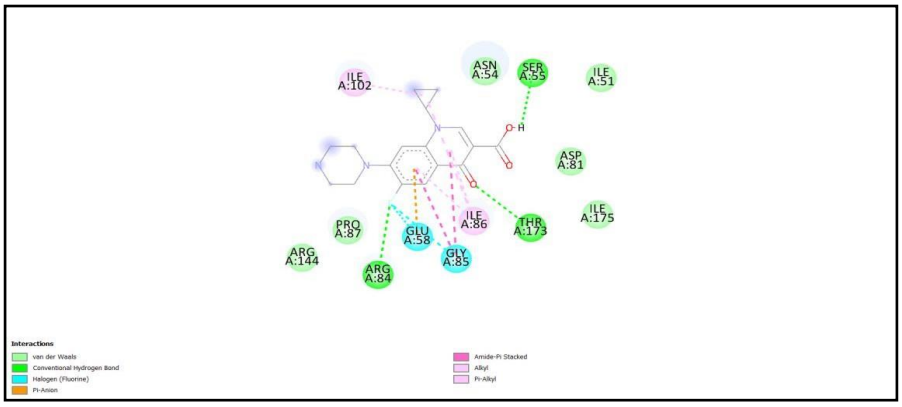


Figure (4-23): Ciprofloxacin interaction with DNA gyrase of S. aureus

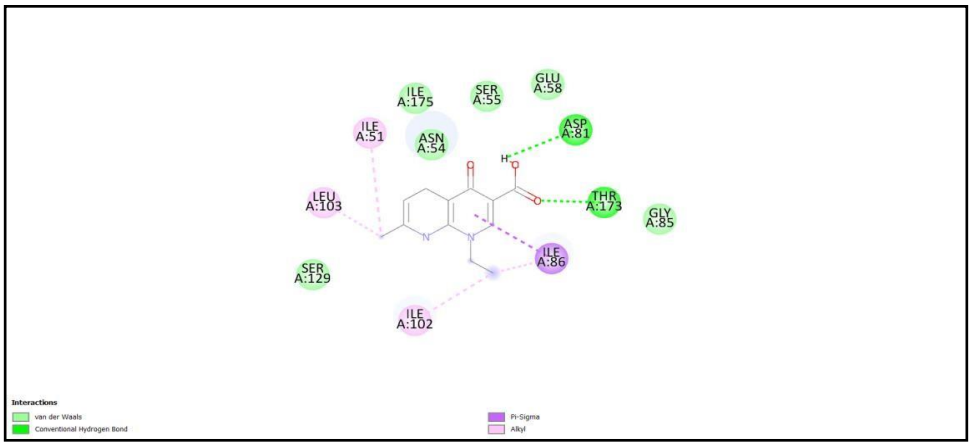


Figure (4-24): Nalidixic acid interaction with DNA gyrase of S. aureus

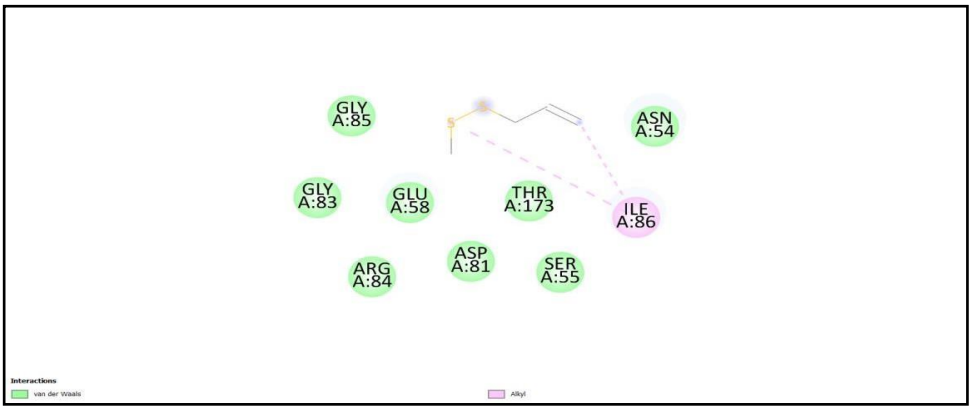


Figure (4-25): Allyl methyl disulfide interaction with DNA gyrase of S. aureus

The position of amino acids in the active site of the receptor can have a significant effect on the binding affinity of the ligand with the receptor. The active site of a receptor is a specific region where a ligand molecule binds and interacts with the receptor through non-covalent interactions. The active site is often composed of a pocket or cleft formed by the specific arrangement of amino acids in the receptor (Maden et al., 2022).

The amino acid residues in the active site can influence the binding affinity by affecting the strength and specificity of the non-covalent interactions with the ligand. For example, a mutation or substitution of an amino acid in the active site can change the shape, size, or electrostatic properties of the binding pocket, which can alter the binding affinity of the ligand (Maden et al., 2022).

In addition, the position and orientation of the amino acids in the active site can also affect the binding affinity by influencing the accessibility and proximity of the ligand to specific functional groups or binding residues in the receptor. Therefore, understanding the role of specific amino acids in the active site and their effect on ligand binding affinity is critical for rational drug design and optimization. Molecular docking and other computational methods can

help predict the optimal binding conformation of the ligand in the active site and identify key amino acid residues that contribute to ligand binding (Maden et al., 2022).

Conclusions

- Diospyrin appeared the highest binding infinity against DNA gyrase of E. coli and S. aureus in comparison with Ciprofloxacin and Nalidixic acid.
- Imidazoline and Allyl methyl disulfide showed the lowest binding infinity against DNA gyrase of E. coli and S. aureus, respectively in comparison with Ciprofloxacin, Nalidixic acid and the rest of phytochemicals compounds.
- All compounds showed variation in the number of amino acids which interacted with the active site of DNA gyrase for both bacteria.

Recommendations

- Use Diospyrin as antibacterial in-vitro and in-vivo study to confirm its activity on DNA gyrase.
- Study the molecular dynamic of the compounds that showed higher binding infinity against DNA gyrase to investigate and understand the behavior of biological systems for these compounds.
- Evaluation the activity of phytochemicals against another targets in pathogenic bacteria in comparison with traditional antibiotics.

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