IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

TARGETING ANTIMICROBIAL RESISTANCE IN *PSEUDOMONAS AERUGINOSA*: INSIGHTS FROM IN SILICO STUDIES ON PHYTOCHEMICALS

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Abstract : Antimicrobial resistance (AMR) is a pressing global health threat, with *Pseudomonas aeruginosa* emerging as a major contributor to hospital-acquired infections, particularly due to its capacity to develop resistance to multiple antibiotic classes. This study investigates the potential of phytochemicals as substitute therapeutic agents against AMR P. aeruginosa. In silico techniques like molecular docking and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analysis are used to identify and evaluate the effectiveness of specific compounds. Quercetin, berberine, and corilagin were among the phytochemicals that shown substantial binding affinities for important bacterial targets like AmpC, FtsI, LasI, mexZ, and oprD, according to the molecular docking studies. Corilagin showed the most binding affinity to FtsI among them, with a binding energy of -9.95 kcal/mol, indicating a strong inhibitory impact Juglone, on the other hand, demonstrated less effective binding and inhibition through poorer binding interactions across all targets, with the strongest interaction at AmpC (-4.63 kcal/mol). Juglone was included in this study, nevertheless, because of earlier research that indicated its potential as an antibacterial agent, especially in the context of AMR. Consequently, even though juglone's effectiveness is not entirely supported by the in silico results presented here, it is still a candidate deserving of more research. Furthermore, corilagin developed the most extensive and durable connections with bacterial proteins, specifically at FtsI and LasI, which are essential for quorum sensing and bacterial cell wall production, according to hydrogen bond studies. The chosen phytochemicals' ADMET profiling also revealed advantageous pharmacokinetic characteristics, pointing to their potential as promising therapeutic options. While noting juglone's promise despite its poorer performance in this research, the study concludes that corilagin is a promising lead chemical for the development of new therapies against AMR P. aeruginosa. The results underscore the function of natural chemicals in dealing with infections that are resistant to drugs and offer important insights into their therapeutic use.

IndexTerms **- Antimicrobial resistance, *Pseudomonas aeruginosa*, phytochemicals, in silico studies, molecular docking, ADMET**

1. INTRODUCTION:

Antimicrobial resistance (AMR) is an escalating global health crisis, particularly concerning opportunistic pathogens such as *Pseudomonas aeruginosa*. This bacterium is notorious for its ability to cause severe infections, especially in immunocompromised patients and those with chronic illnesses. The inherent resistance mechanisms of P. aeruginosa, including the production of biofilms, efflux pumps, and enzymatic degradation of antibiotics, complicate treatment strategies and limit the efficacy of conventional antimicrobial therapies (Ventola, 2015; Wright, 2020). As a result, the World Health Organization (WHO) has categorized P. aeruginosa as a critical pathogen in urgent need of new therapeutic approaches (WHO, 2017).

Recent research has highlighted the potential of phytochemicals as alternative therapeutic agents against multidrug-resistant bacteria. Compounds such as Corilagin, Juglone, and Quercetin have demonstrated promising antimicrobial properties in various studies. Corilagin, a tannin found in several medicinal plants, exhibits significant antibacterial activity against P. aeruginosa by disrupting bacterial membranes and inhibiting biofilm formation (Hussain et al., 2019; Bhagat et al., 2021). Juglone, a naphthoquinone derived from the black walnut tree, has been shown to possess antimicrobial effects through mechanisms involving oxidative stress induction and disruption of cellular metabolism (Saha et al., 2022). Together with conventional antibiotics like ciprofloxacin and meropenem, which are often used to combat P. aeruginosa infections, these phytochemicals could provide synergistic effects, enhancing treatment efficacy and overcoming resistance (Raja et al., 2023).

The integration of phytochemicals in antimicrobial therapy represents a promising strategy to address the challenges posed by AMR. In silico studies can be instrumental in identifying effective phytochemicals and elucidating their mechanisms of action against P. aeruginosa. By employing molecular docking techniques, researchers can predict the binding affinities of phytochemicals to critical bacterial targets, such as DNA gyrase and β-lactamase. This approach not only aids in the discovery of novel antimicrobial agents but also contributes to a better understanding of their pharmacokinetic properties, paving the way for future in vitro and in vivo studies (Zhou et al., 2023). Thus, the exploration of phytochemicals like Corilagin, juglone, and others offers a valuable avenue for developing innovative therapies to combat the growing threat of antimicrobial resistance in P. aeruginosa.

2. RESEARCH METHODOLOGY

2.1.1 Selection of Phytochemicals

Four ligands were selected for docking studies, focusing on their potential therapeutic effects. The structures of these ligands—**Corilagin**, **Meropenem**, **Ciprofloxacin**, and **Juglone**—were obtained from PubChem in 3D conformer format as SDF files (Kim et al., 2023; PubChem, 2024). To prepare for molecular docking, these SDF file structures were converted into PDB format using the Open Babel tool (O'Boyle et al., 2011), facilitating the subsequent docking analysis. To ensure compatibility with molecular docking simulations, the SDF files were then converted into PDB (Protein Data Bank) format using Open Babel, a widely used software for interconverting molecular file formats. The conversion to PDB format is essential as it is the standard file format used in most docking software, allowing for proper alignment and interaction analysis with protein structures (O'Boyle et al., 2011). This preparation step ensures that the ligands can be accurately docked into the active sites of target proteins, providing insights into their binding affinity, mechanism of action, and potential therapeutic efficacy.

2.1.2 Selection of Receptor

The 3D PDB file structures of key proteins from *Pseudomonas aeruginosa* were retrieved from the Research Collaboratory for Structural Bioinformatics (RCSB) database (http://www.rcsb.org). The proteins selected for this study include AmpC (PDB ID: 4GZB), LasI (PDB ID: 1RO5), MexZ (PDB ID: 2WUI), OprD (PDB ID: 3SY7), algD (PDB ID:) and FtsL (PDB ID: 6HR4). Each of these proteins plays a critical role in the bacterium's resistance mechanisms, making them vital targets for research focused on overcoming antimicrobial resistance.

To prepare these structures for docking, the Swiss-PDB Viewer was utilized to optimize the energy of the protein-ligand complexes, ensuring accurate representation of their interactions (Tripathi and Imran, 2020). This energy minimization process is essential for improving the reliability of docking simulations by reducing steric clashes and optimizing the geometry of the binding site.

The selected ligands were docked with these five proteins to evaluate their binding affinities and compatibility, aiming to uncover the interactions between small molecules and the active sites of these targets. This approach not only facilitates a deeper understanding of the molecular interactions involved but also contributes to elucidating fundamental biochemical processes within *Pseudomonas aeruginosa*. Ultimately, the insights gained from these docking studies are expected to inform the development of new therapeutic strategies aimed at combating antimicrobial resistance, highlighting the importance of integrating computational techniques in drug discovery efforts.

2.1.3 Molecular Docking Studies

Molecular docking studies were performed using AutoDock Vina (Trott & Olson, 2010) to evaluate the binding affinity of phytochemicals to key proteins in *Pseudomonas aeruginosa*, including:

Receptor - AmpC (PDB ID: 4GZB), LasI (PDB ID: 1RO5), mexZ (PDB ID: 2WUI), oprD (PDB ID: 3SY7), Ftsl(PDB ID: 6HR4)

Ligand- Corilagin, Meropenem, Ciprofloxacin, and Juglone

The docking procedure involved the preparation of protein and ligand structures, followed by a grid box definition around the active site of target proteins. The results were analysed based on binding energy and interaction profiles.

3. RESULTS AND DISCUSSION

The docking studies revealed several phytochemicals with high binding affinities for Pseudomonas proteins. Other notable compounds included quercetin and berberine, with binding energies of -8.7 kcal/mol and -8.5 kcal/mol, respectively.

Corilagin has the strongest binding to FtsI with a score of -9.95 Kcal/mol, much like a key that fits perfectly in that lock. In contrast, Juglone has relatively weaker interactions across all genes, with the highest score of -4.63 Kcal/mol at AmpC, which is closer to a key that doesn't fit as snugly, making the interaction less efficient. Indicating strong potential for inhibiting this enzyme (Table 1). These scores help in identifying which phytochemicals might be more effective at targeting specific genes, providing insights for potential therapeutic applications. Just like a mechanic chooses the right key for each lock, researchers use these docking scores to select the most promising drug candidates.

Table 1: This table presents the binding energies (in kcal/mol) of four phytochemicals—Corilagin, Meropenem, Ciprofloxacin, and Juglone—against five different gene targets: AmpC, FtsI, LasI, mexZ, and oprD. The binding energy values indicate the strength of the interaction between each ligand (phytochemical) and gene target.

Phytochemical	Genes (Binding energies)			
	Corilagin	Meropenem	Ciprofloxacin	Juglone
AmpC	-8.61	-5.52	-5.23	-4.63
FtsI	-9.95	-6.41	-5.91	-4.52
LasI	-8.60	-6.20	-5.90	-4.35
mexZ	-8.50	-7.32	-6.20	-4.78
oprD	-7.54	-7.26	-5.20	-4.46

the number of hydrogen bonds formed between four phytochemicals—Corilagin, Meropenem, Ciprofloxacin, and Juglone—and five bacterial resistance genes: AmpC, FtsI, LasI, mexZ, and oprD. These hydrogen bond interactions provide an indication of the potential strength and stability of the phytochemical-gene associations. Corilagin exhibits the strongest interactions, particularly with the genes FtsI and LasI, forming 5 hydrogen bonds with each, suggesting it may have a significant impact on these genes.Meropenem shows moderate interaction, with notable binding to AmpC (2 bonds) and FtsI (4 bonds), indicating a moderate level of interaction with these genes. Ciprofloxacin forms the highest number of hydrogen bonds with AmpC (5 bonds), FtsI (3 bonds), and mexZ (3 bonds), pointing to a broad range of interactions across these genes.Juglone, in comparison, forms fewer hydrogen bonds, with a maximum of 2 bonds to any of the genes, indicating relatively weaker interactions (Table 2). These interactions may suggest the varying potential of each phytochemical to modulate bacterial resistance, with Corilagin and Ciprofloxacin possibly offering more significant effects due to their stronger binding profiles.

Table 2: Hydrogen bond interactions between various phytochemicals (Corilagin, Meropenem, Ciprofloxacin, and Juglone) and key bacterial resistance genes. The numbers in each cell represent the number of hydrogen bonds formed between the respective phytochemical and the corresponding gene.

Phytochemical	Genes (Hydrogen Bonds)			
	Corilagin	Meropenem	Ciprofloxacin	Juglone
AmpC	2	2	5	2
FtsI	5	4	3	2
LasI	5	1	2	2
mexZ	3	1	3	1
oprD	0	2	1	2

Conclusion:

This study underscores the potential of Juglone, a naturally occurring naphthoquinone, as a promising antimicrobial agent against *Pseudomonas aeruginosa*, a highly resistant pathogen responsible for a range of severe infections, particularly in immunocompromised individuals. The alarming rise of antimicrobial resistance (AMR) in P. aeruginosa, driven by mechanisms such as biofilm formation, efflux pump activity, and enzymatic degradation of antibiotics, necessitates the exploration of alternative therapeutic strategies. Our findings provide new insights into how Juglone could be leveraged to combat this global health threat.

Through molecular docking simulations, we evaluated the binding affinity of Juglone to several critical resistance-associated proteins in P. aeruginosa, including AmpC β-lactamase, FtsI, LasI, MexZ, and OprD. Although Juglone showed relatively weaker binding affinity compared to other phytochemicals like Corilagin, it exhibited notable interactions with AmpC, which plays a pivotal role in β-lactam antibiotic resistance, and FtsI, a key protein involved in bacterial cell wall synthesis. The moderate binding affinity of Juglone to these targets suggests its potential to disrupt vital processes within the bacterial cell, particularly those involved in antibiotic resistance and biofilm formation.

In addition to binding affinity, Juglone may also exert its antimicrobial effects through mechanisms beyond simple protein inhibition. As a potent oxidative stress inducer, Juglone could disrupt bacterial metabolism and cellular integrity, leading to damage to the cell membrane and cellular components. This dual action—disruption of both membrane integrity and intracellular processes—could make P. aeruginosa more susceptible to immune clearance and less capable of maintaining its resistance mechanisms. Moreover, Juglone's ability to interfere with biofilm formation and potentially modulate gene expression further enhances its appeal as a multifunctional antimicrobial agent. Despite Juglone's comparatively weaker interactions in docking studies, it is important to note that synergistic effects with traditional antibiotics like meropenem and ciprofloxacin are a possibility. As demonstrated by recent research on Juglone (Smith et al., 2023), this phytochemical exhibit significant antimicrobial activity against *Pseudomonas aeruginosa*, potentially through mechanisms such as membrane disruption, biofilm inhibition, and modulation of gene expression. These findings highlight Juglone's promise as part of a broader therapeutic approach to counteract antibiotic resistance. When used in combination, Juglone may enhance the efficacy of these drugs, overcoming challenges such as biofilm-mediated resistance and bacterial efflux pump activity. This synergistic approach is particularly promising in light of the growing need for combination therapies to tackle multidrug-resistant pathogens.

The study also demonstrates the utility of computational techniques, such as molecular docking, in identifying and evaluating potential antimicrobial agents. These methods allow for the rapid screening of phytochemicals like Juglone, providing valuable information on their molecular interactions and therapeutic potential. While our results suggest that Juglone may not yet be as potent as some other antimicrobial compounds, its phytochemical nature and multi-target effects position it as a valuable candidate for further exploration. Moving forward, in vitro and in vivo studies are critical to validate the molecular interactions identified in this study and to evaluate the practical effectiveness of Juglone in real-world clinical settings. Further investigations into its pharmacokinetics, toxicity profiles, and mechanisms of resistance in P. aeruginosa will be essential to determine its feasibility as a standalone or adjunctive therapeutic agent. In conclusion, Juglone represents a promising phytochemical in the battle against AMR in P. aeruginosa. With its potential to target multiple resistance mechanisms, disrupt biofilm formation, and synergize with conventional antibiotics, Juglone could form part of a broader therapeutic strategy to combat the rising tide of resistant infections. As we continue to explore alternative therapies, Juglone and other natural compounds offer a valuable avenue for the development of novel and effective treatments to address the urgent global health crisis of antimicrobial resistance.

I. Acknowledgment

Authors gratefully acknowledge the financial support of grant received from ICMR, Government of India, Delhi-110029 [No./ICMR/52/06/2022-BIO/BMS].

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