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INHIBITORY ACTIVITY OF BIOACTIVE COMPOUNDS OF *Spirulina platensis* COMBINED WITH CINNAMALDEHYDE AGAINTS DNA GYRASE: SINGLE AND MULTI-LIGAND DOCKING

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ABSTRACT

Antibiotic resistance poses a serious challenge in treating bacterial infections, necessitating the development of new antibacterial alternatives. *Spirulina platensis* and cinnamon oil, rich in bioactive compounds, have demonstrated antibacterial potential in vitro, although their molecular mechanisms remain largely unexplored. This research aims to evaluate the interactions of several bioactive compounds derived from *Spirulina platensis* combined with cinnamaldehyde against DNA gyrase, a critical bacterial enzyme, using molecular docking approach. Single and multi-ligand approaches were employed to analyze binding affinity, inhibition constants (Ki), and molecular interactions, including hydrogen bonding and hydrophobic interactions. Docking validation demonstrated high accuracy with an RMSD value of 0.680 Å. Single ligands yielded binding affinities ranging from -6.1 to -7.7 kcal/mol. Multi-ligand combinations significantly enhanced antibacterial activity, with the six-ligand combination showing the most promising results, displaying the lowest binding energy (-19.02 kcal/mol) supported by six hydrogen bonds. Visualization confirmed ligand interactions with the same active site on DNA gyrase, involving critical residues such as ASN54, ASP81, ILE86, SER55, GLY85, GLY125, GLU58, THR173, ARG84, and PRO87. These findings reveal the synergistic effects of multi-ligand interactions that can improve complex stability and antibacterial activity. The study supports the development of natural compound combinations as an effective alternative in addressing antibiotic resistance.

Keywords: *Antibacterial, DNA gyrase, molecular docking, Spirulina platensis, Cinnamon oil*

INTRODUCTION

Bacterial infections pose a significant healthcare challenge due to increasing antibiotic resistance, which compromises treatment effectiveness and prolongs recovery times (Urban-Chmiel et al., 2022). This critical issue has prompted research into discovering new antibacterial compounds from natural sources, with *Spirulina platensis* and Cinnamon oil emerging as promising candidates.

Spirulina platensis, a blue-green microalga, contains bioactive compounds such as gallic acid, chlorogenic acid, quercetin, kaempferol, and coumarin (Mapoung et al., 2020). It has been reported that Spirulina extract demonstrates antibacterial activity against multidrug-resistant bacteria such as *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa*, with a Minimum Inhibitory Concentration (MIC) of ≥ 6.25 mg/mL (Wardoyo et al., 2024). Similarly, Cinnamon oil contains cinnamaldehyde, eugenol, and linalool, with an MIC of 50 µg/mL against *Staphylococcus aureus* (Gilani & Najafpour, 2022; Ellboudy et al., 2023).

While previous research has established the antibacterial properties of these natural sources, the molecular mechanisms remain incompletely understood. In silico approach, particularly molecular docking, have emerged as valuable tools in pharmaceutical research for predicting compound-protein interactions. Single-ligand docking evaluates individual compound potentials (Llop-Peiró et al., 2024), while multi-ligand docking offers more comprehensive approach since exploring potential synergistic effects (Behbahani et al., 2024).

The research focuses on DNA gyrase as a strategic inhibition target, chosen for its broad-spectrum potential. Present in all bacterial species, DNA gyrase inhibitors can potentially provide wide-ranging antibacterial efficacy. These inhibitors cause DNA strand breakage, leading to bacterial cell death, which makes them particularly effective (Dighe & Collet, 2020).

This study aims to address the gap in existing research by investigating the molecular interactions of bioactive compound derived from *Spirulina platensis* combined with cinnamaldehyde from Cinnamon oil using single and multi-ligand docking methods. By examining their molecular mechanisms, the research provide the molecular insight of such those interaction that, offering a promising approach to combating antibiotic-resistant bacterial infections.

METHODS

The PBP protein structure (PDB ID: 4URO) was downloaded from the Protein Data Bank in PDB format (Berman et al., 2002) and was prepared using AutoDock Tools 1.5.7 by removing water molecules, separating native ligands, and adding hydrogen atoms (Forli et al., 2016). Ligand structures were downloaded from PubChem in SDF format (Kim et al., 2016) and were converted to PDB using OpenBabel GUI 2.3.1. Single and multi-ligand molecular docking simulations were performed with AutoDock Vina 1.2.5 via

command prompt (Eberhardt et al., 2021; Trott & Olson, 2010). Method validation was conducted by recalculating the protein with its native ligand, with RMSD criteria less than 2 Å. Complex structure visualization was performed using BIOVIA Discovery Studio Visualizer.

RESULTS AND DISCUSSION

To understand molecular interactions in inhibiting bacteria, particularly targeting DNA gyrase, the molecular docking method was employed. In this study, molecular docking was conducted to compare the effects of single and multi-ligands. During the molecular docking process, validation of the active site coordinates was performed first. The active site coordinates were determined as x: -1.669, y: -0.035, and z: -12.515 in Angstrom (Å). The grid box size was x: 30, y: 38, and z: 24 in Angstrom (Å). These coordinates are also referred to as the grid box. Grid box validation was carried out to determine the Root Mean Square Deviation (RMSD) value, which is considered valid when RMSD is less than 2. A smaller RMSD indicates a more precise predicted ligand position within the enzyme's active site. The enzyme's active site is shown in Figure 1. In this study, an RMSD value of 0.680 Å was obtained.

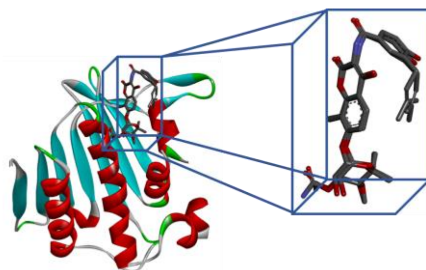

Figure 1. Active site position of enzyme for method validation

In molecular docking analysis, interactions can be observed through hydrogen bonding, hydrophobic interactions, binding affinity, and inhibition constant (Ki). Hydrogen bonding is a crucial intermolecular interaction in molecular docking, which can enhance binding affinity by reducing Gibbs free energy, thereby strengthening the protein-ligand bond (Madushanka *et al*., 2023). In addition to hydrogen bonding, hydrophobic interactions also contribute to the stability of the protein-ligand complex and are more dominant when the ligand has non-polar regions (Fatma *et al*., 2021). Complex stability can also be assessed by the bond length between protein and ligand (Shukla & Trupathi, 2020). Binding affinity from Gibbs free energy is a parameter indicating the spontaneous tendency of protein-ligand equilibrium binding to form a complex. The smaller the Gibbs free energy, the better the binding affinity, as complex formation requires low energy to react

exothermically and spontaneously. The inhibition constant (Ki) represents the ligand concentration needed to inhibit enzyme activity.

Table 1. Binding affinity and inhibition constant of ligand with DNA gyrase

Ligands	Binding affinity (kcal/mol)	Ki (μM)
Ciprofloxacin	-7.8	1.91
Gallic acid	-6.1	3.37×10^{1}
Kaempferol	-7.6	2.68
Chlorogenic acid	-7.1	6.24
Quercetin	-7.7	2.27
Coumarin	-6.2	2.85×10^{1}
cinnamaldehyde	-5.4	1.09×10^{2}
Cinnamaldehyde + Quercetin	-11.58	3.24×10^{-3}
Cinnamaldehyde + Chlorogenic acid + Kaempferol + Quercetin	-16.29	1.14×10^{-6}
Cinnamaldehyde + Gallic acid + Kaempferol + Chlorogenic acid + Quercetin + Coumarin	-19.02	1.13×10^{-8}

Table 2. amino acid residues of ligand with DNA gyrase

Ligands	H-Bond amino acids	Ȧ	Hydrophobic interaction	Ȧ
Ciprofloxacin	ASN54	2.635	ILE86	3.817
	ILE51	2.611	ASN54	4.394
	ASP81	3.384	SER55	4.104
Gallic acid	ARG84	2.969	ASN54	5.440
	GLY85	1.895	SER55	4.478
	GLU58	2.700	ILE86	4.529
	ASP81	2.584		
Kaempferol	GLY85	2.351	ASN54	4.917
	THR173	2.655	ILE86	4.178
	ASP81	2.890	PRO87	4.586
	SER55	3.974	ARG84	4.990
Chlorogenic acid	THR173	2.639	ARG84	5.068
	ASN54	3.216	PRO87	4.120
	SER128	2.510	GLY 125	2.403
	GLY85	2.630		
Quercetin	GLY85	2.566	ILE86	4.976
	GLY125	2.357	ASP57	3.688
	GLU58	2.524	GLU58	4.025
	ASP57	3.688		

Table 2. Continued

Ligands	H-Bond amino acids	Å	Hydrophobic interaction	Å
Coumarin	ARG84	2.691	ILE86	3.602
	GLY85	2.300	ASN54	5.259
			SER55	4.600
			GLY85	4.629
Cinnamaldehyde	No H-Bonds		GLU58	4.239
			ASN54	5.561
			SER55	5.699
			ILE86	4.516
Cinnamaldehyde + Quercetin	GLY85	2.015	ILE86,	4.976
	GLY125	1.897	ASP57	3.588
	GLU58	2.524	GLU58	4.025
	ASP57	2.765	GLY125	2.357
Cinnamaldehyde + Chlorogenic acid + Kaempferol + Quercetin	GLY58	2.360	ARG84	5.068
	GLY125	2.304	PRO87	4.120
	SER128	2.150		
	THR173	2.369		
	ASN54	2.030		
Cinnamaldehyde + Gallic acid + Kaempferol + Chlorogenic acid + Quercetin + Coumarin	GLY85	2.305	ARG84	5.021
	GLY125	2.026	PRO87	4.005
	SER128	2.150		
	THR173	2.304		
	ASN54	2.030		
	ARG84	2.176		

The research results are shown in Table 1 and Table 2. Interactions were compared between antibiotics, single ligands, and multi-ligands. Among the single ligands, ciprofloxacin demonstrated a strong binding affinity with a low inhibition constant, requiring a low concentration to inhibit the target enzyme, with contributions from three hydrogen bonds and three hydrophobic interactions. Ciprofloxacin has been known to have good antibacterial activity in vitro. To provide an alternative antibacterial option and reduce bacterial resistance, research was conducted on alternative compounds. Through molecular docking, among the five ligands from Spirulina platensis (gallic acid, kaempferol, chlorogenic acid, quercetin, and coumarin), quercetin, kaempferol, and chlorogenic acid showed the best affinity and inhibition constant, followed by kaempferol and chlorogenic acid. The excellent affinity of these three compounds was influenced by hydrogen bonding and hydrophobic interactions. Quercetin has four hydrogen bonds and three hydrophobic interactions. Kaempferol has four hydrogen bonds and four hydrophobic interactions. Chlorogenic acid has four hydrogen bonds and three hydrophobic interactions.

Quercetin, which demonstrated the highest binding affinity, underwent multi-ligand docking with cinnamaldehyde. Each ligand from Spirulina platensis was tested in multi-ligand docking with cinnamaldehyde from Cinnamon oil to determine potential activity enhancement when formulating both Spirulina platensis and Cinnamon oil. Table 1 shows that the multi-ligand combination of cinnamaldehyde and quercetin showed an increasingly negative binding affinity of -11.58 kcal/mol, with four hydrogen bonds and four hydrophobic interactions. In addition to quercetin, kaempferol and chlorogenic acid were also added to this combination to investigate whether ligand addition would affect inhibitory activity. This combination resulted in an even lower binding affinity of -16.29 kcal/mol, with the addition of five hydrogen bonds and two hydrophobic interactions. The study also analyzed the combination of all ligands except ciprofloxacin. The combination of all ligands showed the lowest binding affinity of -19.02, which could be influenced by the addition of six hydrogen bonds. Besides the number of hydrogen bonds, bond affinity is also influenced by the hydrogen bond distance (Table 2). The multi-ligand combination results in shorter hydrogen bond distances, making the complex more stable.

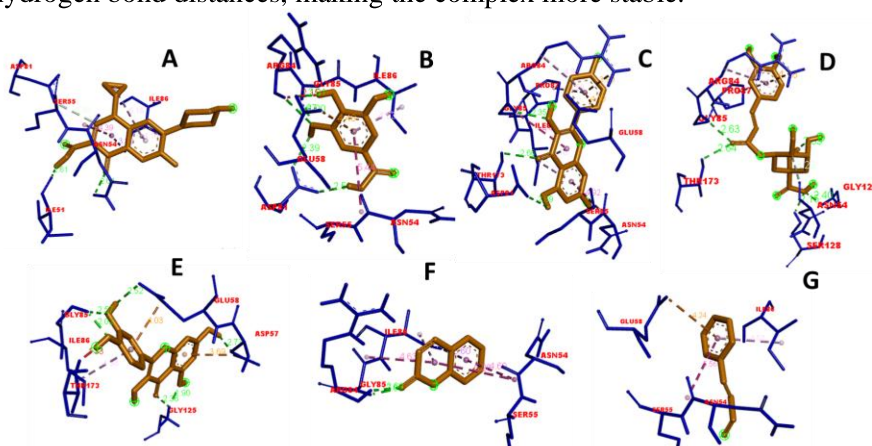

Figure 2. Visualization of DNA gyrase interaction with ciprofloxacin (A), gallic acid (B), kaempferol (C), chlorogenic acid (D), quercetin (E), coumarin (F), cinnamaldehyde (G)

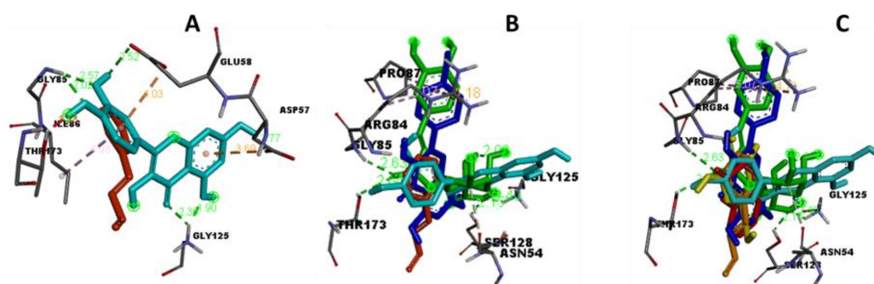

Figure 3. Visualization of DNA gyrase interaction with cinnamaldehyde + quercetin (A), cinnamaldehyde + chlorogenic acid + kaempferol + quercetin (B), cinnamaldehyde + gallic acid + kaempferol + chlorogenic acid + quercetin + coumarin (C)

Figures 2 and 3 illustrate the visualization of complex formation between DNA gyrase and test ligands. The visualization reveals that all ligands bind to the same active site. This is evidenced by the interaction of all ligands with several identical amino acids, including ASN54, ASP81, ILE86, SER55, GLY85, GLY125, GLU58, THR173, ARG84, and PRO87. In Table 1, some ligands exhibit the same number of hydrogen bonds and hydrophobic interactions but demonstrate different binding affinities. Consequently, these multi-ligand interactions exhibit the best binding affinity.

This study demonstrates the application of molecular docking to predict the inhibition activity of a compound in enzyme inhibition. The research results indicate that multi-ligand combinations represent the best inhibitors for DNA gyrase. This is evidenced by the best binding affinity and inhibition constants, along with supporting molecular interactions. The multi-ligand molecular docking analysis reveals the synergistic effect between components found in Spirulina platensis and Cinnamon oil in enhancing their antibacterial activity. Consequently, the combination of these two substances can be considered as an alternative to antibiotics.

CONCLUSION

This study highlights the importance of single-ligand and multi-ligand approaches in developing new antibacterial drugs to address antibiotic resistance. The research demonstrates that multi-ligand combinations, particularly from Spirulina platensis and Cinnamon oil components, have significant potential as antibiotic alternatives in inhibiting DNA gyrase enzyme. Molecular docking analysis revealed that multi-ligand combinations produce the best binding affinity with extremely low inhibition constants, supported by contributions from hydrogen bonding, hydrophobic interactions, and optimal bond distances. This combination shows a greater synergistic effect compared to single ligands, as evidenced by increased complex stability and enhanced antibacterial activity.

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