# In silico Analysis of Phytocompounds from *Plumbago auriculata*, *Gymnema sylvestre*, and *Andrographis paniculata* as Potential Inhibitors of NDM-1 in *Klebsiella pneumoniae*

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**Abstract.** New Delhi metallo-β-lactamase-1 (NDM-1) is a major determinant of antibiotic resistance responsible for hydrolyzing β-lactam antibiotics, including carbapenems, in *Klebsiella pneumoniae*. This study aimed to find potential phytocompound inhibitors of NDM-1 from three medicinal plants, *Andrographis paniculata*, *Gymnema sylvestre*, and *Plumbago auriculata,* by using an in silico computational approach. The NDM-1 three-dimensional structure was obtained from the Protein Data Bank, and phytocompound structures were collected from the IMPPAT database. AutoDock Vina has been used for performing molecular docking, followed by visualization and interaction analysis using AutoDock Tools 1.5.7. Pharmacokinetic and toxicity screening (ADMET and ProTox-III) was performed to assess drug likeness and safety. Docking results revealed different inhibitory potentials among screened phytocompounds. Alpha-Amyrin from *Plumbago auriculata* shows the highest binding affinity (−8.9 kcal/mol), followed by Beta-Amyrin from *Gymnema sylvestre* and *Plumbago auriculata* (−8.8 kcal/mol). All selected phytocompounds showed favourable ADMET profiles and low predicted toxicity, with Beta-Amyrin, Alpha-Amyrin, Daucosterol, and Resiniferonol showing the safest toxicity scores. These findings suggest that selected phytocompounds, particularly Alpha-Amyrin and Beta-Amyrin, may provide promising lead compounds for developing NDM-1 enzyme inhibitors. Despite this, further in vitro and in vivo validation is required to confirm their therapeutic potential.

**Keywords :** *NDM-1, Klebsiella pneumoniae, Molecular Docking, Phytocompounds, ADMET, Drug Discovery*

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## 1 Introduction

The ongoing development of antimicrobial strategies is essential due to the escalating issue of antibiotic resistance and the persistent challenge of bacterial infections. Among the diverse classes of antibiotics, β-lactams offer broad-spectrum action and efficacy in treating various bacterial infections. These antibiotics inhibit the synthesis of bacterial cell walls, ultimately resulting in cell lysis and mortality. These antibiotics include penicillin and cephalosporins. However, the widespread use of β-lactam antibiotics has resulted in the development of resistance mechanisms, mainly through the production of β-lactamase enzymes by bacteria [1]. These enzymes can hydrolyze the β-lactam ring, rendering the antibiotic ineffective and posing a substantial threat to public health [2, 3]. Multidrug resistant pathogens, including *Mycobacterium tuberculosis*, *Escherichia coli*, and the ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species), have evolved diverse strategies towards the effects of β-lactam antibiotics [3]. The synthesis of β-lactamases is particularly alarming among these mechanisms. These enzymes are categorized as metallo-β-lactamases and serine-β-lactamases according to their mechanisms of action and active sites. Metallo-β-lactamases rely on a zinc ion to facilitate the hydrolytic process, whereas serine-β-lactamases utilize a serine residue to hydrolyze the β-lactam ring [4, 5].

The bacterium that has multiple resistance mechanisms is called a “superbug”. Examples of bacteria that carry multiple resistance genes are *P. aeruginosa*, methicillin-resistant *S. aureus*, etc. [6]. NDM-1, a resistance gene conferred by emerging “superbugs,” was first detected in India and has rapidly spread across the globe. NDM-1 has the tendency to hydrolyze almost all β-lactam antibiotics, which leads to the discovery of multidrug resistant bacteria [6]. NDM-1 has remained a central target in antimicrobial research. Scientists have discovered several effective inhibitors, including captopril, thiol compounds, Aspergillomarasmine A, boric acid derivatives, sulphanilamide, succinic acid, and natural bioactive compounds. NDM-1 is categorized as a class B1 broad-spectrum enzyme of β-lactamase. The electron-rich groups of NDM-1 inhibitors interact with two positively charged zinc ions located in the enzyme active sites through ion-dipoles interaction. In the active site, two positively charged zinc ions form electrostatic interactions with the hydroxyl ions, enhancing the hydrogen atom from the coordinate water molecule. This process makes a bridging hydroxide ion with enhanced nucleophilicity, which subsequently targets the carbonyl carbon of the β-lactam ring, leading to the hydrolysis of β-lactam antibiotics [7, 8]. The open conformation and unique electrostatic environment of NDM-1 contribute to its broad substrate specificity and potent catalytic efficiency. The flexible hydrolytic mechanism and various variants of NDM-1 aggravate the global threat of drug-resistant bacterial infections. Despite extensive research, no clinically approved inhibitor yet has been developed [7].

Nowadays, computational techniques like toxicity analysis and molecular docking have become indispensable in modern drug discovery. These techniques are used for efficient and economical assessment of target molecules, significantly minimizing the requirement of laboratories for in vitro and in vivo testing [9]. Molecular docking provides valuable insights

into the binding affinity and inhibitory potential of compounds by simulating the interaction between potential inhibitors and their target proteins. Such approaches are especially beneficial for high-throughput screening of phytochemical libraries to pinpoint bioactive compounds with therapeutic potential [9, 10].

The medicinal plants *Andrographis paniculata*, *Gymnema sylvestre* and *Plumbago auriculata* have a various diversity of bioactive phytoconstituents that show therapeutic tendency. However, the mode of action of their specific bioactive compounds against bacterial β-Lactamases remains largely unexplored. This study aims to investigate the inhibitory effects of phytocompounds from these selected medicinal plants on NDM-1 β-lactamase by using molecular docking analysis. Identification of potent inhibitors could facilitate the development of novel antibacterial agents capable of overcoming β-lactamase mediated resistance. The result of this study can further support the incorporation of these natural biocompounds into the antibacterial drug discovery pipeline, contributing to sustainable strategies against antibiotic resistance.

## 2 Methods

### 2.1 Bioinformatics Tools

The study used the following software and online resources: Open Babel GUI [11], PubChem (www.pubchem.com, accessed August 27, 2025), UCSF Chimaera 1.16, RCSB PDB (http://www.rscb.org/pdb (accessed on August 27, 2025), AutoDock/Vina software [12], Discovery Studio, PROTOX-III (https://tox.charite.de/protox3/) and SWISSADME (http://www.swissadme.ch/).

### 2.2 Ligand Preparation

Molecular docking analysis was conducted on one hundred thirty-nine, two hundred thirty-eight and twenty key phytocompounds derived from the medicinal plants *Andrographis paniculata, Gymnema sylvestre and Plumbago auriculata*, respectively, collected from India. The three-dimensional structures of all the phytocompounds were accessed from the Indian Medicinal Plants, Phytochemistry, and Therapeutics (IMPPAT) database. (https://cb.imsc.res.in/imppat/basicsearch/phytochemical) in PDB format. The control antibiotics (Cefiderocol, Tigecycline, Ceftazidime, Aztreonam, Tetracycline and Avibactam) for *K. pneumoniae* NDM-1 were collected from PubChem (www.pubchem.com, accessed on August 27, 2025) in SDF format [13]. The AutoDock software was used to get a PDBQT file of selected phytocompounds and antibiotics.

### 2.3 Protein Preparation

The 3-D structure of NDM-1 metallo β-Lactamases type-2 protein from K. pneumoniae (PDB ID = 6EFJ) was downloaded from the RCSB Protein Data Bank (http://www.rscb.org/pdb; accessed on August 27, 2025) [14]. Chain A was configured for docking, and a grid box with

dimensions of 30 Å x 30 Å x 30 Å was positioned to encompass the complete protein. Coordinates for the grid box centre were set to x = 25.174 Å, y = 6.705 Å, and z = 14.563 Å.

### 2.4 Molecular Docking of Major *Andrographis paniculata, Gymnema sylvestre and Plumbago auriculata* Phytocompounds

The AutoDock Vina software was utilised to conduct molecular docking between the selected ligands and the NDM-1 metallo β-Lactamases type-2 protein, and the generated file was saved as a pdbqt. Molecular docking was conducted to explore possible ligand conformations and binding modes of ligands at the active site. Docking was performed by using AutoDock Vina version 1.1.2's command-line interface. A total of nine binding modes were assessed in the analysis. The log file generated contained an ascending set of binding modes along with their respective binding energies [12]. The most favourable conformation, corresponding to the lowest docked energy, was selected after the docking process. Protein-ligand interactions were examined by using Discovery Studio 24.1.0 (BIOVIA, San Diego, CA, USA) (https://discover.3ds.com/d (accessed on May 30, 2024)). All non-bonded interactions were analyzed, and the binding affinity of every ligand was evaluated by using a negative score (KJ/mol).

### 2.5 ADMET analysis of phytocompounds

The selected phytocompounds were screened for absorption, distribution, metabolism, excretion, and toxicity (ADMET) characteristics. The 3-D structures of *Andrographis paniculata* selected phytocompounds *(*Daucosterol, Stigmasterol, Andropanoside, Neoandrographolide, Andrographidin A, Andrographidine C, Andrographidine E, Skullcap flavone I), *Gymnema sylvestre* selected phytocompounds *(*Beta-Amyrin, Gymnemic acid IX, Gymnestrogenin, Dammarane, Stigmasterol, Resiniferonol, and Anthraquinone), and *Plumbago auriculata* selected phytocompounds (Alpha-Amyrin, Beta-Amyrin, Beta Sitosterol, Azaleatin, Apigenin, and Luteolin) were represented to SMILES format and then submitted to SWISSADME (http://www.swissadme.ch/) and PROTOX-III (https://tox.charite.de/protox3/) (accessed on September 15, 2025) for ADMET screening. SWISSADME is an internet-based platform applied for forecasting the ADME (Absorption, Distribution, Metabolism, and Excretion) as well as the pharmacokinetic and physicochemical characteristics of a molecule [15, 16, 17, 18, 19]. These factors are important when it comes to assessing the suitability of a molecule for clinical trials. The compounds' LD50 values were used to figure out how toxic they were. Compounds with LD50 values greater than or equal to 50 mg/kg were classified as Class I, those with LD50 values greater than 50 mg/kg but less than 500 mg/kg were classified as Class II, compounds with LD50 values between 500 and 5000 mg/kg were classified as Class III, and compounds with LD50 values greater than 5000 mg/kg were classified as Class IV. Classes I, II, and III had reduced toxicity, whereas Class IV showed no toxicity and Class V and VI represent very low toxicity and non-toxic compounds [20, 21]. Additionally, PROTOX is a computational tool that assesses the oral toxicity of a substance in rodents by calculating its LD50 value and assigning it a toxicity class [15]. Figure 1 depicts a diagram of the experiment.

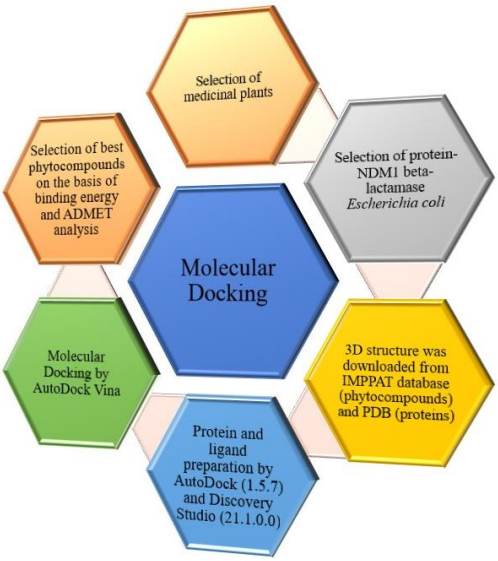



**Fig.1.** Schematic representation of the experiment

# 3 RESULTS

## 3.1. Molecular Docking of 397 Major Phytocompounds from *Andrographis paniculata*, *Gymnema sylvestre*, and *Plumbago auriculata* Against the NDM-1 (PDB ID: 6EFJ)

Molecular docking was conducted by using 6EFJ structure NDM-1 beta-Lactamase and AutoDock to understand the interactions of the major phytocompounds of *Andrographis paniculate, Gymnema sylvestre* and *Plumbago Auriculata*, three medicinal plants from India. The result of molecular docking has shown that several phytocompounds from these medicinal plants has binding energy equivalent to that of selected standard antibiotics. Alpha Amyrin of plant *Plumbago Auriculata* has shown the best binding energy (–8.9 kJ/mol) that is equivalent to those standard antibiotics Cefederocol (-8.5 KJ/Mol), Tigecycline (-8.0 KJ/Mol), Ceftazidime (-7.3 KJ/Mol), Azreonam (-6.7 KJ/Mol) Tetracycline (-6.5 KJ/Mol) and Avibactam (-6.2 KJ/Mol). The remaining phytocompounds that show binding energy equivalent to selected standard antibiotics from Andrographis Paniculata plants are Daucosterol (-8.2 kJ/mol), Stigmasterol (-8.1kJ/mol), Andropanoside (-7.8kJ/mol), Neoandrographolide (-7.7kJ/mol), Andrographidin A(-7.6kJ/mol), Andrographidine C(-7.6kJ/mol), Andrographidine E (-7.5kJ/mol) and Skullcap flavone I(-7.1kJ/mol). Phytocompounds of Gymnema sylvestre shows binding energy affinity include the beta Amyrin (–8.8 kJ/mol), Gymnemic acid IX (–8.7 kJ/mol), Gymnestrogenin (–8.2 kJ/mol), Dammarane (–7.8 kJ/mol), Stigmasterol (–7.5 kJ/mol), Resiniferonol (–6.8 kJ/mol), and Anthraquinone (–6.3 kJ/mol). Phytocompounds from *Plumbago auriculata* shows binding affinity include, β-Amyrin (–8.8 kJ/mol), Beta Sitosterol (–7.6 kJ/mol), Azaleatin (–7.4 kJ/mol), 2-(3,4- (–7.4 kJ/mol), Apigenin (–7.3 kJ/mol), and Luteolin (–7.1 kJ/mol).

After docking phytocompounds with their accession code and interactive amino acids are listed in Table- 1. The three dimensional and two-dimensional interactions of best scoring twenty-one phytocompounds that has best binding energy from the three plants and six antibiotics docked with the 6EFJ protein of NDM-1 beta lactamase are shown in figure 2.

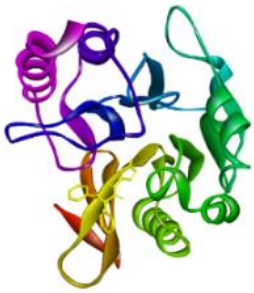
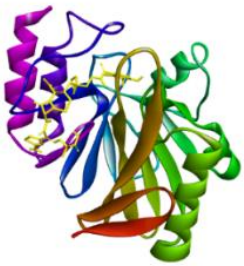
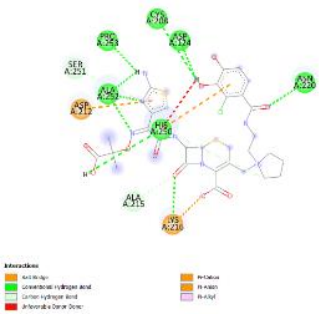
**Table 1.** Intermolecular interactions of the top docked phytocompounds and reference antibiotics with the NDM-1 protein (PDB ID: 6EFJ)

| Sl.no | Phytocompounds/ Control ligands | Ligand type/Accession code/Source | Binding energy (KJ/mol) | No. of Hydrogen bonds | Amino acid residues involved in Hydrogen bond formation | Amino acid residues involved in hydrophobic bond formation |
|---|---|---|---|---|---|---|
| 1 | (2*R*)-2-anilino-2-phenyl-*N*-(2*H*-tetrazol-5-yl) acetamide | Native ligand | 7.2 | 1 | HIS250 | VAL73<br>VAL73<br>HIS250 |
| 2 | Cefiderocol | Antibiotics | 8.5 | 10 | LYS216<br>LYS216<br>ASN220<br>ALA252<br>ALA252<br>ALA252<br>PRO253<br>HIS250<br>ASP124<br>CYS208 | ALA252 |
| 3 | Tigecycline | Antibiotics | 8 | 4 | LYS211<br>LYS211<br>LYS216<br>SER217 | HIS122<br>HIS250 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 4 | Ceftazidime | Antibiotics | 7.3 | 2 | MET67<br>ASP124 | TRP93<br>VAL73<br>LYS211<br>ALA215 |
| 5 | Aztreonam | Antibiotics | 6.7 | 8 | GLN123<br>GLN123<br>HIS189<br>CYS208<br>HIS250<br>GLU152<br>GLU152<br>GLU152 | HIS122 |
| 6 | Tetracycline | Antibiotics | 6.5 | - | - | VAL73 |
| 7 | Avibactam | Antibiotics | 6.2 | 9 | HIS120<br>HIS122<br>GLN123<br>ASP124<br>HIS189<br>CYS208<br>LYS211<br>HIS250<br>ASN22 | - |
| 8 | Daucosterol | IMPHY014838 (*Andrographis paniculata*) | 8.2 | 4 | HIS122<br>GLN123<br>GLU152<br>GLN123 | HIS250 |
| 9 | Stigmasterol | IMPHY014842 (*Andrographis paniculata*) | 8.1 | 1 | GLU152 | VAL73<br>VAL73<br>PHE70<br>PHE70 |
| 10 | Andropanoside | IMPHY014419 (*Andrographis paniculata*) | 7.8 | 3 | GLN123<br>ASN220<br>ASP223 | - |
| 11 | Neoandrographolide | IMPHY007326 (*Andrographis paniculata*) | 7.7 | 1 | - | VAL73 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 12 | Andrographidin A | IMPHY002527 (*Andrographis paniculata*) | 7.6 | 6 | CYS208 LYS211 ASN220 ASN220 ASN220 ASP124 | VAL73 LYS211 ALA215 |
| 13 | Andrographidine C | IMPHY005047 (*Andrographis paniculata*) | 7.6 | 4 | LYS211 ASN220 ASN220 ASP124 | LYS211 ALA215 |
| 14 | Andrographidine E | IMPHY014276 (*Andrographis paniculata*) | 7.5 | 4 | HIS122 GLY219 ASN220 ASP124 | VAL73 PHE70 VAL73 LYS211 ALA215 |
| 15 | Skullcap flavone I | IMPHY004375 (*Andrographis paniculata*) | 7.1 | 2 | HIS122 ASP124 | VAL73 TRP93 LEU65 MET67 |
| 16 | Beta Amyrin | IMPHY012223 (*Gymnema sylvestre*) | 8.8 | 1 | ASP212 | - |
| 17 | Gymnemic acid IX | IMPHY008866 (*Gymnema sylvestre*) | 8.7 | 7 | LYS216 SER217 ASP212 HIS250 ASP212 HIS250 GLN123 | MET154 |
| 18 | Gymnestrogenin | IMPHY001589 (*Gymnema sylvestre*) | 8.2 | 1 | HIS120 | VAL73 TRP93 TRP93 HIS250 VAL73 |
| 19 | Dammarane | IMPHY10964 (*Gymnema sylvestre*) | 7.8 | 0 | - | LEU65 VAL73 TRP93 HIS122 |
| 20 | Stigmasterol | IMPHY014842 (*Gymnema sylvestre*) | 7.5 | 1 | GLU152 | LEU65 MET67 VAL73 VAL73 PHE70 HIS122 |
| 21 | Resiniferonol | IMPHY012388 (*Gymnema sylvestre*) | 6.8 | 2 | ASN220 LEU218 | - |

| | | | | | | |
|---|---|---|---|---|---|---|
| 22 | Anthraquinone | IMPHY007192 (*Gymnema sylvestre*) | 6.3 | 1 | HIS250 | VAL73<br>TRP93<br>TRP93<br>HIS250<br>VAL73 |
| 23 | Alpha Amyrin | IMPHY011619 (*Plumbago auriculata)* | 8.9 | 2 | ASP212<br>ASP212 | VAL73<br>PHE70 |
| 24 | Beta Amyrin | IMPHY012223 (*Plumbago auriculata)* | 8.8 | 1 | SER251 | - |
| 25 | Beta Sitosterol | IMPHY014836 (*Plumbago auriculata)* | 7.6 | 1 | GLU152 | VAL73<br>VAL73<br>PHE70<br>PHE70<br>HIS122<br>HIS122 |
| 26 | Azaleatin | IMPHY012742 *(Plumbago auriculata)* | 7.4 | 7 | HIS122<br>HIS189<br>LYS211<br>ASP124<br>ASP124<br>CYS208<br>LEU218 | HIS250<br>HIS250<br>VAL73 |
| 27 | Apigenin | IMPHY004661 *(Plumbago auriculata)* | 7.3 | 3 | HIS250<br>GLY71<br>CYS208 | TRP93<br>VAL73<br>LEU65<br>MET67 |
| 28 | Luteolin | IMPHY004660 *(Plumbago auriculata)* | 7.1 | 4 | ASP124<br>LYS211<br>LYS211<br>LYS211 | HIS122<br>HIS250<br>HIS250 |

| Sl. No | 3D Structures | 2D Structures |
|---|---|---|
| 1 | Native ligand (2*R*)-2-anilino-2-phenyl-*N* (2*H*-tetrazol-5-yl) acetamide | |
| |  |  |
| 2 | Cefiderocol (Antibiotic) | |
| |  |  |
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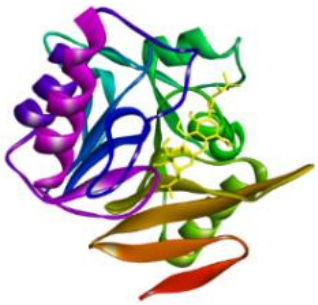
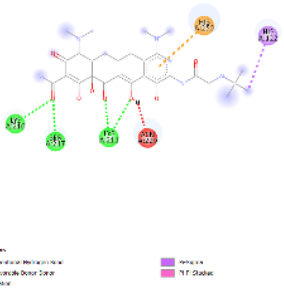
| |  |  |

| | | |
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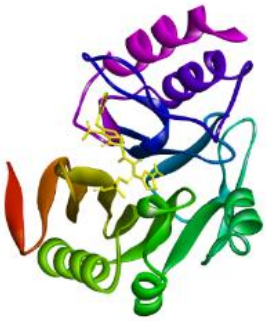
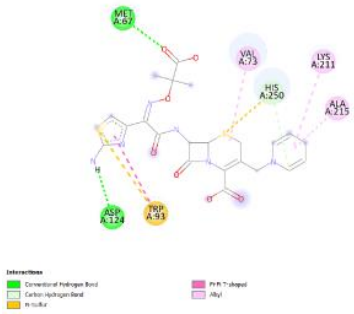
| |  |   |
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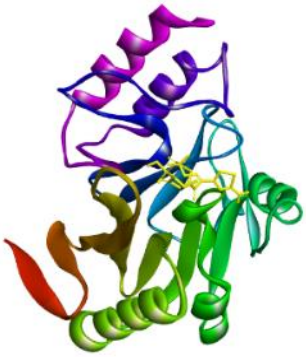
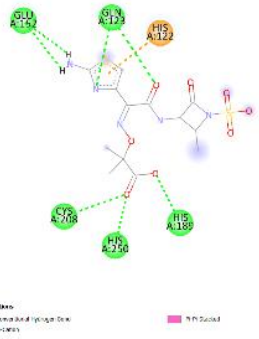
| |  |   |
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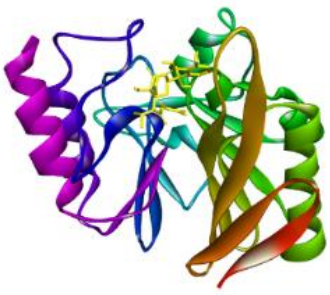
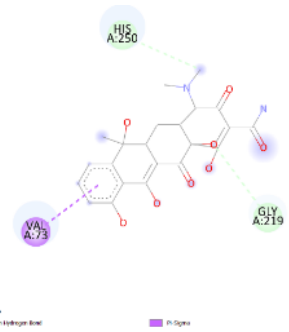
| |  |   |

| | | |
|---|---|---|
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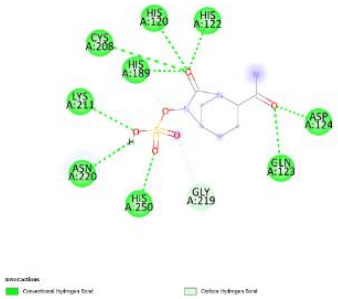
| |  |  |
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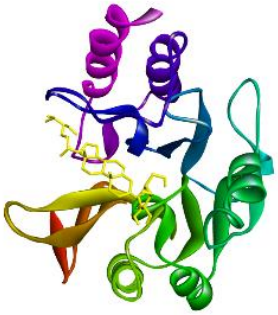
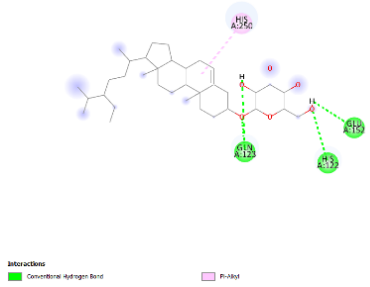
| |  |  |
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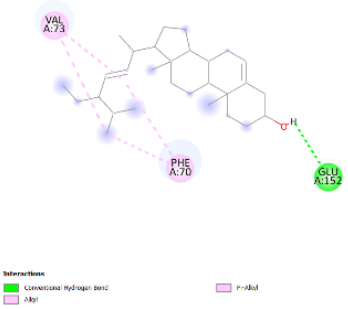
| |  |  |

| | | |
|---|---|---|
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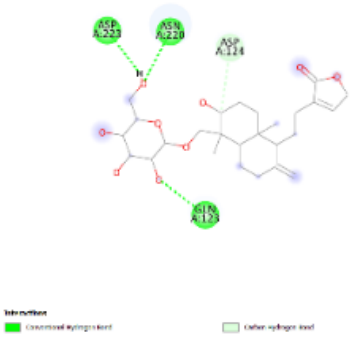
| |  |  |
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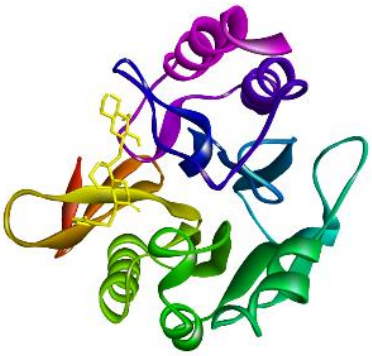
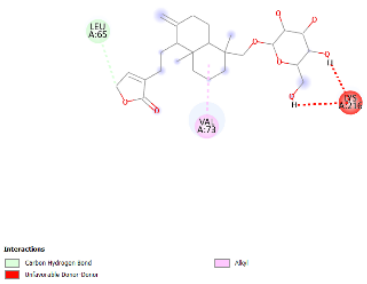
| |  |  |
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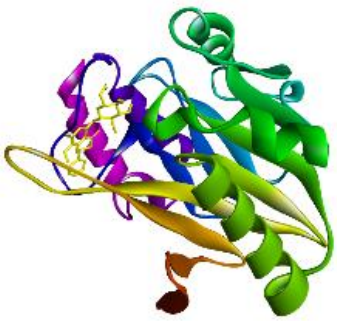
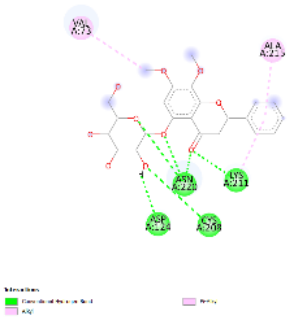
| |  |  |

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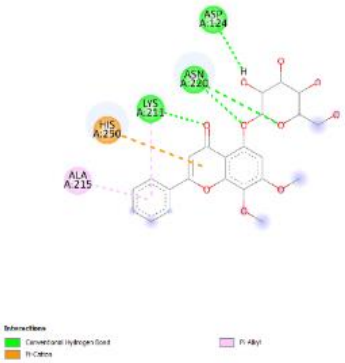
| |  |   |
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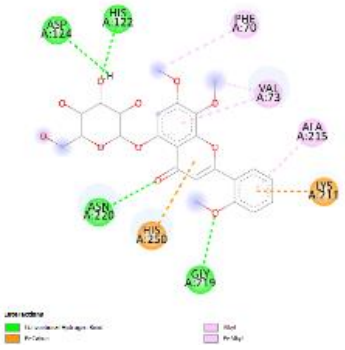
| |  |   |
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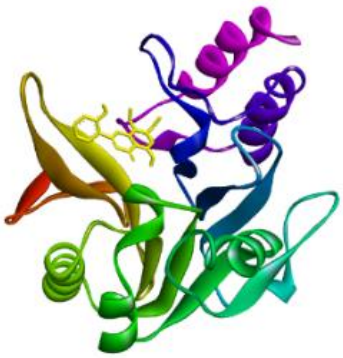
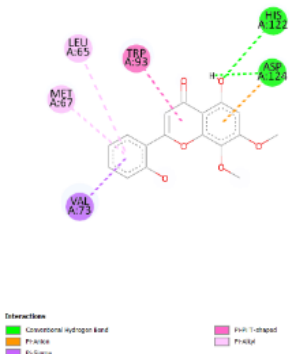
| |  |   |

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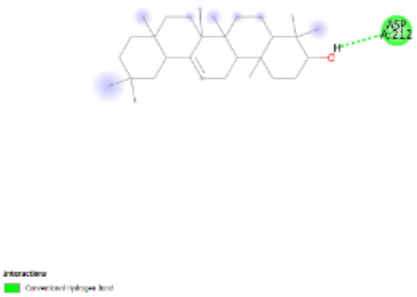
| |  |  |
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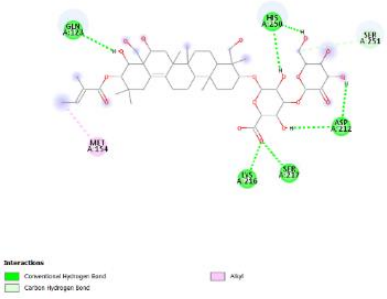
| |  |  |
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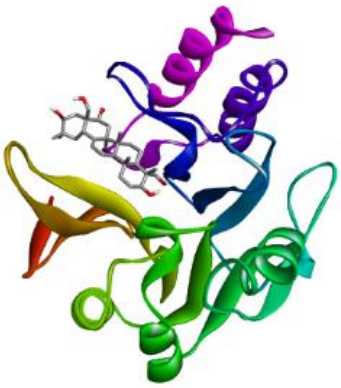
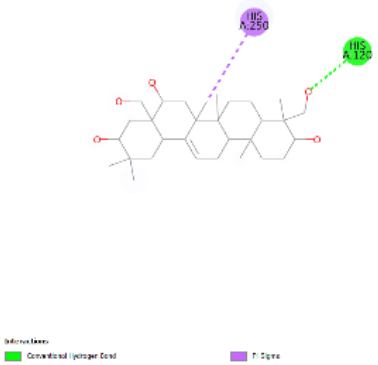
| |  |  |

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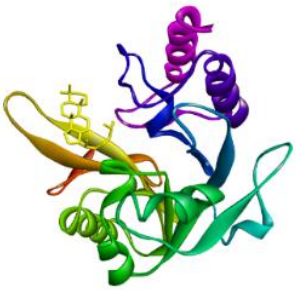
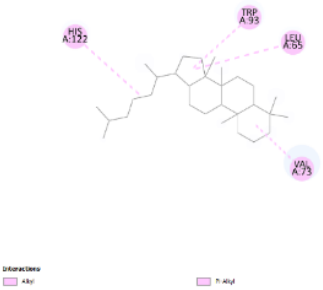
| |  |  |
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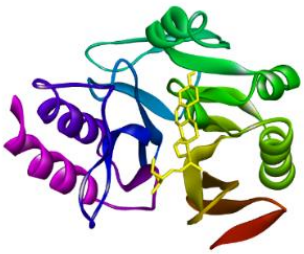
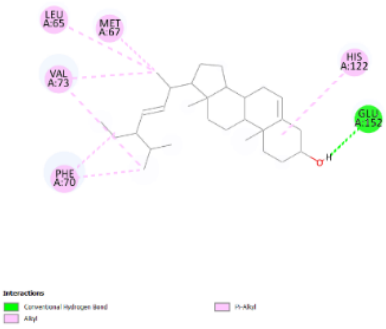
| |  |  |
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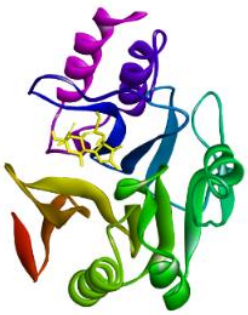
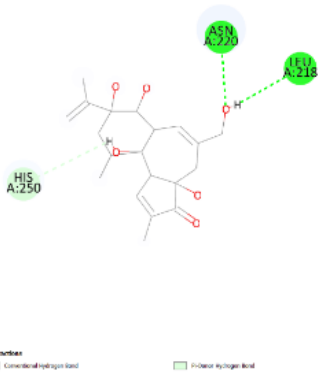
| |  |  |

| | | |
|---|---|---|
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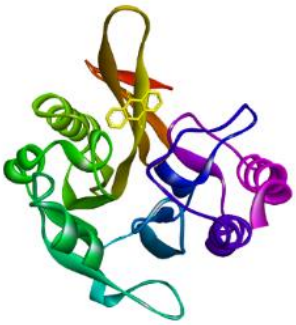
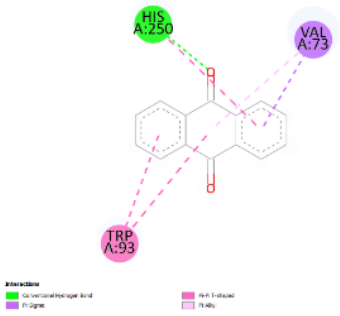
| |  |  |
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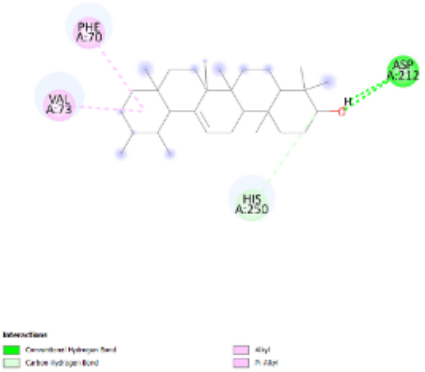
| |  |  |
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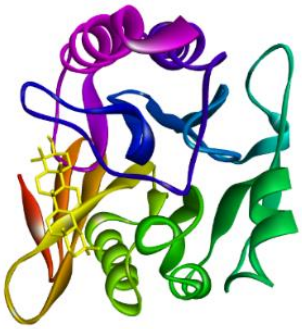
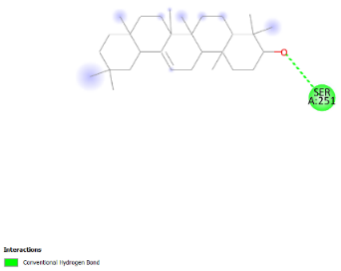
| |  |  |

| | | |
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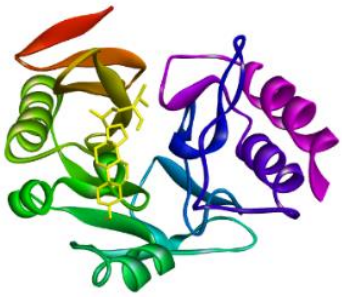
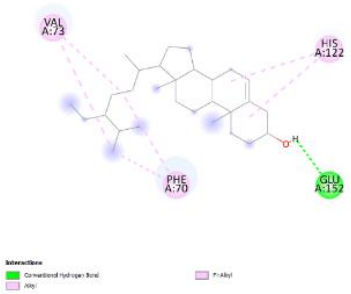
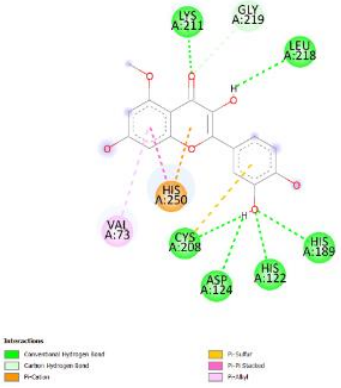
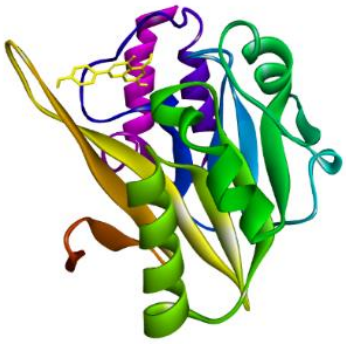
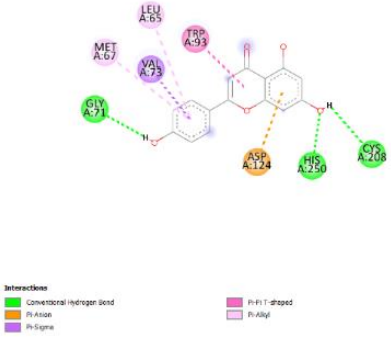
| |  |  |
| | Azaleatin | |
| |  |  |
| | Apigenin | |
| |  |  |

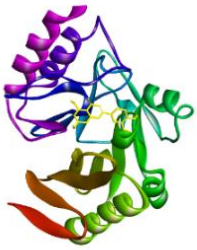
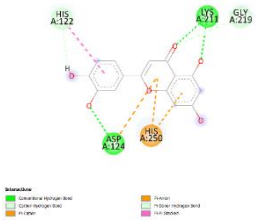
| | Luteolin | |
|---|---|---|
| |  |  |

**Fig. 2.** 2D and 3D molecular interaction diagrams of the best-scoring phytocompounds and standard antibiotics docked with NDM-1 protein. Hydrogen bonds are illustrated in green, and hydrophobic interactions are shown in orange and pink. Native ligand in complex with 6EFJ (NDM-1) has shown hydrogen bonding with the key active site of amino acid residues demonstrating the crucial interaction required for Beta-Lactamase inhibition.

### 3.2 ADMET Analysis

The three-dimensional structure of the *K. pneumoniae* NDM-1 beta-lactamase protein was visualized through Chimera software (version 1.8), as shown in Figure 3. The main results for the physiological, chemical characteristics and toxicological possibility of all twenty – one phytocompounds from the three medical plants *Andrographis paniculate*, *Gymnema sylvestre* and *Plumbago Auriculata* are shown in Table 2. Additionally, the predicted pharmacokinetic characteristics based on ADME parameters are summarized in Table 3. All the selected phytocompounds follows the Lipinski's Rule of Five. Most of their ADMET values are also under acceptable limits, which proves that they have a good-drug like properties. The metabolic activity of the compound was analysed with standard antibiotics. Since there are no results that shows interactions between the phytocompounds and the antibiotics, it confirms that the phytocompounds do not inhibit normal metabolic process. The log S values of the selected phytocompounds were all within the range of (-1 to -9), showing that they have solubility.

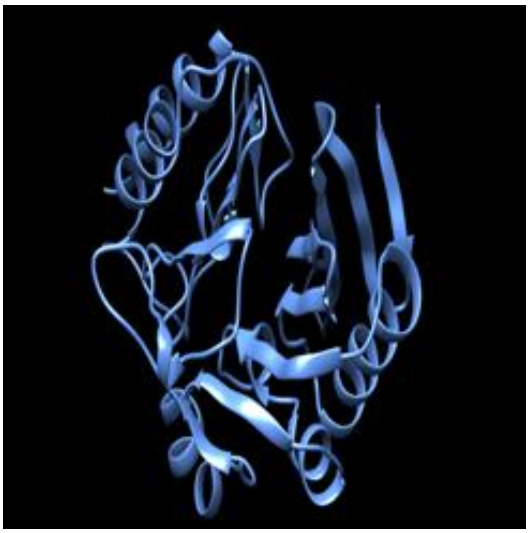


Fig. **3.** The 3D structure of beta-lactamase 6EFJ protein of *K. pneumoniae* observed by Chimera 1.8 software

**Table 2**. Physicochemical properties and predicted of cellular and genetic of dominant phytocompounds from *Andrographis paniculate, Gymnema sylvestre* and *Plumbago Auriculata*

| Source | Name (Accession code) | Lipophilicity | Water | Solubility | Pharmacokinetics | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Log Po/w (iLogP) | Log S (ESOL) | Class | CYP1A2 Inhibitor | CYP2C19 Inhibitor | CYP29 Inhibitor | CYP2D6 Inhibitor | CYP3A4 Inhibitor | Log Kp (Skin permeat ion) |
| Standard co | cefiderocol | -1.54 | -2.62 | Soluble | No | No | No | No | Yes | -12.07cm/s |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ntrol | | | | | | | | | | |
| Standard control | Tigecycline | 2.47 | -3.72 | Soluble | No | No | No | No | No | -9.11cm/s |
| Standard control | Ceftazidime | -2.68 | -1.37 | Very soluble | No | No | No | No | No | -11.23cm/s |
| Standard control | Aztreonam | 0.15 | -2.31 | Soluble | No | No | No | No | No | -8.76cm/s |
| Standard control | Tetracycline | 0.54 | -2.77 | Soluble | No | No | No | No | No | -8.83cm/s |
| Standard control | Avibactam | -0.36 | -0.13 | Very soluble | No | No | No | No | No | -9.22cm/s |
| *Andrographis paniculate* | Daucosterol | 5.17 | -7.70 | Poorly soluble | No | No | No | No | No | -4.32cm/s |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Andrographis paniculate* | Stigmasterol | 5.08 | -7.46 | Poorly soluble | No | No | Yes | No | No | -2.74 |
| *Andrographis paniculate* | Andropanoside | 3.58 | -3.26 | Soluble | No | No | No | No | No | -8.42cm/s |
| *Andrographis paniculate* | Neoandrographolide | 3.37 | -4.01 | Moderately soluble | No | No | No | No | Yes | -7.36 |
| *Andrographis paniculate* | Andrographidin A | 2.50 | -3.01 | Soluble | No | No | No | No | No | -8.63cm/s |
| *Andrographis paniculate* | Andrographidine C | 2.37 | -3.26 | Soluble | No | No | No | No | Yes | -8.43cm/s |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Andrographis paniculate* | Andrographidine E | 3.58 | -3.34 | Soluble | No | No | No | No | Yes | -8.63cm/s |
| *Andrographis paniculate* | Skullcap flavone I | 2.83 | -3.97 | Soluble | Yes | No | Yes | Yes | Yes | -6.12cm/s |
| *Gymnema Sylvestre* | Beta Amyrin | 4.63 | -8.25 | Poorly soluble | No | No | No | No | No | -2.41cm/s |
| *Gymnema Sylvestre* | Gymnemic acid IX | 2.42 | -4.94 | Poorly soluble | No | No | No | No | No | -6.64cm/s |
| *Gymnema Sylvestre* | Gymnestrogenin | 3.44 | -5.86 | Moderately soluble | No | No | No | No | No | -5.79cm/s |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Gymnema Sylvestre* | Dammarane | 5.61 | -9.72 | Insoluble | No | No | No | No | No | -0.22m/s |
| *Gymnema Sylvestre* | Stigmasterol | 5.08 | -7.46 | Poorly soluble | No | No | Yes | No | No | -2.74cm/s |
| *Gymnema Sylvestre* | Resiniferonol | 2.46 | -1.67 | Very soluble | No | No | No | No | No | -8.86cm/s |
| *Gymnema Sylvestre* | Anthraquinone | 1.94 | -3.82 | Moderately soluble | Yes | Yes | No | No | No | -5.6cm/s |
| *Plumbago Auriculata* | Alpha Amyrin | 4.80 | -8.16 | Poorly soluble | No | No | No | No | No | -2.51cm/s |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Plumbago Auriculata* | Beta Amyrin | 4.63 | -8.25 | Poorly soluble | No | No | No | No | No | -2.41cm/s |
| *Plumbago Auriculata* | Beta Sitosterol | 5.05 | -7.90 | Poorly soluble | No | No | No | No | No | -2.20cm/s |
| *Plumbago Auriculata* | Azaleatin | 1.71 | -3.02 | Soluble | Yes | No | No | Yes | Yes | -7.29cm/s |
| *Plumbago Auriculata* | Apigenin | 1.89 | -3.94 | Soluble | Yes | No | No | Yes | Yes | -5.80cm/s |
| *Plumbago Auriculata* | Luteolin | 1.86 | -3.71 | Soluble | Yes | No | No | Yes | Yes | -6.25cm/s |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | |

**Table 3**. SwissADME-predicted pharmacokinetic behaviour of phytocompounds from selected medicinal plants.

| Medicinal Plants\Source | Phytocompounds | GI Absorption | BBB Permeable | P-gp substrate | Lipinski violation | Veber | Egan |
|---|---|---|---|---|---|---|---|
| Standard control | Cefiderocol | Low | No | No | No | No | No |
| Standard control | Tigecycline | Low | No | Yes | No | No | No |
| Standard control | Ceftazidime | Low | No | No | No | No | No |
| Standard control | Aztreonam | Low | No | Yes | Yes | No | No |
| Standard control | Tetracycline | Low | No | Yes | Yes | No | No |
| Standard control | Avibactam | Low | No | No | Yes | Yes | No |
| *Andrographis paniculate* | Daucosterol | Low | No | No | Yes | Yes | Yes |
| *Andrographis paniculate* | Stigmasterol | Low | No | No | Yes | Yes | No |
| *Andrographis paniculate* | Andropanoside | Low | No | Yes | Yes | No | No |
| *Andrographis paniculate* | Neoandrographolide | High | No | Yes | Yes | Yes | Yes |
| *Andrographis paniculate* | Andrographidin A | Low | No | Yes | Yes | No | No |
| *Andrographis paniculate* | Andrographidine C | Low | No | Yes | Yes | No | No |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Andrographis paniculate* | Andrographidine E | Low | No | Yes | Yes | No | No |
| *Andrographis paniculate* | Skullcap flavone I | High | No | No | Yes | Yes | Yes |
| *Gymnema Sylvestre* | Beta Amyrin | Low | No | No | Yes | Yes | No |
| *Gymnema Sylvestre* | Gymnemic acid IX | Low | No | Yes | Yes | No | No |
| *Gymnema Sylvestre* | Gymnestrogenin | High | No | Yes | Yes | Yes | Yes |
| *Gymnema Sylvestre* | Dammarane | Low | No | No | Yes | Yes | No |
| *Gymnema Sylvestre* | Stigmasterol | Low | No | No | Yes | Yes | No |
| *Gymnema Sylvestre* | Resiniferonol | High | No | Yes | Yes | Yes | Yes |
| *Gymnema Sylvestre* | Anthraquinone | High | Yes | No | Yes | Yes | Yes |
| *Plumbago Auriculata* | Alpha Amyrin | Low | No | No | Yes | Yes | No |
| *Plumbago Auriculata* | Beta Amyrin | Low | No | No | Yes | Yes | No |
| *Plumbago Auriculata* | Beta Sitosterol | Low | No | No | Yes | Yes | No |
| *Plumbago Auriculata* | Azaleatin | High | No | No | Yes | Yes | Yes |
| *Plumbago Auriculata* | Apigenin | High | No | No | Yes | Yes | Yes |
| *Plumbago Auriculata* | Luteolin | High | No | No | Yes | Yes | Yes |

### 3.3 ProTox analysis

The ProTox- III server is used to see the toxicity of all selected phytocompounds and the results are shown in Table 4. In total four hundred and forty phytocompounds of all the three plants, only twenty-one phytocompounds among the selected plants has shown one instance of violation and immunological toxicity. After analysation of molecular drug likeness and toxicity data, phytocompounds from all three selected medicinal plants that shows the strongest safe phytocompounds based on toxicity for drug development are Alpha-Amyrin of *Plumbago auriculata* and *Beta Amyrin* of both *Gymnema sylvestre* and *Plumbago auriculata* with Highest LD50 (70000 mg/kg) in Class 6 (low toxicity) and mostly non-toxic in all subcategories , *Daucosterol* of *Andrographis paniculate* and Resiniferonol of *Gymnema sylvestre* with (LD50 (80000 mg/kg) and LD50 (90000 mg/kg respectively)) in Class 6 and mostly non-toxic emerged as the most effective phytocompounds for inhibiting the beta lactamase enzyme.

Table 4. Predicted toxicity profile of Bile Component and Active Phytocompounds based of PROTOX-III analysis

| Sources | Phytocompounds | PROTOX-III | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | LD50 (mg/KG) | class | Hepato-Toxicity | Carcino-Genicity | Immune-Toxicity | Muta-Genicity | Cyto-Toxicity |
| Standard Control | Cefiderocol | 10000 | 6 | Inactive | Inactive | Active | Inactive | Inactive |
| Standard Control | Tigecycline | 3000 | 5 | Active | Inactive | Active | Inactive | Inactive |
| Standard Control | Ceftazidime | 10000 | 6 | Inactive | Inactive | Inactive | Inactive | Inactive |
| Standard Control | Aztreonam | 10000 | 6 | Inactive | Inactive | Inactive | Inactive | Inactive |
| Standard Control | Tetracycline | 4400 | 4 | Active | Inactive | Active | Inactive | Inactive |
| Standard Control | Avibactam | 1500 | 4 | Inactive | Inactive | Active | Inactive | Inactive |
| *Andrographis* | Daucosterol | 8000 | 6 | Inactive | Inactive | Active | Inactive | Inactive |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *paniculate* | | | | | | | | |
| *Andrographis paniculate* | Stigmasterol | 890 | 4 | Inactive | Inactive | Active | Inactive | Inactive |
| *Andrographis paniculate* | Andropanoside | 5 | 1 | Inactive | Inactive | Active | Inactive | Inactive |
| *Andrographis paniculate* | Neoandrographolide | 5 | 1 | Inactive | Inactive | Active | Inactive | Active |
| *Andrographis paniculate* | Andrographidin A | 3000 | 5 | Inactive | Inactive | Active | Active | Inactive |
| *Andrographis paniculate* | Andrographidine C | 5000 | 5 | Inactive | Inactive | Active | Inactive | Active |
| *Andrographis paniculate* | Andrographidine E | 5000 | 5 | Inactive | Inactive | Active | Inactive | Active |
| *Andrographis paniculate* | Skullcapflavone I | 4000 | 5 | Inactive | Inactive | Active | Inactive | Inactive |
| *Gymnema Sylvestre* | Beta Amyrin | 70000 | 6 | Inactive | Inactive | Active | Inactive | Inactive |
| *Gymnema Sylvestre* | Gymnemic acid IX | 1750 | 4 | Inactive | Inactive | Active | Inactive | Inactive |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Gymnema Sylvestre* | Gymnestrogenin | 4300 | 5 | Inactive | Inactive | Active | Inactive | Inactive |
| *Gymnema Sylvestre* | Dammarane | 3660 | 5 | Inactive | Inactive | Active | Inactive | Inactive |
| *Gymnema Sylvestre* | Stigmasterol | 890 | 4 | Inactive | Inactive | Active | Inactive | Inactive |
| *Gymnema Sylvestre* | Resiniferonol | 9000 | 6 | Inactive | Inactive | Active | Inactive | Inactive |
| *Gymnema Sylvestre* | Anthraquinone | 5000 | 5 | Inactive | Inactive | Inactive | Active | Inactive |
| *Plumbago Auriculata* | Alpha Amyrin | 7000 | 6 | Inactive | Inactive | Active | Inactive | Inactive |
| *Plumbago Auriculata* | Beta Amyrin | 7000 | 6 | Inactive | Inactive | Active | Inactive | Inactive |
| *Plumbago Auriculata* | Beta Sitosterol | 890 | 4 | Inactive | Inactive | Active | Inactive | Inactive |
| *Plumbago Auriculata* | Azaleatin | 5000 | 5 | Inactive | Active | Active | Active | Inactive |
| *Plumbago Auriculata* | Apigenin | 2500 | 5 | Inactive | Inactive | Active | Inactive | Active |
| *Plumbago Auriculata* | Luteolin | 3913 | 5 | Inactive | Active | Inactive | Active | Inactive |

## 4. Discussion

New Delhi metallo-β-lactamase-1 (NDM-1) an antibiotic resistance has become a critical threat to global public health concern, particularly due to its power to inactivate most β-lactam antibiotics, including carbapenems [22]. In few years, medicinal plants have received

attention as sources of new therapeutic compounds with antibacterial potential [23]. The adoption of *in silico* screening has demonstrate strong efficacy for identifying lead compounds because computational approaches allow rapid prediction of binding affinity, molecular behaviour, and drug-likeness before laboratory testing [24–25].

In the present study, phytocompounds from *Andrographis paniculata*, *Gymnema sylvestre*, and *Plumbago auriculata* were evaluated against NDM-1 using molecular docking. Among the screened phytocompounds Alpha-Amyrin, Beta -Amyrin shows best binding affinity to β -lactamase compared to standard antibiotics such as Cefiderocol and Tigecycline. Additionally, Alpha-Amyrin shows the best binding affinity (8.9 kl/mol) with a desirable ADMET and toxicity profile, demonstrate strong potential as a lead inhibitor against β -lactamase mediated antibiotic resistance similar to findings in previous docking and inhibitor modelling studies [26–27].

AutoDock Vina has been used for molecular docking analysis effectively predicted ligand-protein interaction. This research aligns with previous research demonstrating the software's accuracy and efficiency in virtual screening [28]. The phytocompounds showing the strongest binding primarily belonged to terpenoid and steroidal structural classes, which are widely reported for their antimicrobial behavior and ability to establish strong hydrophobic and van der Waals interactions within protein active sites [29–30]. Among them, Alpha-Amyrin and Beta -Amyrin demonstrated particularly high binding affinity, which may be attributed to their large hydrophobic scaffolds that enhance nonpolar interactions with the receptor cavity. Additionally, the presence of multiple hydroxyl groups in their structures facilitates the formation of hydrogen bonds with key catalytic residues, contributing to improved ligand stabilization. Their molecular size and steric complementarity further support deeper accommodation within the binding pocket, which likely resulted in more stable and energetically favorable ligand–protein complexes.

ADMET profiling further supported the therapeutic potential of the screened compounds. Most phytochemicals complied with Lipinski's Rule of Five and demonstrated acceptable pharmacokinetic features, which is comparable to previous virtual drug-screening studies using SwissADME and similar computational pipelines [31]. ProTox-III toxicity analysis shows that most phytocompounds safe or moderately safe, supporting their potential for future drug development [32].

While the results are encouraging but this research is limited to computational analysis. Docking scores and ProTox-III toxicity prediction cannot confirm metabolic behaviour and stability, or therapeutic efficacy. Thus, *in vitro* and *in vivo* validation are essential to verify enzyme inhibition, assess cytotoxicity, and explore potential synergistic effects with existing antibiotics [32]. Such laboratory validation is crucial before considering formulation development or clinical translation.

## 5. Conclusion

This study conducted an in-silico evaluation of phytocompounds selected from three different medicinal plants found in India are *Andrographis paniculate, Gymnema sylvestre* and *Plumbago Auriculata* found to identify potential inhibitors of the NDM-1 enzyme responsible for β-lactam antibiotic resistance in *K. pneumoniae*. Molecular docking result shows that several phytocompounds exhibited strong binding affinity toward the NDM-1 active site, comparable to or higher than standard β-lactam antibiotics. Among all screened

phytocompounds Alpha-Amyrin from *Plumbago auriculata* showed the highest binding affinity (–8.9 kcal/mol), followed closely by Beta-Amyrin (–8.8 kcal/mol) from both *Gymnema sylvestre* and *Plumbago auriculata*. Additional compounds, including Daucosterol, Stigmasterol, Gymnemic acid IX, and Neoandrographolide, also displayed favorable docking scores and key interactions with catalytic residues.
ADMET and toxicity screening further indicated that the selected phytocompounds possess acceptable pharmacokinetic behavior with low predicted toxicity, demonstrating favorable gastrointestinal absorption profiles and good drug-likeness features. Solubility varied across compounds, with several exhibiting low solubility but acceptable permeability. Toxicity prediction suggests that Alpha-Amyrin, Beta-Amyrin, Daucosterol, and Resiniferonol possess the most favorable safety profiles, demonstrating high LD50 values and low toxicity classes. Therefore, these phytocompounds stand out as promising safe candidates for further optimization and experimental validation as potential NDM-1 β-lactamase inhibitors.

While these computational results provide encouraging evidence, experimental validation through in vitro enzymatic inhibition assays and in vivo studies is essential to confirm their therapeutic efficacy and safety. Future work may also include molecular dynamics simulations and structure-based optimization to enhance inhibitory potential.

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