Educational Administration: Theory and Practice
2023, 29(4) 3139-3147
ISSN: 2148-2403
https://kuey.net/

Research Article

Targeting Pathogenic Bacterial Proteins: An In-silico Study of Oxalic Acid from *Curvularia pseudorobusta* isolated from *Distimake dissectus* (Jacq.) A.R. Simões & Staples.

MM Kalpashree [1*], K Krishna[2]

[1*]Research Scholar, Yuvaraja's College (Autonomous), University of Mysore, Mysuru-570022
[2]Professor. P.G. Department of Botany, Yuvaraja's College (Autonomous), University of Mysore, Mysuru-570022

***Corresponding Author**: Kalpashree M M
*Research Scholar, Yuvaraja's College (Autonomous), University of Mysore, Mysuru-570022, e-mail: kalpashreemmk@gmail.com

Citation: Kalpashree *et al.*, (2023) Targeting Pathogenic Bacterial Proteins: An In-Silico Study of Oxalic Acid from *Curvularia pseudorobusta* isolated From *Distimake dissectus*, *Educational Administration: Theory and Practice*, *29(4)* 3139-3147 Doi: 10.53555/kuey.v29i4.7817

ARTICLE INFO

ABSTRACT

This study investigates the binding interactions of oxalic acid from *Curvularia pseudorobusta* isolated from *Distimake dissectus* (Jacq.) A.R. Simões & Staples., against targeted bacterial proteins and compares these interactions to those of ampicillin which is used as a positive control. We have analysed protein structures from *Lactobacillus*, *E. coli*, *Pseudomonas*, and *Bacillus* species, viz., 4MKS, 5J9G, 7QLE, 1RX7, 1TLT, 1QJ8, 5OE3, 1EZM, 1IUV, 1NPC, 1AH7, and 1B90.

Oxalic acid exhibited variable binding affinities across different proteins. In *Lactobacillus*, affinities ranged from +2.59 to -4.54 kcal/mol, with dissociation constants from 9.20 mM to 468.58 µM. In *E. coli*, affinities ranged from -1.19 to -4.76 kcal/mol, with constants between 134.36 mM and 325.36 µM. For *Pseudomonas*, affinities varied from -1.13 to -7.67 kcal/mol, with constants ranging from 148.88 mM to 2.38 µM. In *Bacillus*, affinities ranged from -0.27 to -7.67 kcal/mol, with constants from 9.50 mM to 629.86 mM. Ampicillin showed consistently high binding affinities across all proteins, with values from -1.19 to -9.65 kcal/mol and dissociation constants ranging from 1.68 µM to 84.89 nM. The variability in oxalic acid's binding affinities highlights its potential as a selective therapeutic agent. These findings provide insights into oxalic acid's molecular interactions and its comparative efficacy to ampicillin, offering guidance for future drug development and microbial treatment strategies.

Keywords: *Curvularia pseudorobusta, Distimake dissectus,* In-silico, molecular docking, oxalic acid, bacterial proteins.

1. Introduction:

Plants generate a wide array of secondary metabolites (SMs) through various physiological and metabolic processes. These compounds have long been utilized as foundational resources for boosting the immune system and treating a variety of human diseases. Today, over 75% of the global population, particularly in developing countries, depends on plant metabolites or natural products for their primary healthcare needs (Chin *et al.*, 2006). And one of the key achievements of the 20th century was the discovery and advancement of antibiotics. However, this progress was accompanied by the emergence of drug-resistant strains. A growing number of resistant pathogens now cause severe bacterial infections that are challenging to treat. The primary contributors to antibiotic resistance include the misuse of antibiotics, incorrect dosages, and the overuse of antibiotics in livestock for disease prevention or growth promotion. Once established, these resistant bacterial strains can persist in the environment and potentially spread to humans through contact or consumption of animal products (Cui *et al.*, 2012). The diverse range of functional groups of secondary metabolites offer

opportunities to search or target the molecular sites of pathogens, which is an essential condition for drug discovery. Microorganisms are a crucial and reliable source of bioactive compounds. Among them, actinomycetes and fungi have proven to be particularly effective for screening and identifying pharmaceutically valuable compounds (Turgis *et al*., 2012).

Conventional screening method for searching a potential bioactive compound is tedious, time consuming, and cost intensive. Hence, a computational molecular docking method can be adopted to overlap the disadvantage of traditional methods (George *et al*., 2019). Molecular docking significantly reduces the time needed to analyze and identify the most promising bioactive compounds from a large pool. This technique has garnered considerable attention in research, particularly during natural product screening and drug development phases. It has been effectively utilized in numerous drug discovery programs to predict potential binding interactions between a ligand (such as a compound or drug candidate) and a specific receptor (e.g., proteins or nucleic acids involved in biological processes) (Hong *et al*., 2014).

Bioactive compounds exert their antibacterial effects through a multifaceted process, which begins with the physical interaction of the compound with its specific target and leads to changes at the biochemical, molecular, and ultrastructural levels. In silico methods can effectively predict the most likely binding site of a compound on a bacterium. For a successful in silico study, it is crucial to select appropriate targets relevant to the condition being studied and to choose the most suitable model from the Protein Data Bank (Hong *et al*., 2014).

This study aimed to assess the antibacterial activity of oxalic acid, a metabolite of *C. pseudorobusta*, against *Lactobacillus* spp., *E. coli*, *Pseudomonas*, and *Bacillus*. The evaluation focused on observing both morphological and intracellular changes induced by the metabolite. Furthermore, molecular docking studies were conducted with identified compound against twelve different antibacterial targets.

2 Materials and Methods:

2.1 Proteins for Molecular Docking:

The X-ray crystal structures of the target proteins utilized in this study were sourced from the Protein Data Bank (PDB) (http://www.rcsb.org). For the molecular docking studies, we selected three proteins from each of the four bacterial species listed in Table 1.

Table1 Showing list of targeted proteins with their PDB ID

Sl no.	Name of the Bacteria	PDB ID of selected proteins		
1	***Lactobacillus* sps**	4MKS	5J9G	7QLE
2	***E. coli***	1RX7	1TLT	1QJ8
3	***Pseudomonas***	5OE3	1EZM	1IUV
4	***Bacillus***	1NPC	1AH7	1B90

2.2 Target Protein Structure Preparation

2.2.1 Receptor Structure Acquisition: Protein structures were retrieved from the Protein Data Bank (PDB) using their respective PDB IDs. These structures were downloaded in PDB format to ensure compatibility with molecular docking software.

2.2.2 Pre-Docking Preparation: Prior to docking simulations, the receptor proteins were prepared using the following steps:

2.2.3 Removal of Unnecessary Components: Water molecules, heteroatoms, and any co-crystallized ligands not relevant to the study were removed.

2.2.4 Addition of Hydrogen Atoms: Missing hydrogen atoms were added to ensure accurate molecular interactions.

2.2.5 Structure Optimization: The protein structures were optimized to enhance docking accuracy. This step involved minimizing steric clashes and adjusting atom positions to reflect the most stable conformation. The refined receptor structures were then used for evaluating binding interactions with the selected ligands.

2.3 Ligand Structure Preparation

2.3.1 Ligand Sourcing and Structure Retrieval: Ligands, including oxalic acid and ampicillin, were sourced from PubChem. Their canonical SMILES strings were used to obtain 3D structures. For each ligand, the 3D structures were downloaded in PDB format from the Corina Demo software (https://demos.mn-am.com/corina.html).

2.4 Conversion and Preparation:

The PDB format structures of the ligands were converted to PDBQT format, which incorporates essential atom types and charges required for accurate molecular docking simulations. This conversion process ensured that the ligands were properly formatted and equipped with the necessary information for effective and reliable docking studies.

Docking and Visualization Tools

2.4.1 AutoDock 1.5.7:

Docking simulations were conducted using AutoDock 1.5.7, a widely utilized molecular docking software that enabled the evaluation of binding interactions between ligands and receptor proteins. The software offered comprehensive tools for analyzing docking poses, interaction energies, and binding sites. Detailed examination of ligand conformations and affinities within the receptor binding sites was performed through AutoDock's advanced visualization capabilities, ensuring an in-depth understanding of the ligand-receptor interactions**.** Free binding energy (kcal/mol) was calculated and only the best-scored pose was obtained for each compound. The docked pose with the highest docking score has been recognized as the most probable binding conformation of the ligand within the binding site.

2.4.2 Discovery Studio Biovia v24.1.0.23298:

The docking results were further analyzed using Discovery Studio Biovia v24.1.0.23298, which provided comprehensive three-dimensional visualization of ligand-receptor complexes. This software facilitated a detailed examination by offering tools to assess binding poses, interactions between ligands and receptor sites, and potential binding sites. Through its advanced features, Discovery Studio enabled a thorough evaluation of docking conformations, interaction energies, and binding affinities, enhancing the overall analysis of ligand-receptor interactions.

3.0 Result and Discussion:

The in-silico analysis of oxalic acid from the endophyte *Curvularia pseudorobusta* demonstrates varied binding affinities and inhibition constants when interacting with different pathogenic bacterial target proteins compared to ampicillin. The results are summarized in the following observations:

1. *Lactobacillus* Proteins:

Protein 4MKS: Oxalic acid shows a positive binding energy (+2.59 kcal/mol), indicating a weak binding affinity and poor inhibitory potential compared to ampicillin, which has a significantly higher binding energy (+292 kcal/mol) (Fig 1).

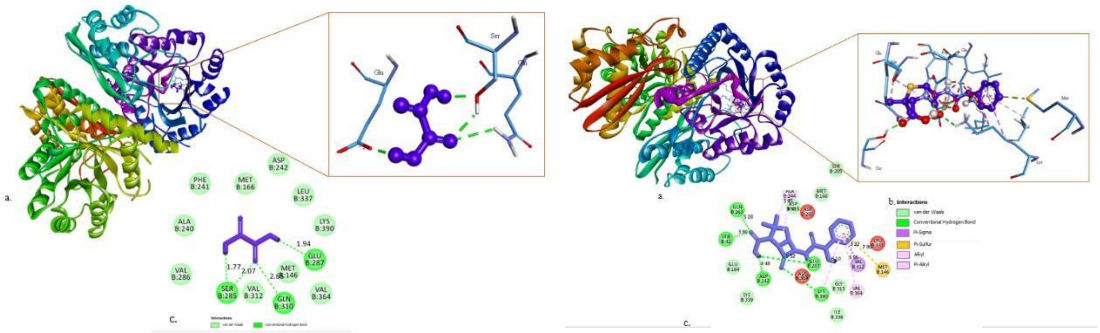

Fig 1. Interaction of 4MKS with Oxalic Acid and Ampicillin

a. 3D Structure of Protein with Bound Ligand b. Interaction of Ligand with Amino Acids c. 2D Interaction Diagram with Distances

Protein 5J9G: Oxalic acid exhibits a binding energy of -4.54 kcal/mol and an estimated inhibition constant of 468.58 µM, suggesting moderate binding affinity. In contrast, ampicillin shows a stronger interaction with a binding energy of -7.44 kcal/mol and a much lower inhibition constant of 3.54 µM (Fig 2).

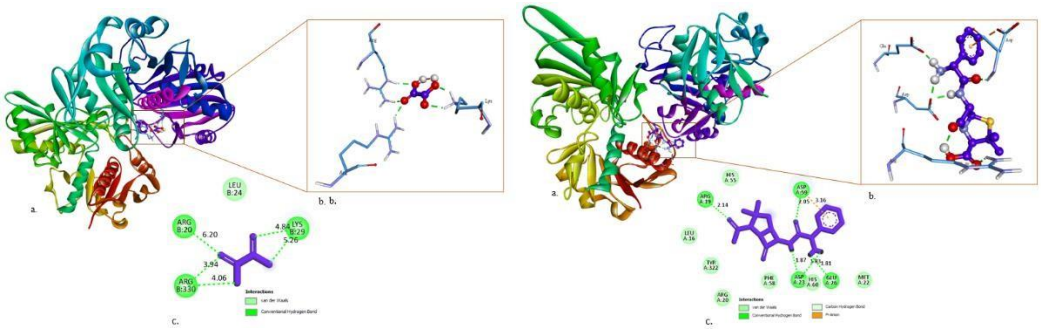

Fig 2. Interaction of 5J9G with Oxalic Acid and Ampicillin

a. 3D Structure of Protein with Bound Ligand b. Interaction of Ligand with Amino Acids c. 2D Interaction Diagram with Distances

Protein 7QLE: Oxalic acid's binding energy is -2.78 kcal/mol with a high inhibition constant of 9.20 mM, indicating weak inhibition. Ampicillin, however, has a stronger binding affinity (-6.68 kcal/mol) and a much lower inhibition constant of 12.78 µM (Fig 3).

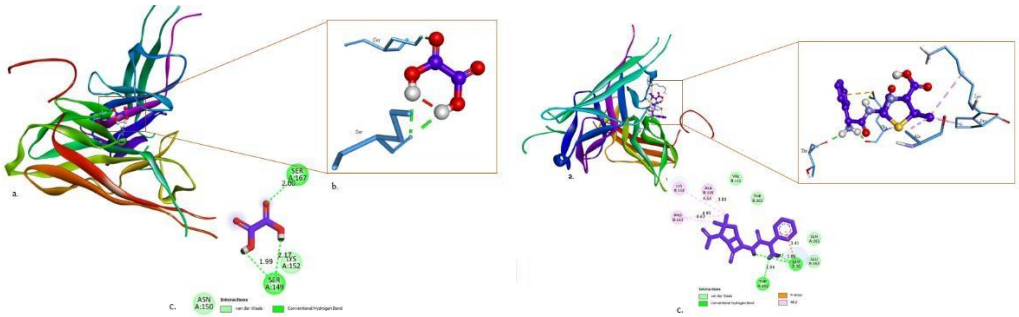

Fig 3. Interaction of 7QLE with Oxalic Acid and Ampicillin

a. 3D Structure of Protein with Bound Ligand b. Interaction of Ligand with Amino Acids c. 2D Interaction Diagram with Distances

2. E. coli Proteins:

Protein 1RX7: Oxalic acid has a binding energy of -4.76 kcal/mol and an inhibition constant of 325.36 µM, showing moderate binding affinity. Ampicillin outperforms with a binding energy of -7.88 kcal/mol and a significantly lower inhibition constant of 1.68 µM (Fig 4).

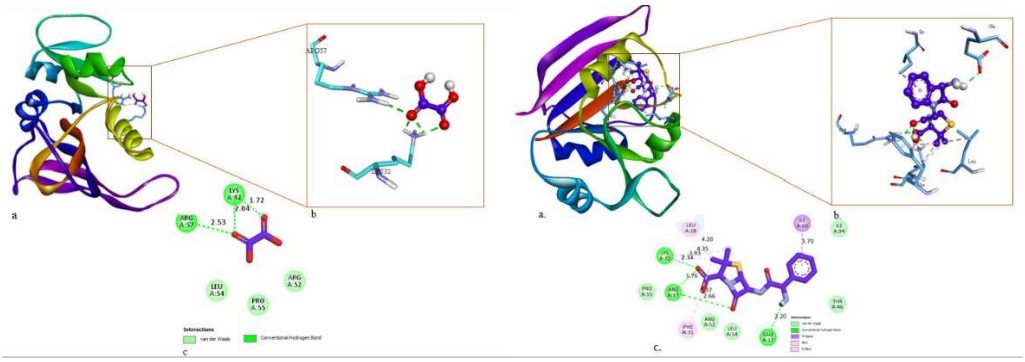

Fig 4. Interaction of 1RX7 with Oxalic Acid and Ampicillin

a. 3D Structure of Protein with Bound Ligand b. Interaction of Ligand with Amino Acids c. 2D Interaction Diagram with Distances

Protein 1TLT: Both oxalic acid and ampicillin have similar binding energies (-1.19 kcal/mol), but their inhibition constants are considerably high, indicating weak binding and inhibition (Fig 5).

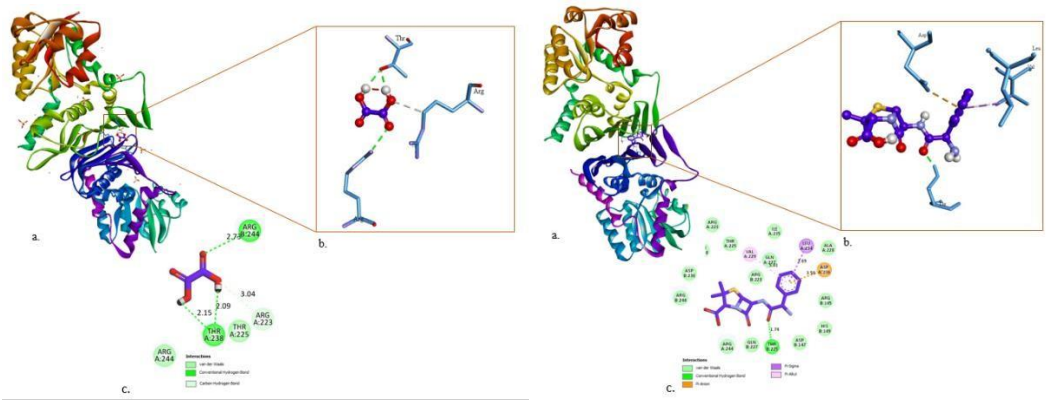

Fig 5. Interaction of 1TLT with Oxalic Acid and Ampicillin

a. 3D Structure of Protein with Bound Ligand b. Interaction of Ligand with Amino Acids c. 2D Interaction Diagram with Distances

Protein 1QJ8: Oxalic acid's binding energy is -2.39 kcal/mol with a high inhibition constant of 017.82 mM, whereas ampicillin shows a stronger binding with a lower inhibition constant of 700.05 µM (Fig 6).

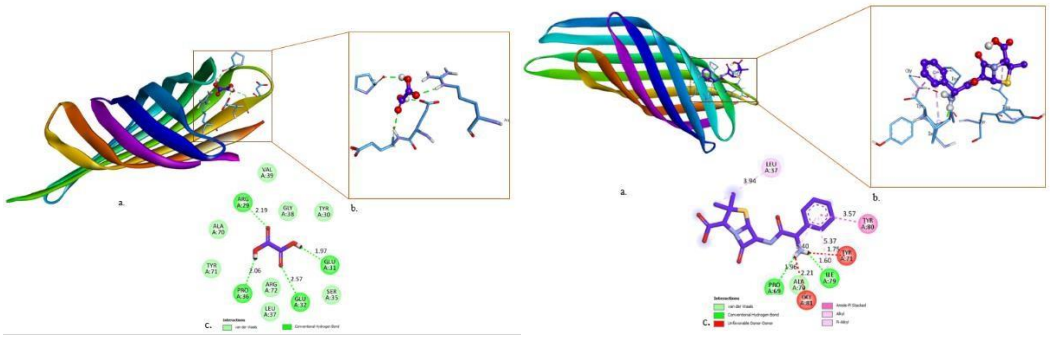

Fig 6. Interaction of 1QJ8 with Oxalic Acid and Ampicillin

a. 3D Structure of Protein with Bound Ligand b. Interaction of Ligand with Amino Acids c. 2D Interaction Diagram with Distances

3. *Pseudomonas* Proteins:

Protein 5OE3: Oxalic acid exhibits a binding energy of -5.04 kcal/mol and an inhibition constant of 202.14 µM, indicating moderate affinity. Ampicillin has a stronger binding affinity (-7.78 kcal/mol) and a lower inhibition constant of 1.99 µM (Fig 7).

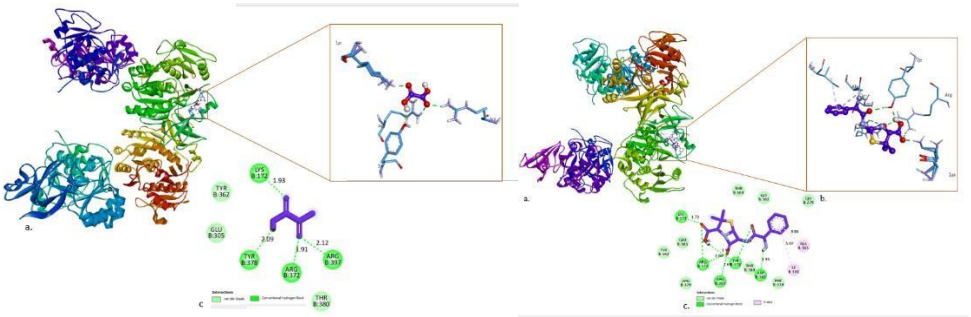

Fig 7. Interaction of 5OE3 with Oxalic Acid and Ampicillin

a. 3D Structure of Protein with Bound Ligand b. Interaction of Ligand with Amino Acids c. 2D Interaction Diagram with Distances

Protein 1EZM: Oxalic acid shows a weak binding energy of -1.13 kcal/mol and a high inhibition constant of 148.88 mM. Ampicillin's data is inconclusive due to a highly positive binding energy (+7.39 kcal/mol) (Fig 8).

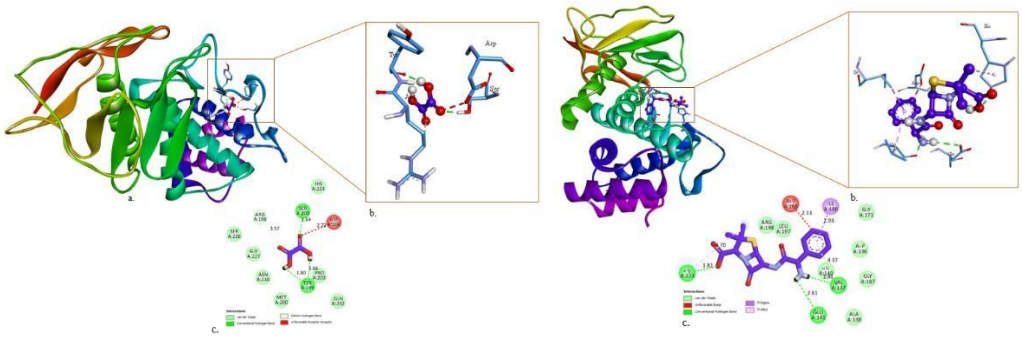

Fig 8. Interaction of 1EZM with Oxalic Acid and Ampicillin

a. 3D Structure of Protein with Bound Ligand b. Interaction of Ligand with Amino Acids c. 2D Interaction Diagram with Distances

Protein 1IUV: Oxalic acid has a very low binding energy of -7.67 kcal/mol with an excellent inhibition constant of 002.38 µM, suggesting a strong inhibitory effect comparable to ampicillin's binding energy of -7.67 kcal/mol and inhibition constant of 2.38 µM (Fig 9).

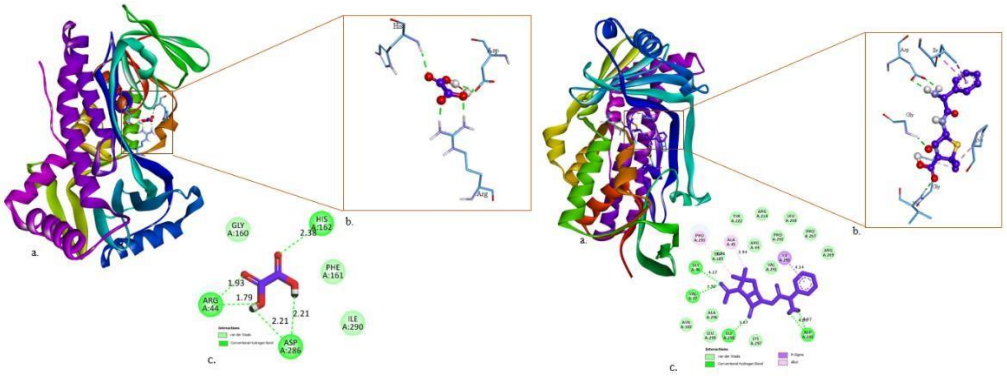

Fig 9. Interaction of 1IUV with Oxalic Acid and Ampicillin

a. 3D Structure of Protein with Bound Ligand b. Interaction of Ligand with Amino Acids c. 2D Interaction Diagram with Distances

4. *Bacillus* Proteins:

Protein 1NPC: Oxalic acid has a low binding energy of -0.71 kcal/mol and a high inhibition constant of 303.24 mM, showing weak inhibition. Ampicillin has a significantly stronger interaction with a binding energy of -6.89 kcal/mol and an inhibition constant of 8.91 µM (Fig 10).

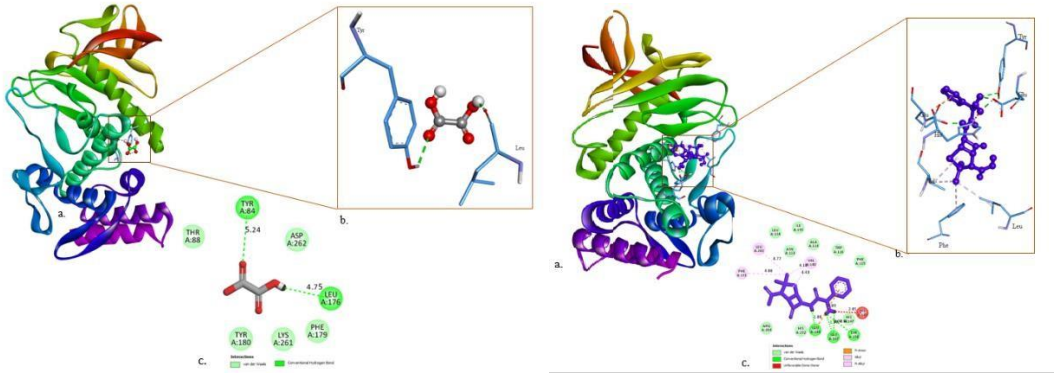

Fig 10. Interaction of 1NPC with Oxalic Acid and Ampicillin

a. 3D Structure of Protein with Bound Ligand b. Interaction of Ligand with Amino Acids c. 2D Interaction Diagram with Distances

Protein 1AH7: The binding energy for oxalic acid is -0.27 kcal/mol with an inhibition constant of 629.86 mM, which is weak compared to ampicillin's binding energy of -9.65 kcal/mol and inhibition constant of 84.89 nM (Fig 11).

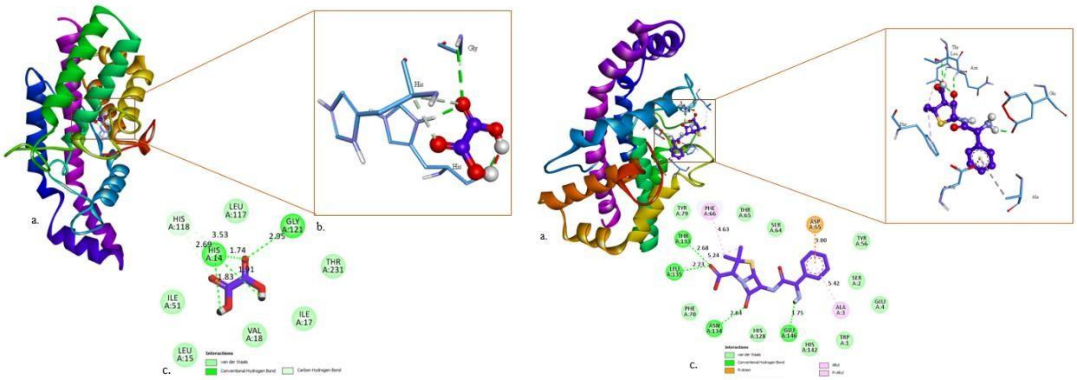

Fig 11. Interaction of 1AH7 with Oxalic Acid and Ampicillin

a. 3D Structure of Protein with Bound Ligand b. Interaction of Ligand with Amino Acids c. 2D Interaction Diagram with Distances

Protein 1B90: Oxalic acid exhibits a binding energy of -1.42 kcal/mol and an inhibition constant of 9.50 mM, reflecting weak inhibition. Ampicillin's binding energy of +42.88 kcal/mol is notably high and inconclusive in terms of effective inhibition (Fig 12).

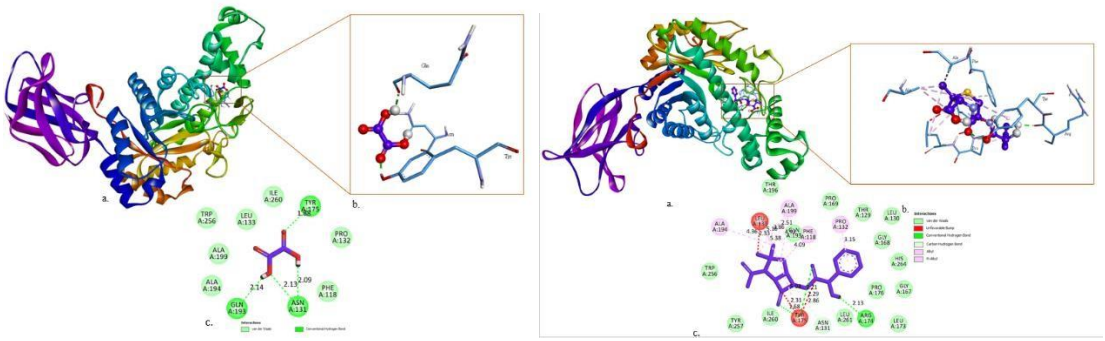

Fig 12. Interaction of 1B90 with Oxalic Acid and Ampicillin

a. 3D Structure of Protein with Bound Ligand b. Interaction of Ligand with Amino Acids c. 2D Interaction Diagram with Distances

The data highlights that oxalic acid generally exhibits weaker binding affinities and higher inhibition constants compared to ampicillin across various bacterial target proteins. Notably, oxalic acid shows promising results for certain *Pseudomonas* and *E. coli* proteins, especially with Protein 1IUV, where it demonstrates substantial binding affinity and inhibitory potential. Conversely, in most cases, ampicillin proves to be a more effective inhibitor with stronger binding affinities and lower inhibition constants. These findings suggest that while oxalic acid has potential as an antibacterial agent, its efficacy is variable and may be dependent on the specific bacterial protein targets.

Discussion

The emergence of drug resistance poses a significant challenge in medical treatment. Over time, particularly in the case of drugs aimed at infectious disease pathogens, their effectiveness can diminish due to selective pressures that lead to the development of resistant strains. This issue not only contributes to the resurgence of diseases but also increases morbidity and mortality rates. In complex diseases, resistance in drugs that target human cells can arise from factors such as epigenetic changes, DNA repair mechanisms, and epithelial-mesenchymal transition (Housman *et al.*, 2014). Common mechanisms associated with drug resistance include drug efflux and inactivation. This ongoing issue highlights the urgent need for continued research and the development of alternative therapies. Additionally, scientists are exploring the underlying biological processes that contribute to resistance in order to discover new strategies to mitigate these effects (Agamah *et al.*, 2020). In silico methods have significantly influenced the discovery of new targets for existing drugs, as well as the prediction of side effects and anatomical therapeutic indicators for approved medications (Keiser *et al.*, 2009; Cameron *et al.*, 2013). This demonstrates that computational approaches have played a crucial role in systematically guiding drug development processes, ultimately shortening the time required for drugs to reach the market (Wu *et al.*, 2013). This is based on the premise that minimizing side effects is achievable when drug candidates are both potent and highly selective (Mogire *et al.*, 2013).

This study evaluated the binding interactions and inhibitory effects of oxalic acid and ampicillin across various bacterial proteins from *Lactobacillus*, *E. coli*, *Pseudomonas*, and *Bacillus*. By analyzing binding energies and estimated inhibition constants, we aimed to assess the potential efficacy of these ligands as antimicrobial agents.

Binding Affinity of Oxalic Acid

Oxalic acid demonstrated a range of binding affinities across the tested bacterial proteins. In *Lactobacillus*, oxalic acid showed a positive binding energy with protein 4MKS (+2.59 kcal/mol), indicating poor binding affinity. Conversely, in other *Lactobacillus* proteins like 5J9G and 7QLE, oxalic acid exhibited moderate to weak binding energies of -4.54 kcal/mol and -2.78 kcal/mol, respectively. The corresponding inhibition constants were 468.58 µM and 9.20 mM, reflecting varying levels of inhibition potency.

In *E. coli*, oxalic acid bound more favourably to protein 1RX7 with a binding energy of -4.76 kcal/mol, translating to an inhibition constant of 325.36 µM. This suggests a more effective inhibition compared to the high inhibition constant of 134.36 mM observed for protein 1TLT, where the binding energy was relatively weak (-1.19 kcal/mol). Similarly, in *Pseudomonas*, oxalic acid exhibited a moderate binding energy of -5.04 kcal/mol with protein 5OE3, resulting in a 202.14 µM inhibition constant, indicating effective inhibition.

Interestingly, in Bacillus, the binding energies of oxalic acid were generally less favourable, with values ranging from -0.27 kcal/mol to -1.42 kcal/mol. These corresponded to high inhibition constants (629.86 mM to 9.50 mM), suggesting that oxalic acid is less effective against *Bacillus* compared to other bacterial species.

Binding Affinity of Ampicillin

Ampicillin, as a positive control, exhibited a broader range of binding affinities and was generally more effective than oxalic acid. In *Lactobacillus*, the binding energy for ampicillin with protein 4MKS was +292 kcal/mol, indicating no effective binding, while other proteins such as 5J9G and 7QLE showed more favourable interactions with binding energies of -7.44 kcal/mol and -6.68 kcal/mol, respectively. The corresponding inhibition constants were notably low (3.54 μM and 12.78 μM), reflecting strong inhibitory effects.

In *E. coli*, ampicillin bound strongly to protein 1RX7 with a binding energy of -7.88 kcal/mol and an inhibition constant of 1.68 μM, suggesting high efficacy. However, for protein 1TLT, the binding energy was weak (-1.19 kcal/mol), resulting in a similarly high inhibition constant (133.45 mM), indicating reduced effectiveness in this case.

In *Pseudomonas*, ampicillin displayed a strong binding affinity with protein 5OE3 (binding energy -7.78 kcal/mol) and a low inhibition constant of 1.99 μM, further confirming its potent inhibitory activity. In *Bacillus*, ampicillin exhibited very strong binding, particularly with protein 1AH7 (binding energy -9.65 kcal/mol) and a remarkably low inhibition constant of 84.89nM, which underscores its exceptional inhibitory potential against this target.

Comparative Analysis

The comparison between oxalic acid and ampicillin highlights that ampicillin generally demonstrates superior binding affinity and inhibitory potential across the bacterial proteins studied. While oxalic acid shows variability in its binding energies and inhibition constants, ampicillin consistently exhibits stronger binding interactions and lower inhibition constants, indicating its higher effectiveness as an antimicrobial agent.

Oxalic acid's variability suggests that its effectiveness as an antimicrobial might be highly dependent on the specific bacterial target and protein structure. Its weaker interactions in *Lactobacillus* and *Bacillus* highlight its limited potential as a broad-spectrum antimicrobial compared to ampicillin.

Implications for Therapeutic Use

The observed differences in binding affinities and inhibition constants emphasize the importance of selecting appropriate antimicrobial agents based on the target bacteria. Ampicillin's strong performance across various proteins makes it a reliable choice for effective antimicrobial therapy. In contrast, oxalic acid may have niche applications depending on the bacterial strain and protein target, but its variable effectiveness limits its broader applicability.

Drug-like compounds often face denial of approval due to unexpected clinical side effects and cross-reactivity observed during trials, leading to a significant increase in attrition rates. These outcomes are closely tied to the selected drug targets. To address this issue, various computational methods have been developed to complement experimental approaches in drug discovery. Identifying targets, particularly for polygenic diseases, is crucial and represents a major bottleneck in the drug development process. The initial step involves the identification and validation of relevant drug targets, which is essential for subsequent stages (Agamah *et al*., 2020).

Future research should focus on exploring the structural basis for the binding interactions of these compounds and evaluating their effects in more complex biological systems. Additionally, the development of new analogs or combination therapies involving oxalic acid could potentially enhance its antimicrobial efficacy.

In summary, while ampicillin stands out as a potent antimicrobial agent with consistent effectiveness, oxalic acid's variable interactions suggest a need for more targeted applications or further optimization to enhance its antimicrobial potential.

Reference:

1. Agamah, F. E., Mazandu, G. K., Hassan, R., Bope, C. D., Thomford, N. E., Ghansah, A., & Chimusa, E. R. (2020). Computational/in silico methods in drug target and lead prediction. *Briefings in bioinformatics*, *21*(5), 1663-1675.
2. Anand, U.; Jacobo-Herrera, N.J.; Altemimi, A.B.; Lakhssassi, N. A (2019), Comprehensive Review on Medicinal Plants as Antimicrobial Therapeutics: Potential Avenues of Biocompatible Drug Discovery. *Metabolites*, *9*, 258.
3. Cameron RT, Coleman RG, Day JP, (2013). Chemical informatics uncovers a new role for moexipril as a novel inhibitor of camp phosphodiesterase-4 (pde4). Biochem Pharmacol 2013;85:1297–305.
4. Chin, Y. W., Balunas, M. J., Chai, H. B., & Kinghorn, A. D. (2006). Drug discovery from natural sources. *The AAPS journal*, *8*, E239-E253.
5. Cui, Y., Zhao, Y., Tian, Y., Zhang, W., Lü, X., & Jiang, X. (2012). The molecular mechanism of action of bactericidal gold nanoparticles on *Escherichia coli*. *Biomaterials*, *33*(7), 2327-2333.
6. George, T. K., Joy, A., Divya, K., & Jisha, M. S. (2019). In vitro and in silico docking studies of antibacterial compounds derived from endophytic *Penicillium setosum*. *Microbial pathogenesis*, *131*, 87-97.
7. Hong, W., Zeng, J., & Xie, J. (2014). Antibiotic drugs targeting bacterial RNAs. *Acta Pharmaceutica Sinica B*, *4*(4), 258-265.

8. Housman G, Byler S, Heerboth S, (2014) Drug resistance in cancer: an overview. Cancers; 6:1769–92
9. Keiser MJ, Setola V, Irwin JJ, (2009). Predicting new molecular targets for known drugs. Nature; 462:175.
10. Mogire RM, Akala HM, Macharia RW, (2017). Target-similarity search using plasmodium falciparum proteome identifies approved drugs with anti-malarial activity and their possible targets. PloS One 2017;12:e0186364.
11. Turgis, M., Vu, K. D., Dupont, C., & Lacroix, M. (2012). Combined antimicrobial effect of essential oils and bacteriocins against foodborne pathogens and food spoilage bacteria. *Food Research International*, *48*(2), 696-702.
12. Wu L, Ai N, Liu Y, (2013). Relating anatomical therapeutic indications by the ensemble similarity of drug sets. J Chem Inf Model 2013; 53:2154–60.