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Medicinal Plant Power Against Superbugs: In-Silico Screening of Phytochemicals Targeting β-Lactamase-Mediated Antibiotic Resistance

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A B S T R A C T

Background: Antibiotic resistance represents a major global public health threat, with multidrug-resistant bacterial strains increasingly described as “silent killers.” The rapid emergence of resistance has significantly reduced the effectiveness of conventional antibiotics, creating an urgent need for alternative or adjunct therapeutic strategies. Herbal medicines have been used for centuries, and according to the World Health Organization, approximately 70–80% of the global population relies on plant-based remedies for primary healthcare. Phytochemicals derived from medicinal plants possess diverse bioactive properties and have demonstrated promising antibacterial potential against resistant pathogens.

Methodology: In this study, an in silico screening approach was employed to evaluate the antibacterial potential of phytochemicals derived from *Allium cepa*, *Acacia nilotica*, and *Azadirachta indica*. Molecular docking was performed against three clinically relevant β-lactamase enzymes: AmpC (PDB ID: 1FSW) from *Escherichia coli*, OXA-48 (PDB ID: 7AUX) from *Klebsiella pneumoniae*, and VIM-2 (PDB ID: 5NI0) from *Pseudomonas aeruginosa*. These enzymes were selected due to their central role in conferring resistance to broad-spectrum β-lactam antibiotics. Ciprofloxacin, ampicillin, and cephalosporin were used as reference drugs for comparative binding analysis. Drug-likeness, physicochemical properties, and pharmacokinetic profiles of the top-ranked phytochemicals were further evaluated using Swiss ADME, and a heat map was generated to visualize their overall performance.

Results: Kaempferol and quercetin from *Allium cepa* exhibited strong binding affinities against the targeted β-lactamases. Phytochemicals such as apigenin, gallocatechin, and cyanidanol from *Acacia nilotica*, and caryophyllin from *Azadirachta indica*, demonstrated higher or comparable binding energies relative to standard antibiotics. Additionally, azadirachtol and acetylnimbandiol showed activity against multiple β-lactamases, indicating broad-spectrum potential.

Conclusion: The findings suggest that selected phytochemicals, including kaempferol, quercetin, cyanidanol, and caryophyllin, hold promise as lead compounds for the development of novel antibacterial agents or antibiotic adjuvants. These compounds may enhance the efficacy of existing antibiotics and help combat resistant bacterial infections. Although the in silico results are encouraging, further in vitro and in vivo studies are essential to validate their therapeutic potential.

Key words: β-Lactamase, phytochemicals, *Klebsiella pneumoniae*

Introduction

The World Health Organization (WHO) has released data that clearly indicate that between 70 and 80% of the world's population uses various plants for therapeutic purposes to meet their basic healthcare needs. From the prehistoric era to the present, around 53,000 different types of plants have been used. Intestinal infections caused by *E.coli* have economic effects on endemic problems .[1] The aim of the study relates to promoting the investigation of phytochemical elements in plant extracts as a means of managing microbial resistance. Eventually, plant extracts as well as their refined components

might be employed as effective medicinal agents to treat *S. aureus* infections without having any noticeable negative effects. Antimicrobial resistance rose to the top of the worldwide death toll, making the development of novel, secure, and effective antibacterial agents critically necessary.[2] Plant-derived compounds can serve as a vital source for novel antibiotic types.[3] Cephalosporins, monobactams, penicillins, and carbapenems are among the many antibiotic drugs that belong to the large class known as β-lactam antibiotics because they all have a β-lactam ring in their molecular structure. These are the most popular antibiotics, and they work by preventing the production of the bacterial cell wall, which causes the germs to lyse and die. Owing to the extensive use of these antibiotics, bacteria have evolved a resistance mechanism against them.[4] This mechanism is often mediated by β-lactamases, which hydrolyze the β-lactam ring of the antibiotics, making them inert. According to recent research, the action of β-lactamases can be effectively countered by combining β-lactam antibiotics with β-lactamase inhibitors. In this study, we have screened plants based on their previous antibacterial properties and further tested whether they can act as beta-lactamase inhibitors or not.[5]They were bound to sit in place of synthetic inhibitors. This helped to analyze and identify potential phytochemicals acting as β-lactamase inhibitors and can be used as an antibiotic .[6]

Currently, using *in silico* techniques have given us validated and economical approaches for medicine discoveries. Neem or *Azadirachta indica* is a member of the family *Meliaceae.* Since ancient times, this plant has been used in conventional medications to cure various illnesses in people. Neem has antibacterial and antifungal properties in its leaves, seeds, and roots.[7] Biological activity is derived from a wide variety of structurally and chemically varied bioactive chemicals, about 140 of which may be discovered in various regions of the plant. Neem is used to extract a variety of biological components, such as phenolic compounds, flavonoids, carotenoids, ketones, and steroids. *A. indica* leaf extracts contain the ability to act for different bacteria like *Streptococcus, E. coli, Pseudomonas,* and *Staphylococcus* **.**[8]

As a native of Egypt, *Acacia nilotica* belongs to the *Fabaceae* family, which is widely distributed over many countries. Its common name is keekar. It is packed with bioactive substances; it can treat a wide range of ailments and infectious problems. Antimalarial and antioxidant effects of *A. nilotica* extracts are favourable. The plant has a large amount of polyphenolic substances, such as catechins, which are believed to possess antioxidants and anti-inflammatory qualities.[9] *A. nilotica* has been demonstrated to inhibit the hepatitis C virus protease and multidrug-resistant bacterial pathogens simultaneously. It can treat coughs, congestion, colds, nerve stimulation, diarrhea, leucorrhoea, hemorrhages, and ophthalmia, as well as provide relief from sclerosis and wound healing. It also possesses antiulcer and anti-inflammatory properties. The mint has diuretic, antihypertensive, and antipyretic properties. Research suggests that *A. nilotica* extract contains antioxidant and insulin-sensitizing properties, potentially reducing obesity and hyperlipidemia. *A. nilotica* seed contains a variety of phytochemicals, making it both edible and nutritious. [10]

Allium cepa is medically valuable and the largest species of its genus. It belongs to the family *Liliaceae*. This plant has worldwide cultivation. *Allium cepa,* commonly called onion, is a widely used edible vegetable with many medicinal advantages. Its most widely used part is the bulb, which is formed by leaf bases when they are at a specific growth stage.[11]

Owing to its flavour and health advantages, the consumption of onions has increased in recent years. *Allium cepa* works well against coughs, sore throats, heart disease, diabetes, osteoporosis, and the common cold. Protein, carbs, sugars, lipids, fibers, water, vitamins (B6 and C), potassium, and trace elements like chromium are all present in good proportion. The plant's varied pharmacological properties result from the increased concentration of sulfur compounds and flavonoids it contains.[12] Onions include flavonoids in the form of glycosides (flavonols) and cyanidin, peonin (anthocyanins), quercetin, kaempferol, and isorhamnetin. Onion skin has a significant concentration of oxidized quercetin derivatives and free and glycosidically bound quercetin. Quercetin, a flavonoid, has demonstrated strong promise as an antioxidant compound, reducing the risk of some forms of cancer and cardiovascular disease. Onions have high quercetin content. But depending on the type and colour of the bulb, its amount varies.

Prior research has demonstrated that *Azadirachta indica* (neem), *Allium cepa* (onion), and *Acacia nilotica* (keekar) have broad-spectrum antibacterial qualities that make them effective against a variety of bacterial infections, such as *Staphylococcus aureus* and *Escherichia coli*[10]. Given the increasing prevalence of antibiotic resistance, these plants are intriguing candidates for additional research into alternative treatments for bacterial illnesses. Scientific data suggests their potential as natural antimicrobial agents that could supplement or improve current antibiotic treatments[8]. Based on the numerous phytochemicals found in these plants, which are well-known for their strong antibacterial qualities, *Acacia nilotica* (keekar), *Allium cepa* (onion), and *Azadirachta indica* (neem) were chosen for antibacterial research. These naturally occurring bioactive substances help the plants fight off bacterial infections like *S.*

aureus and *E. coli*, which makes them an attractive candidate for study.[13]

Materials and Methods

A total of 10 phytochemicals from 3 medicinal plants, Neem (*Azadirachta indica),* Keekar (*Acacia nilotica), and* Onion (*Allium cepa)* were screened from Dr. Duke's Phytochemical and Ethnobotanical Databases (https://phytochem.nal.usda.gov/). 3D structures of phytochemicals were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/) [14], or ZINC database (https://zinc.docking.org) . The 3D structures were opened in PyMol and converted to PDB format and further processed using MGL tools as described by Morris et al. [15]

The protein complexed with the synthetic inhibitor's three-dimensional structure was obtained from PDB IDs 1fsw (AmpC), 7aux (OXA-48), and 5ni0 (VIM-2). PDB is an online library that includes many complex biological compounds such as proteins, DNA, and RNA structures in three dimensions.[16] PyMOL was used to visualize the targeted proteins' three-dimensional structure. Most rendering and representation operations might be completed with a mouse click or menu selection.[17] At the moment, this tool is utilized to identify the residues of the active site that bind with the inhibitor and to visually depict the structures of proteins. This multiplatform program is easy to use and compatible with Windows, Linux, and Mac OS. The Protein Data Bank (PDB) provided the protein for download. To avoid interfering with the docking process, water molecules were eliminated. The protein structure was supplemented with polar hydrogens. Ions, ligands, and other unnecessary molecules were eliminated. Only one protein chain was chosen, containing the active site where the inhibitor was bound. To concentrate on the pertinent binding point, the extra chains were deleted. Following these changes, the protein was stored in PDB format.

After visualizing the protein, a chain having a potential inhibitor attached was selected for the addition of a grid box by using MGL tools (Auto-dock). To guarantee complete sampling of the binding positions of ligand ciprofloxacin, the grid box was centered on the receptor protein from *E.Coli* 1fsw (AmpC) active site and set to 28 Å × 26 Å × 26 Å. A grid box was added on the ligand binding site on the chain and saved in PDBQT format for further docking. To perform molecular docking. Auto Dock Vina v 1.5.6 was used. Similarly, for assuring binding positions of ligand Ampicillin, the grid box was centered on the receptor protein from *Klebsiella pneumoniae* 7aux (oxa-48) active site and set to 20 Å × 22 Å × 22. Next same procedure was repeated for binding positions of ligand cephalosporin; the grid box was centered on the receptor proteins from *Pseudomonas aeruginosa* active site and set to 28 Å × 26 Å × 22. After that, both the setup, i.e., Auto Dock Tools and Auto Dock Vina, were installed. On a disk, a folder named protein was created. It contained several subfolders with the names of ligands. Molecular docking was done by using Vina, and binding energies were visualized.

For further analysis of the best candidates, ADMET analysis was performed by using Swiss ADMET. The heat map was generated to elucidate the pharmacokinetic, physicochemical, and drug-likeness parameters of the top ten molecules regarding docking.

Results

Ten phytochemicals from *Azadirachta indica*, *Acacia nilotica*, and *Allium cepa* were explored for antibacterial activity. The docking method was validated by re-docking the co-crystallized ligand into the active site of selected β-lactamases and achieving a root mean square deviation (RMSD) of less than 2Å, indicating reliability and correct positioning in the docking mechanism. As a result of docking, log files were generated that carried the information on binding energy. The results of ligands were compared with the docking results of the control, and the percentage binding energy was calculated. The effectiveness of target phytochemical inhibitors for various beta-lactamases was determined by comparing them with antibiotics Ciprofloxacin, Ampicillin, and Cephalosporin. Out of 10 phytochemicals, 7 showed efficient binding with beta-lactamases 1fsw (Amp), 9 phytochemicals showed efficient binding with 7aux(OXA-48), and 6 showed efficient binding with 5NI0(VIM-2)[18].

Above-mentioned control binding energies are used as a reference to compare the potential binding of phytochemicals at the inhibitory site of beta-lactamases, where in place where above-mentioned antibiotics were bound.

Table I: Binding Affinities of Control Antibiotics with β-Lactamases.

Control Antibiotic	Drug Bank ID	Beta lactamase	Binding Energy kcal/mol
Ciprofloxacin	DB00537	1fsw (Ampc)	-7.3
Ampicillin	DB00415	7aux(OXA-48)	-7.9
Cephalosporin	DB03313	5NI0 (VIM-2)	-7.1

Phytochemicals were analyzed against three lactamases from three strains. Docking of 1fsw(Ampc) from *E. coli,* 7aux(OXA-48) from *Klebsiella pneumoniae,* 5NI0(VIM-2) from *Pseudomonas aeruginosa* against phytochemicals from *Allium cepa* (Onion), *Acacia nilotica* (Keekar), and *Azadirachta indica (*Neem) showed efficient binding energies as compared to the

control. Three control synthetic antibiotics, Ciprofloxacin, Ampicillin, and Cephalosporin, were analyzed as beta-lactamase inhibitors with different binding energies -7.3 kcal/mol for 1fsw, -7.9kcal/mol for 7aux, and -7.1kcal/mol for 5NI0, respectively. Among various studied phytochemicals, five phytochemicals from different plant sources, as mentioned in Table I, had efficient binding with beta lactamase 7aux when compared to their respective control, Ampicillin (-7.9 kcal/mol). These phytochemicals include Caryophyllin with the highest binding affinity and the least binding energy -9.4kcal/mol among all phytochemicals. The second most efficient phytochemical observed was Quercetin with a binding energy of -9.1kcal/mol. Isofucosterol, Cianidanol and Kaempferol were also observed as potential 7aux beta lactamase inhibitors by comparing their binding energies -8.9kcal/mol, -8.7kcal/mol, and -8.6kcal/mol with the control mentioned above. For beta lactamase 1fsw(Ampc), two phytochemicals, azadirachtol and cianidanol, were most efficient with the least binding energies -8.6kcal/mol and -8.4kcal/mol. These two phytochemicals showed shown highest binding affinity as compared to their respective control ciprofloxacin (-7.3kcal/mol). One phytochemical, Querticin, has effective binding with 5NI0(VIM-2) with potential binding energy -8.7kcal/mol. Other than these eight phytochemicals, five phytochemicals from different plants showed the same binding energy with different enzymes. Azadirachtol and betasisterol had the same binding energy -8.3kcal/mol with 7aux. Similarly, kaempferol, apgenin, and cianidanol also have the same binding energy-8.3kcal/mol with another enzyme 5NI0(VIM-2). Quercetin and kaempferol showed equivalent binding affinity with 1fswwith 1fsw by exhibiting the same binding energy -8.1kcal/mol. The other three effective phytochemicals for 1fsw are apgenin, gallocatechin, and acetylnimbnadiol with the same binding energies -8kcal/mol. When compared to their respective controls, gallocatechin has shown less binding energy -7.9kcal/mol and effective binding for 7aux as well as for 5NI0 with binding energy -7.6kcal/mol and had efficient binding affinity. Azadirachtol was the only phytochemical that has the same binding energy -7.1kcal/mol as the control cephalosporin when used as an inhibitor for 5NI0 lactamase.

The heatmap correlation of the ten docked molecules, in Figure 5, revealed that several candidates had good drug-likeness and good pharmacokinetic profiles, while a few of them had parameter deviations. Molecules A, C, F, and H had notably good profiles, with molecular weights less than 500 Da, decent TPSA (<140 Å²), moderate LogP values (2–4), and no more than a single Lipinski violation, indicating good oral absorption potential. These molecules also displayed well-balanced hydrogen bond donors/acceptors and rotatable bonds, increasing their possibilities for good permeability and bioavailability. On the other hand, Molecule D and Molecule J were far from drug-likeness filters, mostly because they possess high TPSA and several rule infringements (Ghose or Muegge), which can weaken absorption and permeability. Molecule B and Molecule G had moderate LogP values but poorer solubility (negative Z-scores), indicating possible bioavailability issues in spite of favorable docking scores. Molecule E and Molecule I indicated mixed outcomes, having sufficient drug-likeness but marginal pharmacokinetic attributes like greater molecular flexibility. Molecule A, C, F, and H are the most promising leads overall, whereas Molecule D and J are less likely due to physicochemical or drug-likeness constraints.

Table II: Binding Affinities of Phytochemicals against β-Lactamases.

Beta lactamase Protein	Phytochemicals	PubChem ID	Plant Source	Binding Affinity (kcal/mol)
1fsw (Ampc)	Querticin	5280343	*Allium cepa*	-8.1
	Kaempferol	5280863	*Allium cepa*	-8.1
	Cianidanol	9064	*Acacia nilotica*	-8.4
	Apgenin	5280443	*Acacia nilotica*	-8
	Gallocatechin	65084	*Acacia nilotica*	-8
	Azadirachtol	23256847	*Azadirachta indica*	-8.6
	Acetyl Nimbandiol	52952216	*Azadirachta indica*	-8
7aux (OXa-48)	Querticin	5280343	*Allium cepa*	-9.1
	Isofucosterol	5281326	*Allium cepa*	-8.9
	Kaempferol	5280863	*Allium cepa*	-8.6
	Cianidanol	9064	*Acacia nilotica*	-8.7
	Apgenin	5280443	*Acacia nilotica*	-8.4
	Gallocatechin	65084	*Acacia nilotica*	-7.9
	Caryophyllin	10494	*Azadirachta indica*	-9.4
	Betasisterol	222284	*Azadirachta indica*	-8.3
	Azadirachtol	23256847	*Azadirachta indica*	-8.3
5NI0(VIM-2)	Querticin	5280343	*Allium cepa*	-8.7
	Kaempferol	5280863	*Allium cepa*	-8.3
	Cianidanol	9064	*Acacia nilotica*	-8.3
	Apgenin	5280443	Acacia nilotica	-8.3
	Gallocatechin	65084	Acacia nilotica	-7.6
	Azadirachtol	23256847	Azadirachta indica	-7.1

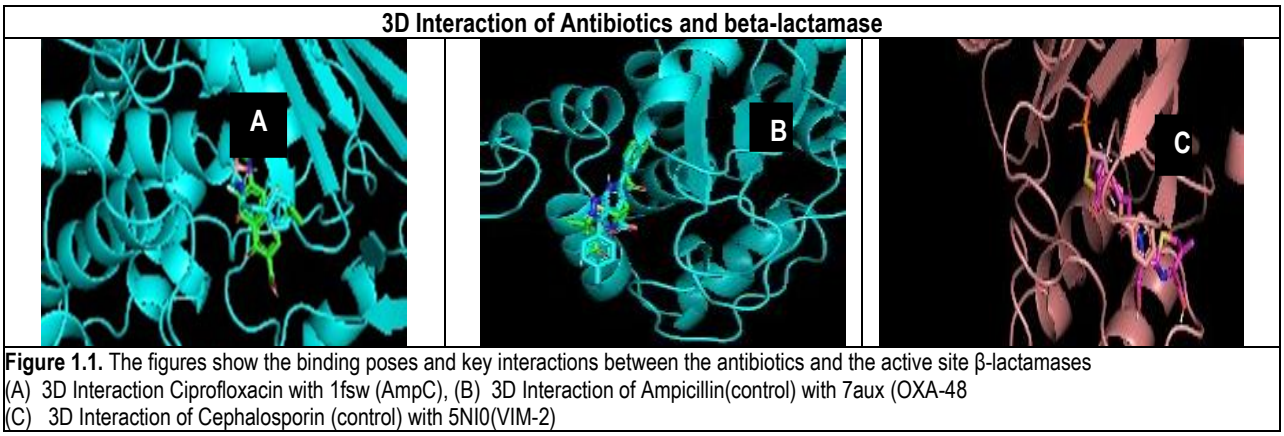

Figure 1.1. The figures show the binding poses and key interactions between the antibiotics and the active site β-lactamases
(A) 3D Interaction Ciprofloxacin with 1fsw (AmpC), (B) 3D Interaction of Ampicillin(control) with 7aux (OXA-48
(C) 3D Interaction of Cephalosporin (control) with 5NI0(VIM-2)

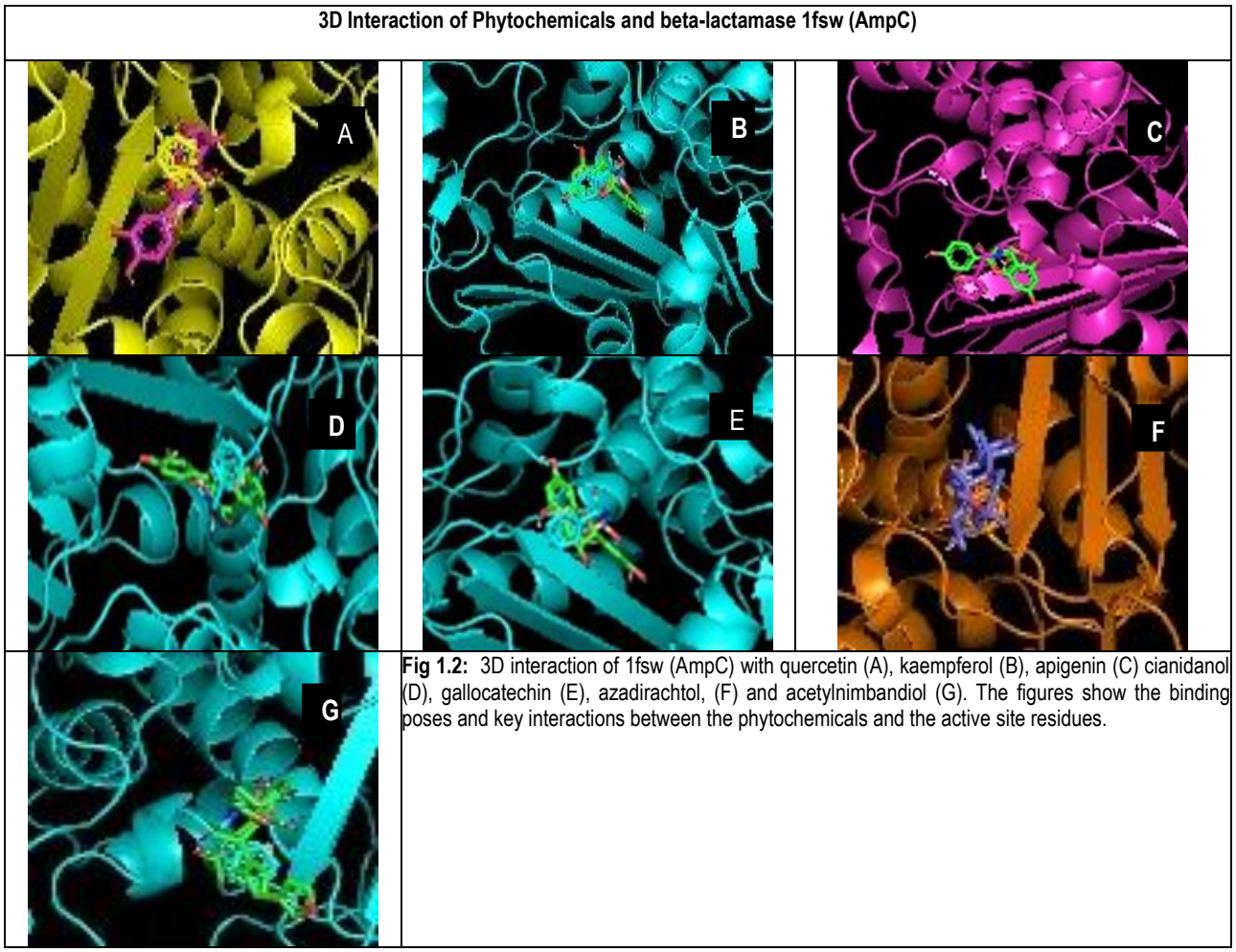

Fig 1.2: 3D interaction of 1fsw (AmpC) with quercetin (A), kaempferol (B), apigenin (C) cianidanol (D), gallocatechin (E), azadirachtol, (F) and acetylnimbandiol (G). The figures show the binding poses and key interactions between the phytochemicals and the active site residues.

Discussion

Most healthcare systems acknowledge that bacterial resistance is a serious medical problem. Multidrug-resistant (MDR) infections and resistance-determining genes, typically in combination, are proliferating at a rate never seen before. The Gram-positive strains of *Staphylococcus* spp. are well-known resistance carriers with significant therapeutic implications. Many Gram-negative strains have developed resistance to *Escherichia coli, Klebsiella pneumonia,* and *Pseudomonas aeruginosa* to almost all antibiotics.[19] Based on the main factors that promote resistance, their resistance epidemiology identifies three areas as hotspots for high-impact resistance. Although there is an evident medical need for new antibiotics that don't have cross-resistance problems. The pipelines for antibacterial research and development are almost empty, making it difficult to produce the new medicines needed to keep up with the rapid

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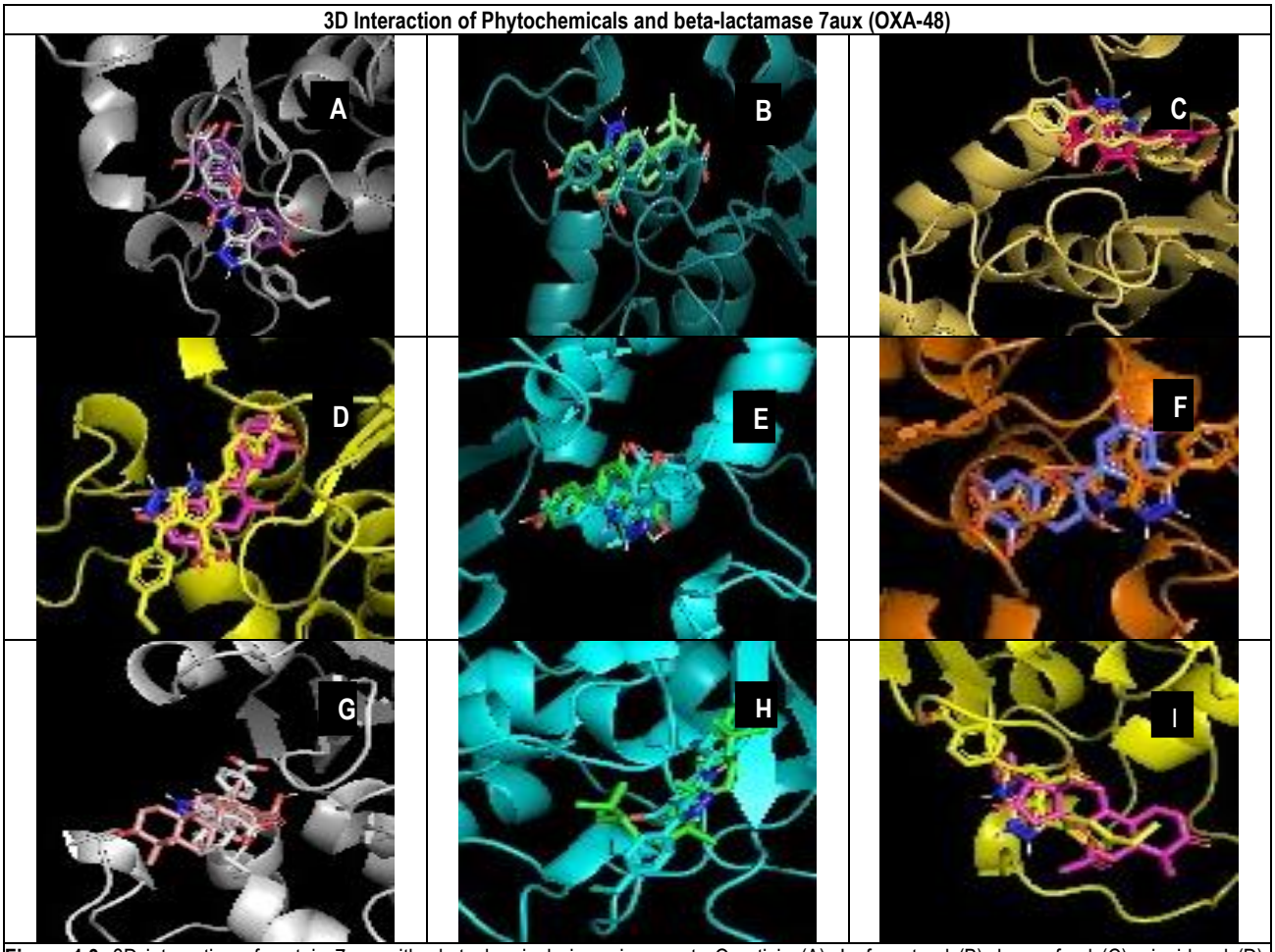

Figure 1.3. 3D interaction of protein 7aux with phytochemicals in various parts Querticin (A), Isofucosterol (B), kaempferol (C), cianidanol (D), apgenin (E), gallocatechin (F), caryophyllin (G), betasisterol (H) and azadirachtol (I). The figures show the binding poses and key interactions between the phytochemicals and the active site residues.

rise and spread of multidrug-resistant bacteria.[20] Only coordinated international efforts can reduce the likelihood that infectious diseases will become incurable in the modern world.[21] For patients and the healthcare system, the cost of these treatment setbacks and delays is the main factor contributing to the negative effects of antibiotic resistance. The primary factor causing delays in the administration of effective treatment is resistance, which frequently leads to mismatches between antibiotic susceptibility test results after empirical treatment .[22] According to a study, for example, patients with infections caused by ESBL-producing strains of *K. pneumoniae* and *E. coli* received the appropriate antibiotic treatment a median of 72 hours after the infection was suspected, whereas matched controls infected with non-ESBL-producing strains of *K. pneumoniae* and *E. coli* received treatment a median of 11.5 hours later. A meta-analysis established a significantly higher likelihood of postponing the initiation of effective medication in cases of ESBL-associated bacteremia.[23] Treatment delivery delays for patients with carbapenem-resistant *K. pneumoniae* bacteremia have also been shown to be caused by antibiotics that act against such bacteria *in vitro.* [24] Microbial infections can now be prevented and treated using synthetic antimicrobial agents thanks to their discovery. However, interest in studying phytochemicals as a synthetic antimicrobial compound substitute has renewed due to the emergence of antibiotic-resistant bacterial strains, the decline in the efficacy and safety of antimicrobials, and the search for novel antimicrobials to counteract the emergence of incurable diseases by conventional antimicrobial agents. Although numerous investigations on the antibacterial properties of *Allium cepa*, *Acacia nilotica*, and *Azadirachta indica* have been conducted, an important group of plant phytochemicals with antibacterial properties has been identified. These studies have used phytochemicals such as various phenolic compounds, alkaloids, saponins, iridoids and secoiridoids, polyacetylenes, glucosinolates, terpenoids, sulfinate, limonoids (tetranortepenoids), and anthranoids against resistant strains .[25]

However, over the last ten years, there has been a discernible rise in the development and application of computational *(in silico)* methods to generate and evaluate pharmacological

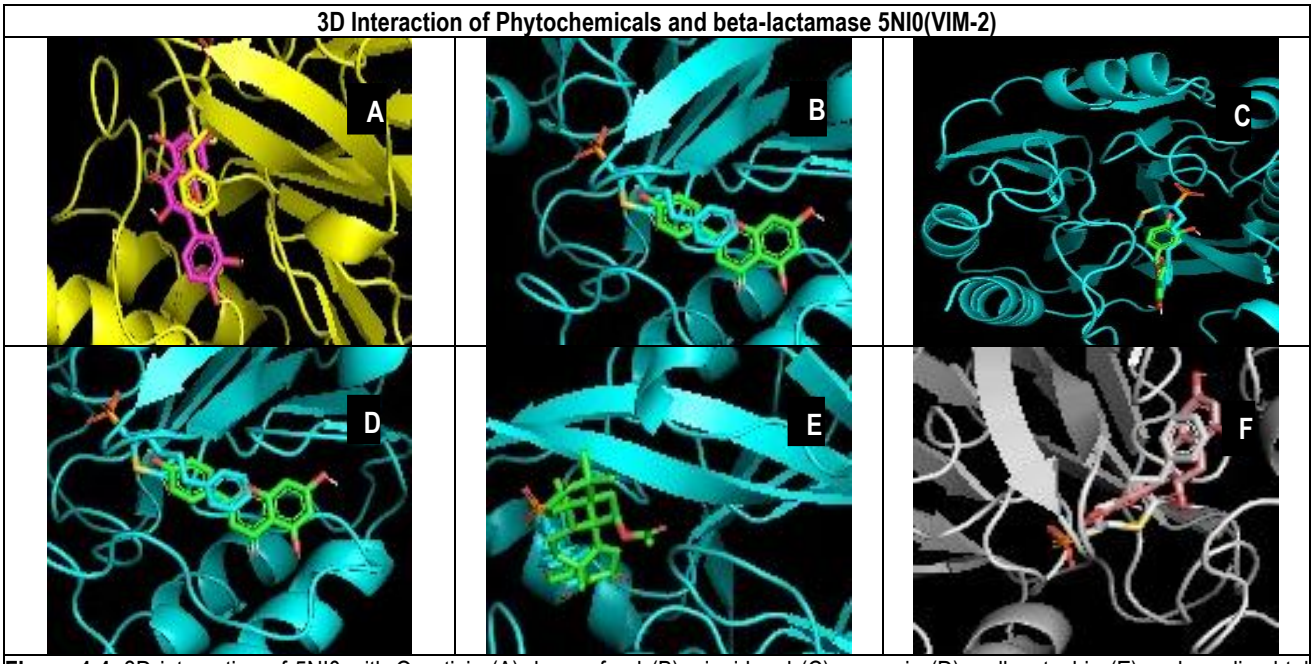

Figure 1.4. 3D interaction of 5NI0 with Querticin (A), kaempferol (B), cianidanol (C), apgenin (D), gallocatechin (E) and azadirachtol (F). The figures show the binding poses and key interactions between the phytochemicals and the active site residues.

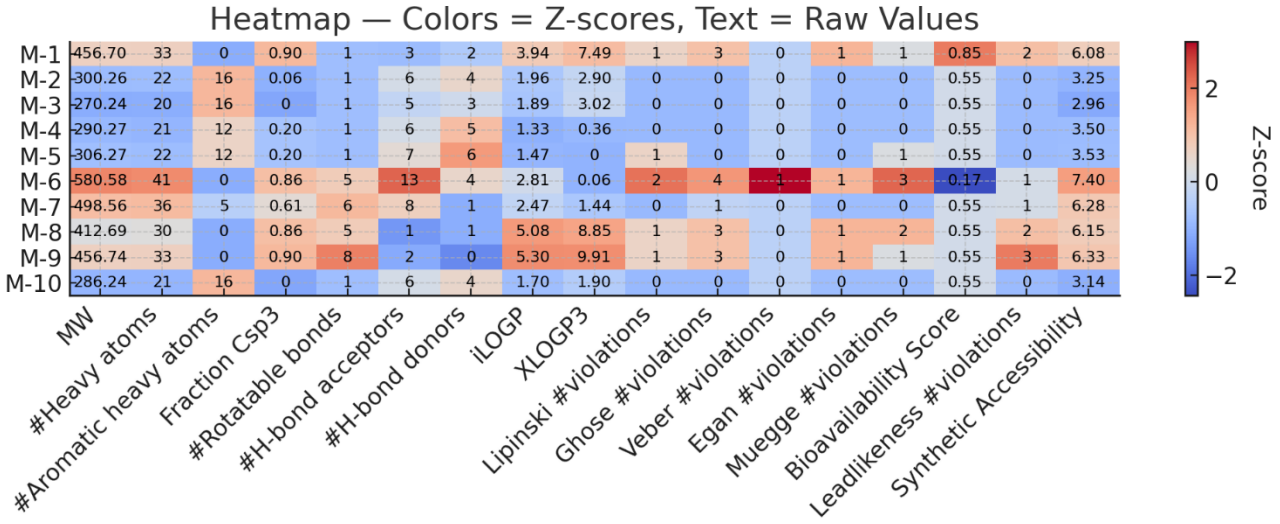

Figure 5. Heatmap representing Z-score standardization within each parameter is indicated by the color intensity (blue = below average, red = above average), allowing direct comparison between molecules regardless of scale. Raw values are also shown in each cell for understandability.

theories. Examples of *in silico* technology include databases, homology models, pharmacophores, quantitative structure-activity correlations, and other molecular modeling techniques. Included are network analysis tools, computer-based data analysis tools, machine learning, and data mining. *In silico* techniques are usually used with *in vitro* data generation to create and validate the model. These models are extensively employed in the identification and development of novel compounds with affinity for a target, as well as in the physicochemical characterization, absorption, distribution, metabolism, excretion, and toxicity properties.[26] Still, there has been a discernible surge in the development and use of computational *(in silico*) methods for the generation and testing of pharmacological hypotheses over the last ten years.

In silico docking of phytochemicals of *Allium cepa* with 1fsw (AmpC) showed different binding energies. Two phytochemicals, kaempferol (-8.1) and quercetin (-8.1), showed the least binding energy as compared to the control binding energy of Ciprofloxacin (-7.3kcal/mol). These two phytochemicals can be used as an antibiotic against *E. Coli* strains producing AmpC beta-lactamases. Phytochemical

screening of phytochemicals of *Acacia nilotica* showed different binding energies, and binding efficiency was analyzed by comparing with the Control energies. Among phytochemicals, apgenin (-8kcal/mol), cianidanol (-8.4kcal/mol), gallocatechin (-8kcal/mol), three phytochemicals, apgenin, cianidanol, and gallocatechin showed the least binding energies and high binding affinity with the target lactamase inhibitory site, thus reflecting their ability to inactive AmpC beta lactamase and can be used as an antibiotic for *E. coli*. The next screening was done with the phytochemicals of *Azadirachta indica.* two phytochemicals acetynimbandiol (-8kcal/mol), azadirachtol (-8kcal/mol) showed least binding energies. Their binding energy depicts their antibacterial activity against *E. coli* beta-lactamase AmpC.

Klebsiella pneumoniae produces β-lactamase 7aux (OXA-48) that contributes to its resistance to various antibiotic treatments.[27] Different plants' phytochemicals of *Allium cepa* iso-fucosterol (-8.9), kaempferol (-8.6kcal/mol), and quercetin (-9.1kcal/mol) showed less binding energies as compared to reference or control treatment binding energies, Ampicillin (-7.9kcal/mol). Isofucosterol, quercetin, and kaempferol showed the least binding energies and thus reflect their use as antibiotics against *Klebsiella pneumoniae* beta-lactamases 7aux (OXA-48). Phytochemicals of *Acacia nilotica (*keekar*),* apgenin (-8.4kcal/mol), and cianidanol (-8.7kcal/mol) have less binding energies than the control and exhibit potential for using them as antibiotics against β-lactamase of *Klebsiella pneumoniae.* Further *in silico* screening of phytochemicals of *Azadirachta indica, including* caryophyllin, azadirachtol, and betasisterol, two phytochemicals, azadirachtol and betasisterol, showed similar least binding energies (-8.3kcal/mol) as compared to control Ampicillin (-7.9kcal/mol), and Caryophyllin (-9.4kcal/mol) showed most least binding energy and can be categorized as the most effective phytochemical with antibacterial potential.

Pseudomonas aeruginosa produces beta-lactamase 5NI0 (VIM-2), which contributes to antibiotic resistance in many treatments.[28] Different phytochemicals were *in silico* screened from plants Allium *cepa*, *Acacia nilotica,* and *Azadirachta indica.* Among phytochemicals of *Allium cepa,* quercetin (-8.7kcal/mol) and kaempferol (-8.3kcal/mol) showed the least binding energies compared to the control treatment Cephalosporin (-7.1kcal/mol). Kaempferol and Quercetin can be used as an antibiotic against VIM-2 β-lactamase of *Pseudomonas aeurignosa.* Phytochemicals of *Acacia nilotica* (keekar) apgenin (-8.3kcal/mol), cianidanol (-8.3kcal/mol), and gallocatechin (-7.6kcal/mol) showed more binding energies than the control, and these can be used as potential antibiotics. Screening of 5NI0 with Phytochemicals of *Azadirachta indica,* only azadirachtol (-7.1kcal/mol binding affinity equal to control Cephalosporin. The phytochemicals exhibit a high affinity for the active site of beta-lactamase, which may result in efficient inhibition of the enzyme. This interaction could potentially prevent the enzyme from carrying out its function, thereby enhancing the effectiveness of antibiotics that are otherwise resistant to beta-lactamase activity. Despite the encouraging *in silico* results, more experimental confirmation is needed to confirm these phytochemicals' antibacterial efficacy *in vitro* and *in vivo*. These kinds of investigations are essential for verifying their biological activity and guaranteeing their potential for therapeutic application under actual circumstances. The genuine antibacterial potential of these drugs can only be accurately evaluated through these extensive studies.

Conclusion

. *In silico* screening of various phytochemicals gave us the best phytochemicals that can be used as antibacterial agents against different beta-lactamases. Kaempferol and quercetin of *Allium cepa* showed the most efficient activity for 1fsw (AmpC) beta-lactamase of *E. coli*. Apgenin, cianidanol, and gallocatechin of *Acacia nilotica* were identified with the least binding energies and best binding efficiencies. From phytochemicals of *Azadirachta indica,* acetyl nimbandiol, and azadirachtol showed efficient binding. For beta lactamase 7aux (OXA-48) of *Klebseilla pneumoniae,* kaempferol, quercetin, and isofucosterol of *Allium cepa* were identified with less binding energies. From *Acacia nilotica* apgenin and cianidanol showed the least binding energy and efficient binding. Among phytochemicals of *Azadirachta indica,* caryophyllin has the most efficient binding with a binding energy of -9.4kcal/mol. Other phytochemicals, azadirachtol and beta-sisterol, showed similar binding efficiency. *Allium cepa* phytochemicals kaempferol and querticin showed similar activity as for beta-lactamase 5NI0 of *Pseudomonas aeruginosa* as for lactamase 1fsw of *E. coli*. *Acacia nilotica* gave three effective phytochemicals, apgenin, cianidanol, and gallocatechin for 5NI0. *Azadirachta indica* has only one phytochemical, azadirachtol, exhibiting similar binding energy as the Control treatment.

Phytochemical treatments are safer approaches because their doses can be optimized, and their side effects are reduced. Phytochemical treatments provide safer treatments, eliminating adverse reactions. *In silico* phytochemical screenings are less expensive than allopathic treatments because they require no experimental trials in a lab. This cost effectiveness helps researchers to develop more drugs and keep work continuous. These techniques are environmental friendly, minimizing the effects of harvesting. This technique gave us an enhanced understanding of molecular interactions, so we can discover

drugs with more efficient activities. This technique reduces the need for the development of more complex compounds in the future. It upgrades the approval mechanism of drugs and accelerates their marketing paths. This technique enhances the accessibility of drugs in local masses that cannot afford allopathic drugs, plant extracts for treatments.

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