Full Paper

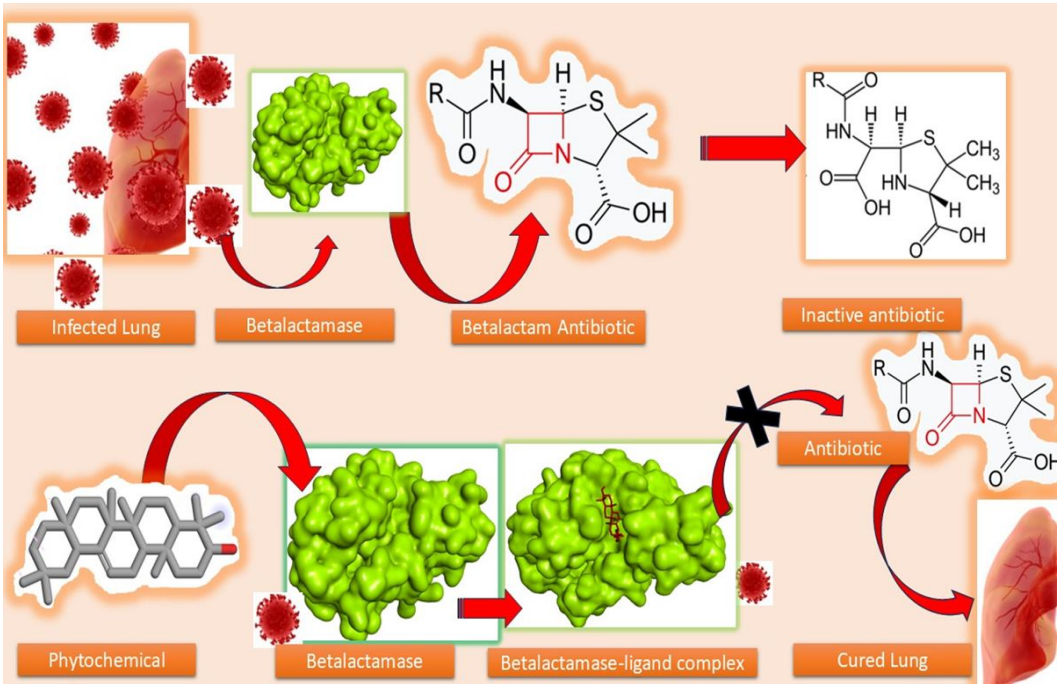
SciRad SCIENTIAE RADICES

Inhibition of class A beta lactamases of *K. pneumoniae* by the selected phytochemicals- Computational study

Bindu T. K.[1], Vinod P. Raphael[2], Reeja Johnson[3]✉, Hidhayathunnisa A. V.[4], Syamily Sivadas[5]

[1,2]Department of Chemistry, Government Engineering College Thrissur, Kerala, India-680009
[3]Department of Chemistry, St. Thomas College (Autonomous), Thrissur, India-680001 (Affiliated to University of Calicut)
[4]Department of Chemistry, Mother Arts and Science College Peruvallur, India-680508 (Affiliated to University of Calicut)
[5]Department of Chemistry, St. Mary's College Thrissur, India-680020 (Affiliated to University of Calicut)

✉ Correspondence to: reejajoh@gmail.com



Abstract: The rise of beta-lactamase-producing bacteria poses a significant threat in healthcare. These bacteria render common antibiotics ineffective, necessitating stronger options with potentially harsher side effects. To combat these resistant strains, researchers are exploring two avenues: developing new antibiotics and creating drugs that inhibit beta-lactamase activity. This study focused on the

anti-beta-lactamase potential of 27 phytochemicals against class A Extended-Spectrum Beta-Lactamases (ESBLs) of *Klebsiella pneumoniae*, specifically CTX-M-15, SHV-1, SHV-2 and KPC-2, using molecular docking simulations. Docking analysis and ADME (Absorption, Distribution, Metabolism, and Excretion) predictions were performed using CB-Dock v.2 and SwissADME web servers, respectively. Phytochemicals derived from *Brideliastipularis*, *Andrographis paniculata*, quercetin derivatives, and withanolides were screened for their inhibitory activity against beta-lactamases. Interestingly, withanolides, a class of steroidal compounds, displayed high inhibition potential. Among these, withanolide B exhibited remarkable inhibitory activity against three beta-lactamase enzymes, with the SHV-2-withanolide B complex demonstrating a binding score of -10.2 kcal/mol. This study primarily discusses phytochemicals with high binding scores and favorable drug-like properties.

Keywords: ESBL, *Klebsiella pneumoniae*, phytochemicals, molecular docking, ADME

Received: 2025.09.09
Accepted: 2025.10.20
Published: 2025.11.04
DOI: 10.58332/scirad2025v4i4a01

Introduction

Beta-lactam antibiotics are some of the most powerful weapons in our fight against bacterial infections [1-3]. However, bacteria are constantly evolving ways to resist these drugs, and one of the most common mechanisms is the production of enzymes called beta-lactamases [4,5]

Beta-lactam antibiotics have a specific ring structure, essential for their function. They act like scissors, cutting open this ring and rendering the antibiotic ineffective. There are four main classes of beta-lactamases (A, B, C, and D), each with different molecular structures [6,7]. Three enzymes namely A, C, and D destroy the antibiotic with the help of a special amino acid called serine. This creates a temporary bond between the enzyme and the antibiotic. Then, water comes in and breaks the antibiotic apart permanently. Class B beta-lactamases work differently. These are like tiny factories equipped with zinc ions as tools. These zinc ions help activate a water molecule, turning it into a highly reactive weapon that directly attacks the antibiotic's ring structure and dismantles it [8-13].

Class A beta-lactamases are particularly concerning because they are like antibiotic hitchhikers. They often live on tiny packages of DNA called plasmids, which can jump between bacteria. This makes them easily shared, allowing resistance to spread quickly [14]. These enzymes are common in Gram-negative bacteria that cause hospital infections,

nicknamed the ESKAPE pathogens. This group of pathogens includes familiar foes like *E. coli* and *K. pneumoniae*. Class A enzymes especially love to break down penicillins. Just like any army facing new weapons, bacteria developed ways to resist these new strategies. Some Class A enzymes mutated, gaining the ability to break down the newer oxyimino-cephalosporins. Others learned to evade the drugs designed to stop them.

Extended spectrum beta lactamases (ESBL) [15,16], are enzymes which can render certain antibiotics ineffective. Scientists classify ESBLs into different groups based on their structure and origin. Notably, they are also carbapenemases, even more powerful resistance enzymes, which can further complicate treatment. In this work a main concern is given for class A ESBLs of *K. pneumoniae* such as CTX-M, SHV, and KPC enzymes.

Clavulanate, sulbactam, tazobactam, avibactam, vaborbactam, relebactam, and durlobactam are a class of drugs called beta-lactamase inhibitors [17,18]. While they have minimal antibacterial activity on their own, they can significantly inhibit the action of certain beta-lactamase enzymes produced by bacteria. Beta-lactamase inhibitors, in combination with specific antibiotics, can restore their effectiveness against the resistant bacteria.

Phytochemicals as beta lactamase inhibitors

Withanolides, comprising a group of at least 300 naturally occurring steroids, are predominantly found as secondary metabolites within Nightshade family genera, like the tomatillo. Structurally, they feature a steroid backbone linked to a lactone or one of its derivatives. Computational techniques employed by H S K et al revealed that both Withanolide A and Withanolide R exhibit notable binding efficiency on *Stenotrophomonas maltophilia* beta lactamase [19]. *In silico* studies by Zainab Bibi et al. identified butein, monodemethylcurcumin, and rosmarinic acid (all polyphenols) as potential inhibitors of the metallo-β-lactamase NDM-1 [20]. An *in silico* study by FaeghehEtminani et al. evaluated potential Staphylococcus aureus β-lactamase inhibitors from *Rosmarinus officinalis*, *Ocimum basilicum*, *Eucalyptus globulus*, and *Thymus vulgaris*. Among the studied phytochemicals, carvotanacetone from *E. globulus*, α-terpineol from *R. officinalis* and *T. vulgaris,* and 3,7-dimethyloct-1,5-dien-3,7-diol from *O. basilicum* showed the strongest binding affinities [21]. Sweet basil (*Ocimum basilicum*) essential oil contains mostly methyl cinnamate, which inhibited CTX-M type ESBL β-lactamases in a study by Nagwa A. Shoeib et al. Methyl cinnamate's efficacy (IC50 11.6 µg/mL) was comparable to clavulanic acid (IC50 8.1 µg/mL), a standard inhibitor. This suggests that methyl cinnamate is a potential new treatment for β-lactamase-producing bacteria [22].

Present study

This computational study aimed to evaluate the potential of various phytochemicals derived from well-known medicinal plants like *Bridelia stipularis* and *Andrographis paniculata*, both known for their therapeutic properties. Additionally, quercetin derivatives and withanolides were included in the *in silico* investigation.

The stem bark and leaves of *Bridelia stipularis* are reported to contain bioactive steroids and triterpenoids [23,24]. In this work, the flavonoids isolated from the methanolic extract of leaves of *Bridelia stipularis* were identified as three known flavonoids: 7-O-methyl luteolin, apigenin, and 5, 7, 2′, 5′ tetrahydroxyflavone and a six ring structured molecule beta-amyrin. These compounds were investigated computationally for their interaction with beta-lactamases.

Andrographolide, the major bioactive compound extracted from *Andrographis paniculata*, possesses several beneficial properties [25,26]. Among its analogues, 14-deoxyandrographolide exhibits immunomodulatory and anti-atherosclerotic effects, while neoandrographolide has anti-inflammatory, anti-infective, and anti-hepatotoxic properties. In this study, we investigated the beta-lactamase inhibition capacities of these andrographolide analogues. In a separate investigation, we found out that [27] Bulnesene and Bisabolene epoxide are also present in the methanolic extract of *Andrographis paniculata* leaves. Bulnesene and its derivatives are known for their anti-allergic properties, while Bisabolene derivatives possess anti-cancer effects. In addition to andrographolides, these compounds were selected for further computational analysis to check for the beta-lactamase inhibition capacity.

Quercetin, along with several related molecules, have been shown to have anticancer, anti-inflammatory, anti-angiogenesis, anti-oxidant, anticoagulant properties. These related molecules including rutin, quercitrin, isoquercitrin, isorhamnetin, tamarixetin, rhamnetin, and hyperoside were selected for anti beta-lactamase studies[28–30].

Withanolides are a fascinating group of naturally occurring molecules found primarily in plants of the Nightshade family, including the tomatillo [31,32]. They are considered as secondary metabolites, meaning they are not essential for the plant's basic survival but may play a role in defense. Withanolides have been shown to possess antitumor, cytotoxic, and apoptotic (cell death) properties, suggesting potential in cancer treatment. Additionally, they exhibit anti-inflammatory, immune-modulating, and antimicrobial activity [33,34]. Withanolides have received the most attention due to its wide range of these beneficial activities. This suggests exciting possibilities for using withanolides in future drug

development. Nine withanolides have been selected for computational investigation in the present work.

Results and discussion

Out of twenty-seven compounds selected for *in silico* investigation, we found that 14 compounds exhibited high binding affinities to various class A beta-lactamases of *K. pneumoniae*. Eleven compounds showed significant binding affinities towards the SHV-2 enzyme with the PDB ID: 1N9B. Although receptors 1N9B and 4ZAM have nearly identical sequences and conformations, the former exhibited high binding affinity (> 9.0 kcal/mol binding energy) towards various phytochemicals. The most active compound in *B. stipularis* was beta amyrin which showed -9.5 kcal/mol with the SHV-2 enzyme. The KPC-2 enzyme of the bacterium also displayed a strong binding affinity with withanolides. Five withanolides and one quercetin derivative (Rhamnazin) effectively inhibited the CTX-M-15 beta-lactamase of *K. pneumoniae*.

Table 1. Binding scores of various phytochemicals (kcal/mol) on the beta lactamases of *K. pneumoniae*

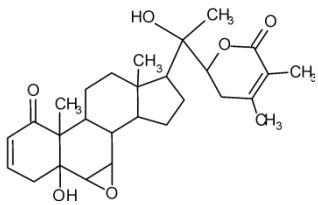
	4ZAM	1N9B	4Zbe	7BDS
Phytochemicals from *Brideliastipularis*				
7-O-methyl luteolin (5318214)*	-8.3	-8.4	-7.9	-8.5
Apigenin (5280443)	-8.6	-8.6	-7.7	-8.0
5,7,2′,5′ tetrahydroxyflavone (5487756)	-8.0	-8.1	-7.7	-7.5
Beta amyrin (73145)	-8.6	-9.5	-8.1	-8.2
Phytochemicals from *Andrographis paniculata*				
Bisabolene epoxide (91749653)	-7.6	-10.2	-6.2	-6.0
Alpha bulnesene (94275)	-5.9	-5.6	-6.5	-6.2
Andrographolide (5318517)	-8.3	-8.6	-7.1	-7.2
1,4-deoxyandrographolide (11624161)	-7.9	-8.3	-7.2	-6.8
Neoandrographolide (9848024)	-8.2	-8.1	-8.4	-8.0
Quercetin derivatives				
Quercetin (5280343)	-8.0	-8.1	-8.3	-8.3
Quercitrin (5280459)	-8.5	-9.0	-8.5	-7.9

Isoquercetin (5280804)	-8.0	-8.8	-8.1	-7.9
Rhamnetin (5281691)	-8.5	-8.6	-8.5	-8.4
Isorhamnetin (5281654)	-7.8	-9.1	-8.1	-8.6
Rhamnazin (5320945)	-8.1	-8.1	-8.0	-9.0
Rutin (5280805)	-8.1	-9.2	-8.7	-9.1
Tamarixetin (5281699)	-7.9	-8.4	-8.4	-8.4
Hyperoside (5281643)	-7.9	-8.4	-8.8	-8.1
Withanolides				
Withanolide A (11294368)	-9.2	-9.9	-9.3	-8.0
27-hydroxywithanone (21574483)	-8.2	-9.7	-8.4	-9.3
Dihydrowithaferin A (418033)	-9.0	-8.5	-8.8	-8.2
Viscolactone B (57403080)	-8.9	-8.6	-9.2	-9.7
Withaferin A (265237)	-9.2	-9.6	-8.8	-8.5
Withanolide B (14236711)	-9.7	-10.2	-10.1	-9.0
Withanone (21679027)	-8.9	-9.3	-8.5	-8.4
Withanoside IV (71312551)	-8.7	-9.4	-9.1	-8.8
Withanoside V (10700345)	-8.8	-10.1	-9.7	-9.5

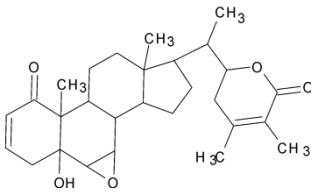
*PubChem CID

In general, quercetin derivatives (flavonoids) and withanolides showed high binding affinities (>9.0 kcal/mol) on the beta-lactamases. These binding affinities were significantly greater than those of standard beta-lactamase inhibitors (Table 1). The highest binding affinity of -10.2 kcal/mol was exhibited by the 1N9B-withanolide B complex. Withanoside V and Withanolide B also displayed binding affinities of -10.1 kcal/mol with SHV-2 and KPC-2 enzymes of *K. pneumoniae*. Detailed analysis of protein-ligand complexes showing magnitude of binding energies greater than or equal to 9 kcal/mol will be discussed in subsequent paragraphs.

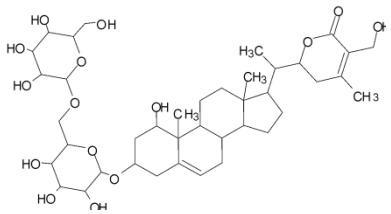
Molecular structures of compounds which displayed high binding scores on the various beta lactamase enzymes are given in the Figure 1.



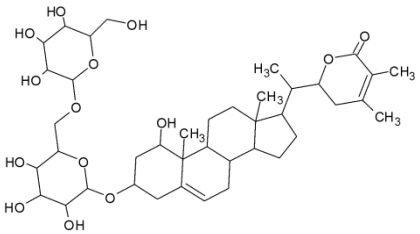
Withanolide A



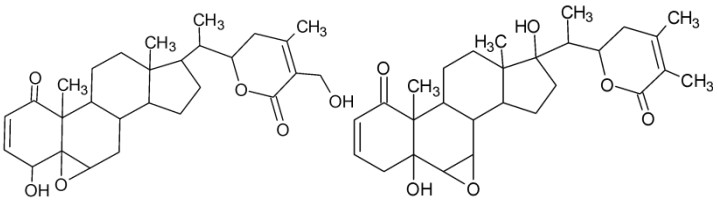
Withanolide B



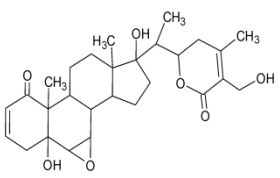
Withanoside IV



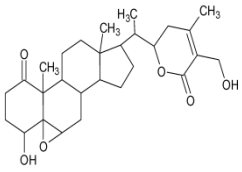
Withanoside V



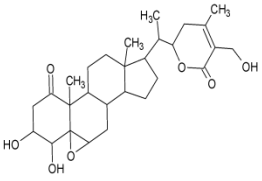
Withaferin A Withanone



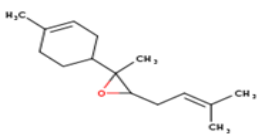
27-hydroxy withanone



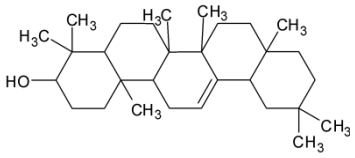
DihydrowithaferinA



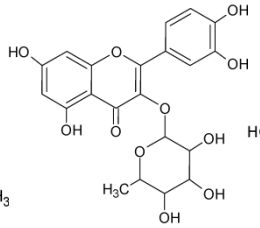
Viscolactone B



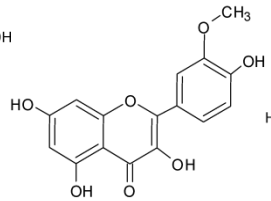
Alpha bisabolene epoxide



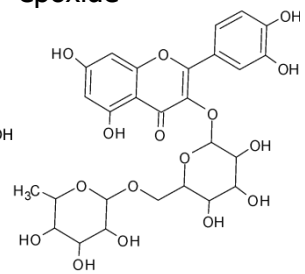
Beta amyrin



Quercitrin



Isorhamnetin



Rutin

Fig. 1. Structures of molecules which displayed high binding scores on the various beta lactamase enzymes

Inhibition studies of Phytochemicals present in *Bridelia stipularis*

Among the four medicinal compounds present in the *Bridelia stipularis* plant, only beta amyrin displayed a very high binding score on the beta lactamase (-9.5 kcal/mol). Other compounds displayed binding energies ranging from 7.5-8.6 kcal/mol. Apigenin, a trihydroxyflavone having antioxidant and anti-inflammatory properties, showed good binding scores (i.e., -8.6 kcal/mol) on SHV-1 and SHV-2 beta lactamases of *K. pneumoniae*. 7-O-

methyl luteolin was active on the CTM-X receptor and showed -8.5 kcal/mol binding energy. Other interactions between the phytochemicals and the receptors showed only moderate binding scores.

The main interactions displayed by the beta-amyrin-1N9B complex are the strong three pi-alkyl interactions originating from the Tyr105 residue of the receptor. The large, non-planar ring system of beta-amyrin did not make any hydrophilic interactions with the binding pocket and showed a binding energy of -9.5 kcal/mol. Beta-amyrin also showed good binding affinity on the SHV-1 enzyme (-8.6 kcal/mol). Fig. 2 represents the 2D and 3D interactions of beta-amyrin with the SHV-2 enzyme (PDB: 1N9B)

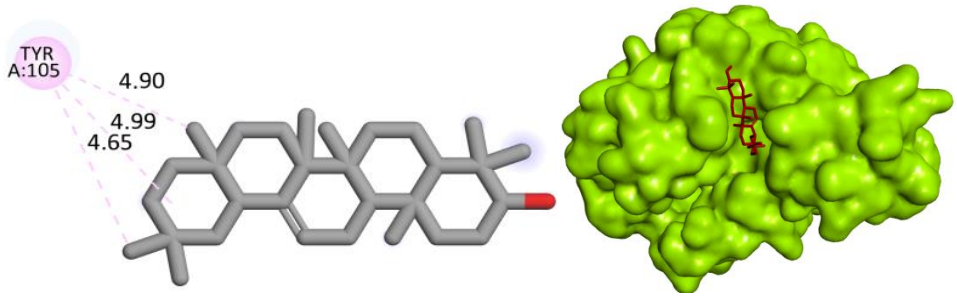


Fig. 2. 2D and 3D interaction plots of beta-amyrin with the SHV-2 enzyme (1N9B)

While beta-amyrin displayed a high binding score on SHV-2 enzyme, its ADME properties deviated from the average behavior of drug-like molecules. The SwissADME server predicted that the molecule's lipophilicity and insolubility fall outside the recommended range. Additionally, the molecule violated Lipinski's Rule of Five with an MLogP greater than 4.15 (Table 2).

Inhibition studies of Phytochemicals present in *Andrographis paniculata*

The main phytochemicals in *A. paniculata*, such as bisabolene epoxide, bulnesene, and various andrographolides, were screened against the various beta-lactamases of *K. pneumoniae*. Bisabolene epoxide, a bicyclic compound, showed high activity against the SHV-2 enzyme (PDB: 1N9B), with a maximum binding score of -10.2 kcal/mol. Interestingly, it exhibited poor binding affinity towards the other three beta-lactamase enzymes. Alpha-bulnesene displayed weak inhibitory effects on the beta-lactamases. Andrographolides, well-known phytochemicals with diverse medicinal properties, showed only moderate binding scores against the class A beta-lactamases of *K. pneumoniae*.

The structural evaluation of the 1N9B-bisabolene epoxide complex revealed sixteen alkyl hydrophobic interactions and one non-conventional hydrogen interaction. Gly283 made one carbon-hydrogen bond with the epoxide oxygen atom of the molecule (2.3 Å). These sixteen alkyl interactions occurred between the amino acid residues Val224, 261; Ala248, 280; Leu221, 225, 250; and Ile221, 231, 246, and various hydrophobic regions of the molecule (3.66-5.41 Å). The interaction plots of the receptor-ligand complex are shown in Fig. 3 which illustrate the most suitable confirmation of the molecule in the binding site. SwissADME predicted favorable drug-like properties for bisabolene epoxide. All parameters fell within acceptable ranges, indicating compliance with Lipinski's rule. The molecule has good gastrointestinal absorption potential as per ADME analysis.

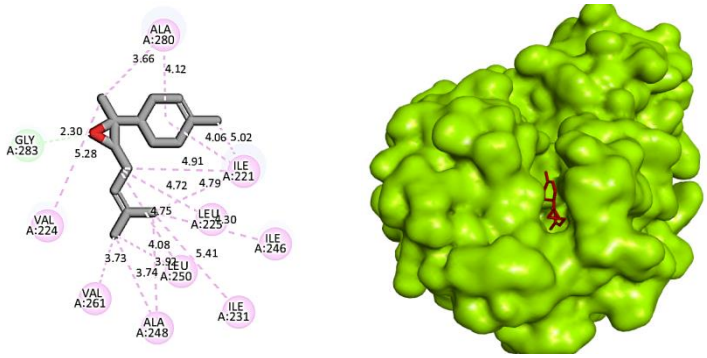


Fig. 3. 2D and 3D interaction plots of the 1N9B-bisabolene epoxide complex

Inhibition studies of Quercetin and its derivatives

Quercetin exhibited moderate binding scores towards all beta-lactamases tested. In contrast, quercitrin is strongly bound to the SHV-2 enzyme, displaying a binding energy of -9.0 kcal/mol. The binding energies of isoquercetin and rhamnetin varied between -7.9 and -8.8 kcal/mol across different receptors. Isorhamnetin and rhamnazin displayed very good binding scores on the SHV-2 and CTX-M enzymes, respectively, with binding energies of -9.1 kcal/mol and -9.0 kcal/mol for these proteins. Rutin inhibited both SHV-2 and CTX-M-15 enzymes, with binding scores of -9.2 kcal/mol and -9.0 kcal/mol, respectively. Quercetin derivatives such as hyperoside and tamarixetin showed only moderate binding scores on various beta-lactamases. Interestingly, all quercetin derivatives with high inhibitory efficiencies preferentially occupied the hydrophobic binding site of the 1N9B receptor.

Though rutin showed the highest binding score among all quercetin derivatives, the ADME properties of the molecule were not up to the mark. It showed three violations against Lipinski's rule, and the drug-like parameters such as average size and polarity of the molecule showed considerable deviation from the normal values. So it was decided to

exclude the binding analysis of this molecule with the 1N9B receptor. The second most important complex was 1N9B-isorhamnetin which displayed -9.1 kcal/mol binding energy.
The isorhamnetin-1N9B complex is stabilized primarily by a combination of classical hydrogen bonds and hydrophobic interactions. The carbonyl group (C=O) of the Gly245 residue forms a hydrogen bond with the hydroxyl group of the molecule (2.53 Å). Two alkyl hydrophobic interactions were observed between Ala248 and Leu250 (3.74, 4.06 Å) with the phytochemical. Additionally, eight pi-alkyl bonds contribute to the stability of the receptor-ligand complex. Ile279, Ile221, Ile246, and Ala280 residues participated in the pi-alkyl interaction (4.45-5.38 Å)

The most stable conformation of isorhamnetin within the SHV-2 enzyme's binding pocket and the 2D interaction plot of the enzyme-substrate complex are shown in Fig. 4.

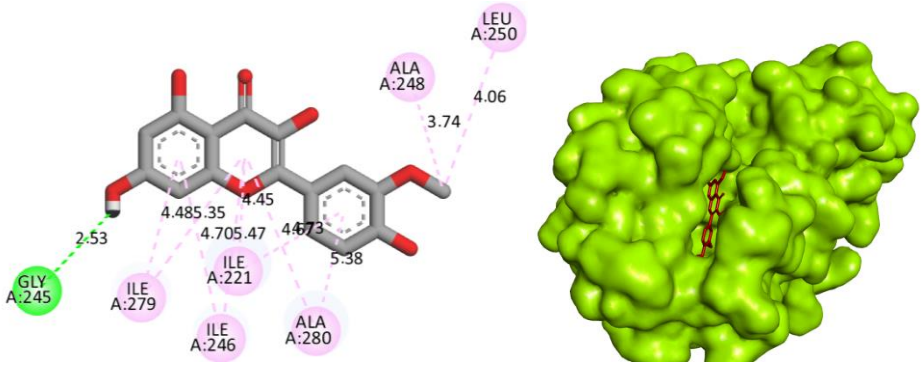


Fig. 4. 2D and 3D interaction plots of the 1N9B- isorhamnetin complex

Binding affinities of withanolides

Nine withanolides were evaluated for their binding affinities against the four class A beta-lactamase enzymes of *K. pneumoniae*. Notably, twenty out of the thirty-six investigated enzyme-ligand complexes displayed binding scores greater than or equal to -9.0 kcal/mol. This suggests a strong inhibitory potential of withanolides against these beta-lactamases. Ten complexes exhibited binding affinities exceeding -9.5 kcal/mol, and three even surpassed -10.0 kcal/mol. The SHV-2 enzyme (1N9B) appeared to be particularly susceptible to inhibition by withanolides, primarily through interactions with its hydrophobic binding site.

Withanolide A and Withanolide B demonstrated very high binding affinities towards the SHV-1, SHV-2, and KPC enzymes. The 1N9B-withanolide B complex exhibited the strongest binding affinity among all the withanolide complexes, reaching -10.2 kcal/mol. Closely following were the 4ZBE-withanolide B and 4ZAM-withanolide B complexes, with

binding affinities of -10.1 kcal/mol and -9.7 kcal/mol, respectively. Withanolide A also interacted effectively with the hydrophobic pocket of 1N9B, achieving a binding score of -9.9 kcal/mol.

Withanone and 27-hydroxy withanone displayed binding energies of -9.3 kcal/mol and -9.7 kcal/mol against the SHV-2 enzyme, respectively. Interestingly, Withanoside V showed significantly higher activity than Withanoside IV on SHV-2, KPC, and CTX-M-15 enzymes. Withanoside V reached its peak binding score of -10.1 kcal/mol in the SHV-2 complex.

In conclusion, all investigated withanolides displayed very good binding scores against at least one class A beta-lactamase of *K. pneumoniae*, highlighting their potential as inhibitors for this important class of enzymes. The structural analyses of the most stable withanolide complexes for each enzyme category are discussed in the subsequent sections.

Withanolide B binds to the SHV-1 enzyme (PDB: 4ZAM) primarily through interactions with its hydrophilic pocket. A strong conventional hydrogen bond formed between Ser130 of the enzyme and a hydroxyl group of the withanolide molecule at a distance of 3.01 Å. This interaction contributes to the binding energy of the 4ZAM-withanolide B complex, which is -9.7 kcal/mol. Additionally, two alkyl interactions with Tyr105 and Val216 (at distances of 5.09 Å and 4.22 Å, respectively) and a pi-alkyl bond (4.76 Å) between Ala237 and the third six-membered ring system of the withanolide molecule further stabilized the complex. Fig. 5 shows the interaction plots of the 4ZAM-withanolide B complex.

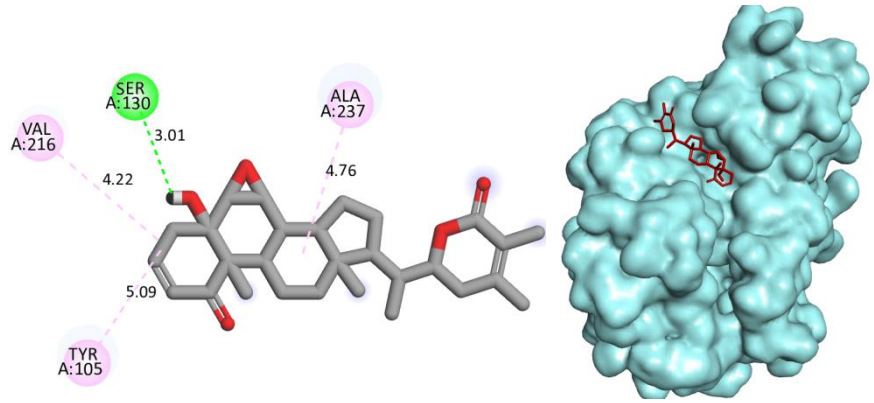


Fig. 5. 2D and 3D interaction plots of the SHV-1- Withanolide B complex

Withanolide B, withanoside V and withanolide A exhibited the strongest inhibitory effects on the SHV-2 enzyme (PDB: 1N9B), with binding energies of -10.2 kcal/mol, -10.1 kcal/mol and -9.9 kcal/mol respectively. The 1N9B-withanolide B complex is stabilized by a combination of interactions: a non-conventional hydrogen bond between Gln277 (2.43 Å) and a carbonyl group of withanolide B, and thirteen alkyl interactions. Notably, withanolide B preferentially occupies the hydrophobic binding site of 1N9B. Amino acid residues Val261,

Leu286, Ile246, Ala280, Ile221, Val224, Ala217, and Leu220 (3.68-5.43 Å) participated in these alkyl hydrophobic interactions. Ring systems 1, 3 (six-membered), and 4 (five-membered) of the withanolide molecule are primarily responsible for these interactions. Fig. 6 depicts the 2D and 3D interaction plots of the 1N9B-withanolide B complex.

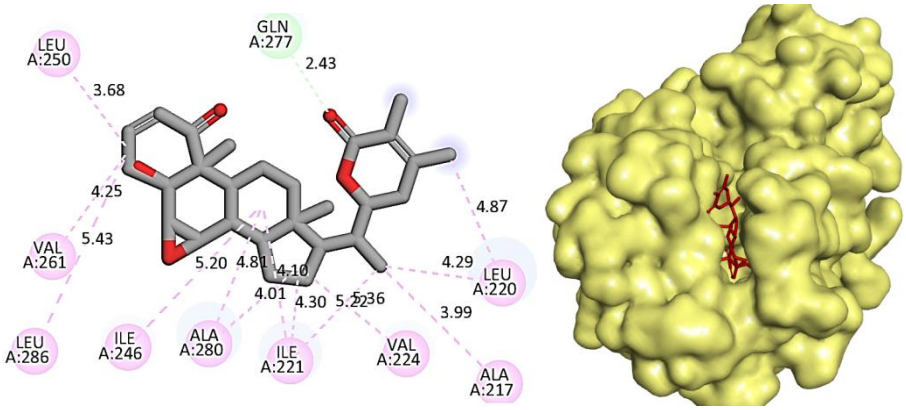


Fig. 6. 2D and 3D interaction plots of the SHV2- Withanolide B complex

Withanoside V's large size and high polarity make it a poor drug-like compound according to ADME evaluations. Furthermore, it violates Lipinski's rule in three ways (Table 2). Due to its enhanced hydrophilicity, withanoside V likely has poor gastrointestinal (GI) absorption. Consequently, withanoside V is not a good candidate for further drug development.

Withanolide A also displayed strong inhibitory activity (-9.9 kcal/mol) against the SHV-2 enzyme (PDB: 1N9B) through one conventional hydrogen bond (between Gln277 and the carbonyl group at 2.44 Å) and thirteen alkyl interactions in the hydrophobic binding site of SHV-2 beta-lactamase (Fig. 7). The amino acid residues Leu250, Val261, Ile246, Leu286, Val224, Ala280, Ala217, Leu220, and Ile221 were participated in these hydrophobic interactions (3.82-5.32 Å).

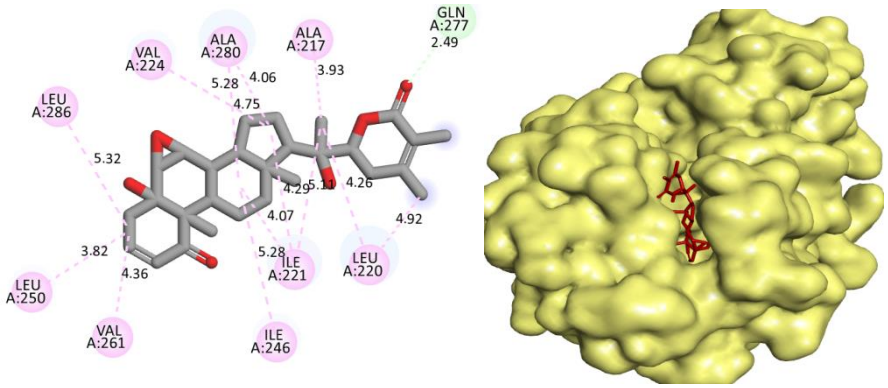


Fig. 7. 2D and 3D interaction plots of the SHV-2- Withanolide A complex

The KPC enzyme (PDB ID: 4ZBE) was significantly inhibited by withanolide B, exhibiting a binding score of -10.1 kcal/mol. Other withanolides, including withanoside V, withanolide A, viscolactone B, and withanoside IV, also showed very good binding scores (-9.7, -9.3, -9.2, and -9.1 kcal/mol, respectively). This section focuses on the binding analysis of the most stable complex, KPC-withanolide B. Withanolide B occupies a primarily hydrophilic region of the KPC-2 enzyme. The receptor-ligand complex was primarily stabilized by two classical hydrogen bonds. One hydrogen bond was formed between a hydroxyl group of the withanolide molecule and the Ser69 amino acid residue (2.59 Å). The other originated from Asn169 (2.89 Å) and interacted with the epoxide oxygen of the withanolide B. Additionally, Trp104 participated in a pi-sigma interaction (3.83 Å) with the withanolide molecule. Three alkyl interactions were also observed between Val239, Leu166, Trp104, and the alkyl groups of this molecule. The interaction plots of the 4ZBE-withanolide B complex are displayed in Fig. 8.

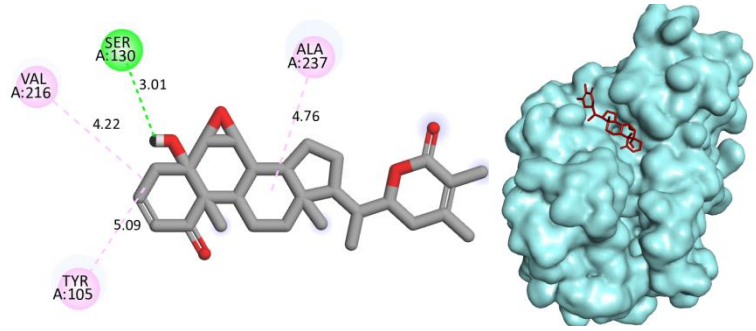


Fig. 8. 2D and 3D interaction plots of the KPC-2 Withanolide B complex

Among the studied phytochemicals, viscolactone B displayed the strongest inhibitory effect on the CTX-M-15 (PDB ID: 7BDS) with a binding score of -9.7 kcal/mol. Withanoside V, dihydroxy withanone, and withanolide B also exhibited good binding scores, exceeding or equal to -9.0 kcal/mol. Viscolactone B preferentially occupied the hydrophilic binding site of 7BDS. The protein-ligand complex was primarily stabilized by a strong hydrogen bond formed between a hydroxyl group of viscolactone B and Asn132 (2.39 Å). Additionally, three pi-alkyl hydrophobic interactions were observed between Tyr105 and two rings of the steroid molecule. However, the complex also exhibited one unfavorable interaction between Ala219 and a hydroxyl group of viscolactone B. Fig. 9 shows the interaction plots of the CTX-M-15-viscolactone B complex

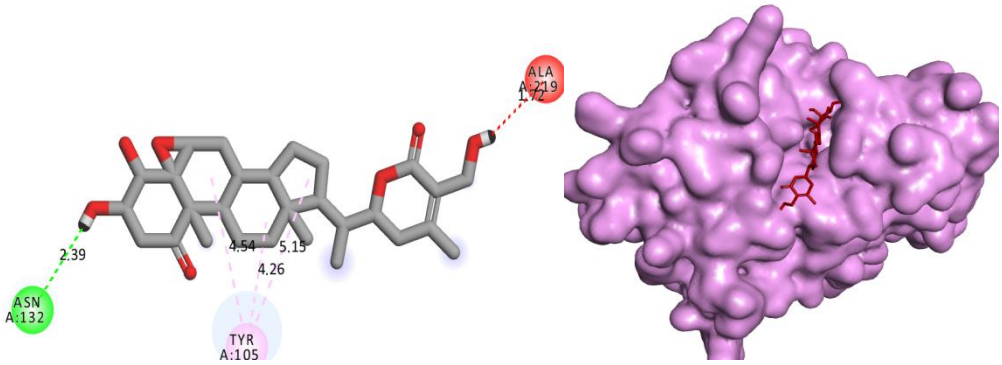


Fig. 9. 2D and 3D interaction plots of the 7BDS- Viscolactone B complex

Table 2. ADMET properties of phytochemicals shows high docking scores with beta lactamases of *K. pneumoniae*

ADME Parame-ters	Alpha Bisabolene epoxide	Withanolide B	Withanoside V	Withanolide A	Viscolactone B	Beta-amyrin	Isorhamnetin	Rutin
GI absorption	High	High	Low	High	High	Low	High	Low
BBB permeant	Yes	No	No	No	No	No	No	No
P-gp substrate	No	Yes	Yes	Yes	Yes	No	No	Yes
Consensus Log $P_{o/w}$	3.91	4.22	1.35	3.33	2.71	7.18	1.65	-1.29
Solubility in water	soluble	Moderately soluble	soluble	soluble	soluble	Poorly soluble	soluble	soluble
Lipinski Rule	Yes; 0 violation	Yes; 0 violation	No; 3 violations: MW>500, NorO>10, NHorOH>5	Yes; 0 violation	Yes; 0 violation	Yes; 1 violation: MLOGP>4.15	Yes; 0 violation	No; 3 violations: MW>500, NorO>10, NHorOH>5
Predicted Toxicity Class	5	2	2	2	4	6	5	5

Prediction of toxicity of phytochemicals

The oral toxicities of phytochemicals that exhibited high binding efficiency with the β-lactamases of *Klebsiella pneumoniae* were assessed using the ProTox 3.0 webserver. The compounds selected for computational toxicity prediction included Alpha-Bisabolene epoxide, Withanolide B, Withanoside V, Withanolide A, Viscolactone B, Beta-amyrin, Isorhamnetin, and Rutin. According to the webserver, toxicity classes range from 1 to 6, with toxicity

decreasing from Class 1 (most toxic) to Class 6 (least toxic). Table 2 presents the toxicity classifications of the selected phytochemicals. Among them, Withanolides A, B, and V demonstrated notable toxicities (Class 2), including respiratory, cardiac, immune, and cytotoxic effects. In contrast, Alpha-Bisabolene epoxide, Viscolactone B, Beta-amyrin, Isorhamnetin, and Rutin were predicted to be less toxic or non-toxic.

Comparison of the binding affinities of phytochemicals and therapeutics

The inhibitory power of beta-lactamase inhibitors used in allopathic medicine is provided in Table 3. In general, these inhibitors displayed lower binding scores compared to phytochemicals. Relebactam showed good inhibition efficiency on the SHV-2 enzyme of *K. pneumoniae* (-8.5 kcal/mol). Interestingly, all the inhibitors possessed a beta-lactam skeletal ring in their molecular structure, which is the same target site recognized by the bacteria for antibiotic degradation. Despite having lower binding scores, beta-lactamase inhibitors can still be effective. They compete with the antibiotic for the enzyme's active site. This competition allows some of the antibiotic molecules to bind to the enzyme and become inactivated, while others remain free to act on the bacterial cells and inhibit their growth. However, small mutations in the genes of bacterial strains can lead to alterations in the conformation of the beta-lactamase enzymes. These changes can decrease the efficacy of the inhibitors, and consequently, the potency of the antibiotics. The efficacy of phytochemicals compared to conventional beta-lactamase inhibitors needs to be proven by *in vitro* and *in vivo* studies.

Table 3. Binding scores of various therapeutical compounds on the beta-lactamases of *K. pneumoniae*

Therapeutic inhibitor	4ZAM	1N9B	4Zbe	7BDS
Clavulanate	-6.2	-6.3	-6.1	-6.0
Sulbactam	-6.1	-6.2	-5.4	-5.3
Tazobactam	-5.9	-6.0	-6.4	-5.7
Avibactam	-7.0	-7.2	-7.0	-6.7
Relebactam	-6.5	-8.5	-6.2	-6.4
Durlobactam	-7.7	-7.4	-7.5	-6.5

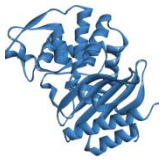
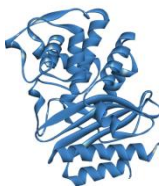
Material and methods

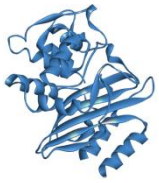
Beta-lactamases

In this study we took four class A beta-lactamase enzymes of *Klebsiella pneumoniae* which are derived by the most common Extended Spectrum of beta-lactamase encoding genes. This includes Sulfhydryl Reagent variable enzymes (SHV-1 and SHV-2), *Klebsiella pneumoniae* carbapenemase (KPC-2) and Cefotaxime-Munich (CTX-M-15) type.

The 3D structure of various receptor enzymes, PDB ids, sequence, resolution of the structure and method of characterization are provided in Table 4. The sequences of SHV-1 and SHV-2 enzymes are almost the same but they differ in the 213th moiety. In SHV-1 it is glycine residue while it is Serine in SHV-2. This leads to the conformational changes in the structures of SHV enzymes. The KPC-2 enzyme and CTX-M-15 enzymes have 264 and 265 amino acids in the primary structure and have entirely different sequences.

Table 4. Structural features of beta lactamases of *K. Pneumoniae* from Protein Data Bank

No.	Enzyme (PDB Id); sequence length,Method, Resolution	Sequence	Structure
1	SHV-1 (4ZAM) 265/XRD/1.42 Å	SPQPLEQIKLSESQLSGRVGMIEMDLASGRTLTAWRADER FPMMSTFKVVLCGAVLARVDAGDEQLERKIHYRQQDLVDY SPVSEKHLADGMTVGELCAAAITMSDNSAANLLLATVGGP AGLTAFLRQIGDNVTRLDRWETELNEALPGDARDTTTPAS MAATLRKLLTSQRLSARSQRQLLQWMVDDRVAGPLIRSVL PAGWFIADKTGA**G**ERGARGIVALLGPNNKAERIVVIYLRDT PASMAERNQQIAGIGAALIEHWQR	
2	SHV-2 (1N9B) 265/XRD/0.90 Å	SPQPLEQIKLSESQLSGRVGMIEMDLASGRTLTAWRADER FPMMSTFKVVLCGAVLARVDAGDEQLERKIHYRQQDLVDY SPVSEKHLADGMTVGELCAAAITMSDNSAANLLLATVGGP AGLTAFLRQIGDNVTRLDRWETELNEALPGDARDTTTPAS MAATLRKLLTSQRLSARSQRQLLQWMVDDRVAGPLIRSVL PAGWFIADKTGA**S**ERGARGIVALLGPNNKAERIVVIYLRDT PASMAERNQQIAGIGAALIEHWQR	
3	KPC-2 (4ZBE) 264/XRD/1.80 Å	TNLVAEPFAKLEQDFGGSIGVYAMDTGSGATVSYRAEERFP LCSSFKGFLAAAVLARSQQQAGLLDTPIRYGKNALVPWSPI SEKYLTTGMTVAELSAAAVQYSDNAAANLLLKELGGPAGLT AFMRSIGDTTFRLDRWELELNSAIPGDARDTSSPRAVTESL QKLTLGSALAAPQRQQFVDWLKGNTTGNHRIRAAVPADW AVGDKTGTCGVYGTANDYAVVWPTGRAPIVLAVYTRAPNK DDKHSEAVIAAAARLALEGLG	

4	CTX-M-15 (7BDS) 265/XRD/0.91 Å	ADVQQKLAELERQSGGRLGVALINTADNSQILYRADERFA MCTSKVMAAAAVLKKSESEPNLLNQRVEIKKSDLVNYNPIA EKHVNGTMSLAELSAAALQYSDNVAMNKLIAHVGGPASVT AFARQLGDETFRLDRTEPTLNTAIPGDPRDTTSPRAMAQT LRNLTLGKALGDSQRAQLVTWMKGNTTGAASIQAGLPAS WVVGDKTGSGGYGTTNDIAVIWPKDRAPLILVTYFTQPQP KAESRRDVLASAAKIVTD	

Computational studies

Crystal structures of class A beta-lactamases from *K. pneumoniae* were retrieved from the Protein Data Bank (PDB) and prepared for docking using Discovery Studio software [35]. Water molecules and co-crystallised ligands were removed from the protein crystal structures and hydrogen atoms were added using Discovery Studio.

This work aimed to evaluate the potential of various phytochemicals derived from well-known medicinal plants such as *Bridelia stipularis* and *Andrographis paniculata*, both recognized for their therapeutic properties. Additionally, quercetin derivatives and withanolides were included in the *in silico* investigation. Most of the phytochemicals selected for this study have previously demonstrated medicinal activities, including antibacterial, anti-inflammatory, and anticancer effects. In total, 27 phytochemicals were screened for their efficacy against selected β-lactamase receptors. Phytochemical structures were obtained from the PubChem database. Docking simulations were performed using the web server CB-Dock version 2 which utilises cavity detection guided blind docking method [36]. The prepared crystal structure of the protein in pdb format and the structure of the ligand in sdf format was uploaded to the webserver. The resulting protein-ligand complexes were then analyzed with Biovia Discovery Studio software

The binding affinities of known beta-lactamase inhibitors used clinically were also evaluated against the β-lactamase enzymes for comparison with the various potencies of phytochemicals. Drug-likeness parameters like logP, blood-brain barrier (BBB) penetration, P-glycoprotein (Pgp) substrate potential, and adherence to Lipinski's rule were assessed using the SwissADME web server [37]. Toxicities of the phytochemicals which showed high docking scores with beta lactamases of *K. Pneumoniae* were predicted computationally using ProTox. 3 webserver[38].

The overall drug-like behavior was then analyzed using the bioavailability radar provided by the server.

Conclusions

Twenty-seven phytochemicals were screened for their binding efficacy on four ESBLs of *K. pneumoniae* using molecular docking studies. The compounds came from four major categories*: B. stipularis*, *A. paniculata*, quercetin derivatives, and withanolides.

Beta-amyrin, a six-membered ring structured molecule from *B. stipularis*, inhibited the SHV-2 enzyme with a maximum binding score of -9.5 kcal/mol. However, it showed slight deviations from ideal drug-likeness properties in ADME studies. The most effective compound from *A. paniculata* was Bisabolene epoxide, showing a binding energy of -10.2 kcal/mol on the SHV-2 enzyme.

Out of nine quercetin derivatives, isorhamnetin was the most promising candidate for inhibiting the SHV-2 enzyme (-9.1 kcal/mol). Notably, the steroidal phytochemicals, withanolides, showed very good binding scores against the ESBLs in general. Withanolide B displayed superior inhibition potential on SHV-1 (-9.7 kcal/mol), SHV-2 (-10.2 kcal/mol), and KPC-2 (-10.1 kcal/mol) enzymes. Withanolide A also effectively inhibited the SHV-2 enzyme with a binding score of -9.9 kcal/mol. The CTX-M-15 beta-lactamase showed the best interaction potential with viscolactone B (-9.7 kcal/mol).

Isorhamnetin, bisabolene epoxide, withanolide A, withanolide B, and viscolactoneB all showed good ADME properties according to computational studies. But the computational toxicity prediction showed that the three withanolides A, B and V are toxic in nature. Overall, these phytochemicals displayed very good binding scores compared to therapeutic beta-lactamase inhibitors. This study identified highly stable ESBL-ligand complexes for each investigated class A beta-lactamase.

In vitro and *in vivo* studies are necessary to confirm the effectiveness and oral toxicities of these molecules in combating beta-lactamases and potentially improving the efficacy of beta-lactam antibiotics.

References

[1] Hamed, R. B.; Gomez-Castellanos, J. R.; Henry, L.; Ducho, C.; McDonough, M. A.; Schofield, C. J. The Enzymes of β-Lactam Biosynthesis. *Nat. Prod. Rep.*, **2013**, *30* (1), 21–107. DOI: 10.1039/C2NP20065A.

[2] Fiddian-Green, R. G.; Silen, W. Mechanisms of Disposal of Acid and Alkali in Rabbit Duodenum. *Am J Physiol*, **1975**, *229* (6), 1641–1648. DOI: 10.1152/ajplegacy.1975.229.6.1641.

[3] Lima, L. M.; Silva, B. N. M. D.; Barbosa, G.; Barreiro, E. J. β-Lactam Antibiotics: An Overview from a Medicinal Chemistry Perspective. *European Journal of Medicinal Chemistry*, **2020**, *208*, 112829. DOI: 10.1016/j.ejmech.2020.112829.

[4] Rossolini, G. M.; Arena, F.; Pecile, P.; Pollini, S. Update on the Antibiotic Resistance Crisis. *Current Opinion in Pharmacology*, **2014**, *18*, 56–60. DOI: 10.1016/j.coph.2014.09.006.

[5] Read, A. F.; Woods, R. J. Antibiotic Resistance Management. *Evolution, Medicine, and Public Health*, **2014**, *2014* (1), 147–147. DOI: 10.1093/emph/eou024.

[6] Sawa, T.; Kooguchi, K.; Moriyama, K. Molecular Diversity of Extended-Spectrum β-Lactamases and Carbapenemases, and Antimicrobial Resistance. *j intensive care*, **2020**, *8* (1), 13. DOI: 10.1186/s40560-020-0429-6.

[7] Öztürk, H.; Ozkirimli, E.; Özgür, A. Classification of Beta-Lactamases and Penicillin Binding Proteins Using Ligand-Centric Network Models. *PLoS ONE*, **2015**, *10* (2), e0117874. DOI: 10.1371/journal.pone.0117874.

[8] Makena, A.; Brem, J.; Pfeffer, I.; Geffen, R. E. J.; Wilkins, S. E.; Tarhonskaya, H.; Flashman, E.; Phee, L. M.; Wareham, D. W.; Schofield, C. J. Biochemical Characterization of New Delhi Metallo-β-Lactamase Variants Reveals Differences in Protein Stability. *Journal of Antimicrobial Chemotherapy*, **2015**, *70* (2), 463–469. DOI: 10.1093/jac/dku403.

[9] Shaikh, S.; Fatima, J.; Shakil, S.; Rizvi, S. Mohd. D.; Kamal, M. A. Antibiotic Resistance and Extended Spectrum Beta-Lactamases: Types, Epidemiology and Treatment. *Saudi Journal of Biological Sciences*, **2015**, *22* (1), 90–101. DOI: 10.1016/j.sjbs.2014.08.002.

[10] Mora-Ochomogo, M.; Lohans, C. T. β-Lactam Antibiotic Targets and Resistance Mechanisms: From Covalent Inhibitors to Substrates. *RSC Med. Chem.*, **2021**, *12* (10), 1623–1639. DOI: 10.1039/D1MD00200G.

[11] Flores-Kim, J.; Dobihal, G. S.; Fenton, A.; Rudner, D. Z.; Bernhardt, T. G. A Switch in Surface Polymer Biogenesis Triggers Growth-Phase-Dependent and Antibiotic-Induced Bacteriolysis. *eLife*, **2019**, *8*, e44912. DOI: 10.7554/eLife.44912.

[12] Elmonir, W.; Abd El-Aziz, N. K.; Tartor, Y. H.; Moustafa, S. M.; Abo Remela, E. M.; Eissa, R.; Saad, H. A.; Tawab, A. A. Emergence of Colistin and Carbapenem Resistance in Extended-Spectrum β-Lactamase Producing Klebsiella Pneumoniae Isolated from Chickens and Humans in Egypt. *Biology*, **2021**, *10* (5), 373. DOI: 10.3390/biology10050373.

[13] Zaman, T. U.; Alrodayyan, M.; Albladi, M.; Aldrees, M.; Siddique, M. I.; Aljohani, S.; Balkhy, H. H. Clonal Diversity and Genetic Profiling of Antibiotic Resistance among

Multidrug/Carbapenem-Resistant Klebsiella Pneumoniae Isolates from a Tertiary Care Hospital in Saudi Arabia. *BMC Infect Dis*, **2018**, *18* (1), 205. DOI: 10.1186/s12879-018-3114-9.

[14] Philippon, A.; Slama, P.; Dény, P.; Labia, R. A Structure-Based Classification of Class A β-Lactamases, a Broadly Diverse Family of Enzymes. *Clin Microbiol Rev*, **2016**, *29* (1), 29–57. DOI: 10.1128/CMR.00019-15.

[15] Fils, P. E. L.; Cholley, P.; Gbaguidi-Haore, H.; Hocquet, D.; Sauget, M.; Bertrand, X. ESBL-Producing Klebsiella Pneumoniae in a University Hospital: Molecular Features, Diffusion of Epidemic Clones and Evaluation of Cross-Transmission. *PLoS ONE*, **2021**, *16* (3), e0247875. DOI: 10.1371/journal.pone.0247875.

[16] Wang, G.; Huang, T.; Surendraiah, P. K. M.; Wang, K.; Komal, R.; Zhuge, J.; Chern, C.-R.; Kryszuk, A. A.; King, C.; Wormser, G. P. CTX-M β-Lactamase–Producing *Klebsiella Pneumoniae* in Suburban New York City, New York, USA. *Emerg. Infect. Dis.*, **2013**, *19* (11), 1803–1810. DOI: 10.3201/eid1911.121470.

[17] Bush, K.; Bradford, P. A. β-Lactams and β-Lactamase Inhibitors: An Overview. *Cold Spring Harb Perspect Med*, **2016**, *6* (8), a025247.DOI: 10.1101/cshperspect.a025247.

[18] González-Bello, C.; Rodríguez, D.; Pernas, M.; Rodríguez, Á.; Colchón, E. β-Lactamase Inhibitors To Restore the Efficacy of Antibiotics against Superbugs. *J. Med. Chem.*, **2020**, *63* (5), 1859–1881. DOI: 10.1021/acs.jmedchem.9b01279.

[19] H, S. K.; Jade, D.; Harrison, M. A.; Sugumar, S. Identification of Natural Inhibitor against L1 β-Lactamase Present in Stenotrophomonas Maltophilia. *J Mol Model*, **2022**, *28* (11), 342. DOI: 10.1007/s00894-022-05336-z.

[20] Bibi, Z.; Asghar, I.; Ashraf, N. M.; Zeb, I.; Rashid, U.; Hamid, A.; Ali, M. K.; Hatamleh, A. A.; Al-Dosary, M. A.; Ahmad, R.; et al. Prediction of Phytochemicals for Their Potential to Inhibit New Delhi Metallo β-Lactamase (NDM-1). *Pharmaceuticals*, **2023**, *16* (10), 1404. DOI: 10.3390/ph16101404.

[21] Etminani, F.; Etminani, A.; Hasson, S. O.; Judi, H. K.; Akter, S.; Saki, M. In Silico Study of Inhibition Effects of Phytocompounds from Four Medicinal Plants against the Staphylococcus Aureus β-Lactamase. *Informatics in Medicine Unlocked*, **2023**, *37*, 101186. DOI: 10.1016/j.imu.2023.101186.

[22] Shoeib, N. A.; Al-Madboly, L. A.; Ragab, A. E. *In Vitro* and *in Silico* β-Lactamase Inhibitory Properties and Phytochemical Profile of *Ocimum Basilicum* Cultivated in Central Delta of Egypt. *Pharmaceutical Biology*, **2022**, *60* (1), 1969–1980. DOI: 10.1080/13880209.2022.2127791.

[23] Murthy, H. N.; Dalawai, D.; Mamatha, U.; Angadi, N. B.; Dewir, Y. H.; Al-Suhaibani, N. A.; El-Hendawy, S.; Al-Ali, A. M. Bioactive Constituents and Nutritional Composition of *Bridelia Stipularis* L. Blume Fruits. *International Journal of Food Properties*, **2021**, *24* (1), 796–805. DOI: 10.1080/10942912.2021.1924776.

[24] Sini, A.; Bindu, T.; Raphael, V. P.; Shaju, K. In Vitro and in Silico Studies of Methanol Extract of Bridelia Stipularis on S. Aureus and E. Coli. *Indian Journal of Chemistry*, **2024**, *63* (5), 437–443.

[25] Okhuarobo, A.; Ehizogie Falodun, J.; Erharuyi, O.; Imieje, V.; Falodun, A.; Langer, P. Harnessing the Medicinal Properties of Andrographis Paniculata for Diseases and beyond: A Review of Its Phytochemistry and Pharmacology. *Asian Pacific Journal of Tropical Disease*, **2014**, *4* (3), 213–222. DOI: 10.1016/S2222-1808(14)60509-0.

[26] Fardiyah, Q.; Ersam, T.; Suyanta; Slamet, A.; Suprapto; Kurniawan, F. New Potential and Characterization of Andrographis Paniculata L. Ness Plant Extracts as Photoprotective Agent. *Arabian Journal of Chemistry*, **2020**, *13* (12), 8888–8897. DOI: 10.1016/j.arabjc.2020.10.015.

[27] Sini, A.; Bindu, T. K.; Raphael, V. P.; Shaju, K. S.; Sebastian, S. Growth Inhibition of P. Aeruginosa by Methanol Extract of Bridelia Stipularis and Identification of Active Components Using in Silico Studies. *Futur J Pharm Sci*, **2024**, *10* (1), 96.DOI: 10.1186/s43094-024-00668-4.

[28] Alsharairi, N. A. Quercetin Derivatives as Potential Therapeutic Agents: An Updated Perspective on the Treatment of Nicotine-Induced Non-Small Cell Lung Cancer. *IJMS*, **2023**, *24* (20), 15208. DOI: 10.3390/ijms242015208.

[29] Alizadeh, S. R.; Ebrahimzadeh, M. A. Quercetin Derivatives: Drug Design, Development, and Biological Activities, a Review. *European Journal of Medicinal Chemistry*, **2022**, *229*, 114068. DOI: 10.1016/j.ejmech.2021.114068.

[30] Materska, M. Quercetin and Its Derivatives: Chemical Structure and Bioactivity-a Review. *Polish journal of food and nutrition sciences*, **2008**, *58* (4).

[31] Modi, S. J.; Tiwari, A.; Ghule, C.; Pawar, S.; Saste, G.; Jagtap, S.; Singh, R.; Deshmukh, A.; Girme, A.; Hingorani, L. Pharmacokinetic Study of Withanosides and Withanolides from Withania Somnifera Using Ultra-High Performance Liquid Chromatography-Tandem Mass Spectrometry (UHPLC-MS/MS). *Molecules*, **2022**, *27* (5), 1476. DOI: 10.3390/molecules27051476.

[32] White, P. T.; Subramanian, C.; Motiwala, H. F.; Cohen, M. S. Natural Withanolides in the Treatment of Chronic Diseases. In *Anti-inflammatory Nutraceuticals and Chronic Diseases*; Gupta, S. C., Prasad, S., Aggarwal, B. B., Eds.; Advances in Experimental

Medicine and Biology; Springer International Publishing: Cham, 2016; Vol. 928, pp 329–373. DOI: 10.1007/978-3-319-41334-1_14.

[33] Choudhary, M. I.; Yousuf, S.; Atta-ur-Rahman. Withanolides: Chemistry and Antitumor Activity. In *Natural Products*; Ramawat, K. G., Mérillon, J.-M., Eds.; Springer Berlin Heidelberg: Berlin, Heidelberg, 2013; pp 3465–3495. DOI: 10.1007/978-3-642-22144-6_150.

[34] Saleem, S.; Muhammad, G.; Hussain, M. A.; Altaf, M.; Bukhari, S. N. A. Withania Somnifera L.: Insights into the Phytochemical Profile, Therapeutic Potential, Clinical Trials, and Future Prospective. *Iranian Journal of Basic Medical Sciences*, **2020**, *23* (12). DOI: 10.22038/ijbms.2020.44254.10378.

[35] BIOVIA, Dassault Systèmes, Discovery Studio Visualizer, **2024**; San Diego: Dassault Systèmes, **2024**

[36] Yang Liu, et al . CB-Dock2: improved protein-ligand blind docking by integrating cavity detection, docking and homologous template fitting. Nucleic Acids Research, **2022**.

[37] Daina, A., Michielin, O. & Zoete, V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep* **7**, 42717 (2017). DOI: 10.1038/srep42717

[38] Banerjee P., Kemmler E., Dunkel M., Preissner R.: ProTox 3.0: a webserver for the prediction of toxicity of chemicals Nucleic Acids Res (Web server issue **2024**); NAR