

Article

Structure-Based Identification of Ponganone V from *Pongamia pinnata* as a Potential KPC-2 β -Lactamase Inhibitor: Insights from Docking, ADMET, and Molecular Dynamics

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Abstract

Carbapenem-resistant Enterobacterales (CREs) pose a critical threat to global public health, largely driven by the enzymatic activity of *Klebsiella pneumoniae* carbapenemase-2 (KPC-2), a class A serine β -lactamase that hydrolyzes most β -lactam antibiotics. While β -lactamase inhibitors like avibactam offer temporary relief, emerging KPC variants demand novel, sustainable inhibitory scaffolds. This study aimed to identify and characterize potential natural inhibitors of KPC-2 from *Pongamia pinnata*, leveraging a comprehensive in silico workflow. A curated library of 86 phytochemicals was docked against the active site of KPC-2 (PDB ID: 3DW0). The top-performing ligands were subjected to ADMET profiling (pkCSM), and 100 ns molecular dynamics simulations (GROMACS) to evaluate structural stability and interaction persistence, using avibactam as control. Ponganone V exhibited the most favorable binding energy (-9.0 kcal/mol), engaging Ser70 via a hydrogen bond and forming π - π interactions with Trp105. Glabrachromene II demonstrated a broader interaction network but reduced long-term stability. ADMET analysis confirmed high intestinal absorption, non-mutagenicity, and absence of hERG inhibition for both ligands. Molecular dynamics simulations revealed that Ponganone V maintained compact structure and stable hydrogen bonding throughout the 100 ns trajectory, closely mirroring the behavior of avibactam, whereas Glabrachromene II displayed increased fluctuation and loss of compactness beyond 80 ns. Principal Component Analysis (PCA) further supported these findings, with Ponganone V showing restricted conformational motion and a single deep free energy basin, while avibactam and Glabrachromene II exhibited broader conformational sampling and multiple energy minima. The integrated computational findings highlight Ponganone V as a potent and pharmacologically viable natural KPC-2 inhibitor, with strong binding affinity, sustained structural stability, and minimal toxicity. This study underscores the untapped potential of *Pongamia pinnata* phytochemicals as future anti-resistance therapeutics and provides a rational basis for their experimental validation.

Keywords: KPC-2 β -lactamase; carbapenem resistance; *Pongamia pinnata*; molecular dynamics; antibiotic resistance



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1. Introduction

The accelerating emergence of multidrug-resistant (MDR) bacterial infections, especially those driven by carbapenem-resistant Enterobacteriaceae (CRE), poses one of the

gravest threats to global public health [1–3]. Among the most formidable resistance mechanisms is the production of *Klebsiella pneumoniae* carbapenemase (KPC), a class A serine β -lactamase capable of hydrolyzing a broad spectrum of β -lactam antibiotics, including last-resort carbapenems [4–6]. The spread of KPC-producing strains has significantly compromised current antibiotic therapy and led to increased morbidity, mortality, and healthcare burdens worldwide. Despite the introduction of β -lactamase inhibitors such as avibactam, the persistence of resistance and limitations in efficacy necessitate the urgent identification of novel, safe, and potent KPC inhibitors [7–9]. Natural products, particularly phytochemicals derived from medicinal plants, represent a rich and largely untapped source of novel antimicrobial agents [10,11]. Among these, *Pongamia pinnata* (L.) Pierre, a leguminous tree native to the Indian subcontinent and parts of Southeast Asia, has garnered attention for its extensive ethnopharmacological and bioactive profile. Traditionally used to treat skin ailments, inflammation, and microbial infections, *P. pinnata* has been shown to contain a broad spectrum of phytoconstituents including flavonoids (e.g., pongamol, karanjin, lanceolatin B), terpenoids, and alkaloids with proven pharmacological properties [12–14]. Modern investigations have reinforced these traditional claims, demonstrating that various extracts of *P. pinnata* possess potent antibacterial activity against a wide range of clinical pathogens including *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* [15,16]. Particularly, flavonoid-rich extracts such as those containing karanjin and pongamol have shown significant inhibition of resistant bacterial strains [17], suggesting their potential as scaffolds for novel antibiotics or resistance-modifying agents. We selected *Pongamia pinnata* because it is a chemically rich medicinal plant known to contain drug-like flavonoids particularly karanjin, pongamol and related chromenes that have demonstrated broad-spectrum antibacterial activity, including against *Klebsiella pneumoniae*. These phytochemicals offer favorable pharmacological scaffolds and have been repeatedly highlighted in phytochemical surveys as promising antimicrobial leads. In addition, plant-derived molecules are established sources of β -lactamase inhibitors and provide structural diversity that complements synthetic libraries. Given the urgent need for new KPC inhibitors, and the absence of prior evaluations of *P. pinnata* compounds against KPC-2, a focused in silico screen of this well-characterized species presents a rational and cost-effective strategy to identify high-priority candidates for subsequent experimental validation. In recent years, structure-based docking tools such as AutoDock Vina, ADMET prediction platforms including pkCSM, and molecular dynamics simulations with GROMACS have continued to be widely used and well-validated in modern drug-discovery studies. These methods remain reliable in 2025 because they are regularly improved, supported by extensive published work, and form the standard workflow for identifying promising inhibitor candidates before laboratory testing [18–21]. Including these approaches therefore provides a scientifically sound and practical first step for screening *P. pinnata* compounds against KPC-2.

Currently, with artificial intelligence in drug design, virtual screening and molecular dynamics simulation (MDS) play essential roles in rationally discovering possible drugs [22]. Using these methods, scientists can quickly predict how small molecules interact structurally and dynamically with proteins which saves time and money from performing wet lab experiments [23]. Furthermore, pharmacokinetic profiling using ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) tools, such as SwissADME and ProTox-II, helps in early elimination of compounds with poor drug-likeness or high toxicity risk [24]. To test the therapeutic value of *Pongamia pinnata* phytochemicals, we used an extensive in silico method, retrieving the compounds from the IMPPAT database. High-throughput molecular docking was used to pick out the leading candidates which were then tested for stability and compared to avibactam in 100 ns molecular dynamics simulations. The lead

compounds were further assessed with ADMET and toxicity prediction models to check their suitability and safety for medicine. Not only does this technique help select notable natural chemicals against key drug resistance, but it also adds to the surge in interest to reuse ancient plant compounds in modern antibiotic research programs.

2. Methodology

2.1. Molecular Docking

2.1.1. Receptor Preparation

Molecular docking was performed against the KPC (*Klebsiella pneumoniae* carbapenemase) β -lactamase enzyme, a major contributor to carbapenem resistance in Gram-negative bacteria. The crystal structure of KPC-2 (PDB ID: 3DW0) was retrieved from the RCSB Protein Data Bank. Receptor preparation involved removal of water molecules, ligands, and non-essential ions using UCSF Chimera 1.19 [25]. Hydrogen atoms were added, and Gasteiger charges were assigned to the structure. Key catalytic residues—Ser70, Lys73, Ser130, Glu166, and Asn170—known to be crucial for substrate binding and hydrolysis, were used to define the active site for docking [26].

2.1.2. Ligand Preparation

Eighty-six different phytochemicals from *Pongamia pinnata* were chosen since they were said or expected to have antimicrobial benefits. Selected ligands were imported from the IMPPAT database and properly arranged for docking in Open Babel 3.1.1 and UCSF Chimera. Energy optimizations were done using the General AMBER Force Field (GAFF). The number of hydrogen atoms was increased, torsions were set and each compound was optimized in 3D to create PDBQT files for flexible docking [25,27].

2.1.3. Docking Protocol

Docking simulations were conducted using InstaDock Version 1.801, an AutoDock Vina 1.2.7 based docking software optimized for speed and accuracy [28]. The grid box was centered over the active site of KPC-2 with the following coordinates: center_x = -6.5248 , center_y = 8.2880 , center_z = 15.9251 , and dimensions size_x = 38.1457 , size_y = 37.1314 , and size_z = 35.1382 . The exhaustiveness parameter was set to 8 to ensure sufficient conformational sampling, and nine binding poses were generated for each ligand. The best conformation for each compound was selected based on the lowest binding energy and favorable orientation within the active site pocket. To validate the docking workflow and serve as a reference for binding affinity comparison, avibactam (CID: 9835049), a clinically approved β -lactamase inhibitor, was docked under identical parameters. Avibactam was selected as the reference not for structural similarity, but because it is the clinically approved KPC-2 inhibitor currently used in therapy. This functional benchmark allows direct comparison of the natural candidates against a validated inhibitor, and no flavonoid-based KPC-2 inhibitors have been reported to serve as structural controls.

2.2. ADMET Analysis

Using the pkCSM website (<https://biosig.lab.uq.edu.au/pkcsm/>, accessed on 28 September 2025) [29] which applies graph signatures, the selected phytochemicals' pk (pharmacokinetic) & toxicity profiles were estimated. Assessing absorption involved making predictions about water solubility, Caco-2 cell permeability, how much would be absorbed in the human small intestine and interaction with P-glycoprotein. This research focused on volume of distribution, the ability of the drug to cross the blood–brain barrier (BBB) and its rate of uptake into the central nervous system (CNS). Metabolic predictions covered cytochrome P450 enzyme interactions, particularly the inhibition and substrate status for major CYP

isoforms. Excretion analysis focused on total systemic clearance and renal OCT2 substrate status. Toxicological evaluation included predictions for hepatotoxicity, mutagenic potential via the Ames test, and cardiotoxicity risk through hERG I and II channel inhibition. These comprehensive in silico predictions offer valuable preliminary insights into the drug-likeness, safety, and therapeutic viability of the screened phytochemicals, thereby guiding their selection for further dynamic simulation studies.

2.3. Molecular Dynamics Simulations

To investigate the dynamic stability and interaction behavior of the top two ligand–KPC complexes obtained by docking, molecular dynamics (MD) simulations were carried out by using GROMACS 2020.3. The force field used for the protein was applied from the force field of the CHARMM36 force field, while the parameters for the ligand were created using the force field of the CHARMM 36 General Force Field (CGenFF) in order to ensure compatibility. Each complex was placed into a cubic simulation box, with a minimum distance of 1.0 nm between the protein and edges of the box to avoid artifacts caused by periodic boundary interactions. The system was solvated with TIP3P water molecules and the counterions were added to achieve electroneutrality. Initial energy minimization was performed with the steepest descent algorithm for the removal of steric clashes and unfavorable contacts. The system was then equilibrated in two phases, a phase of 100 ps NVT (constant number of particles, volume, and temperature) at 300 K using the V-rescale thermostat and a phase of 100 ps NPT (constant number of particles, pressure, and temperature) using the Parrinello-Rahman barostat. Production MD simulations were performed for 100 nanoseconds for each complex. A 100 ns time duration was selected because all systems were at equilibrium long before 20 ns and this timescale is widely sufficient to capture stable conformational and binding behavior in enzyme–ligand complexes. This protocol was an efficient way to get good dynamic sampling without sacrificing computation. Post-simulation analysis consisted of calculation of the following parameters: root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (R_g), solvent accessible surface area (SASA), and hydrogen bonding profiles, to assess stability and intactness of the bound structures [30,31].

3. Results

3.1. Molecular Docking

Molecular docking was performed to assess the binding affinity and efficiency of selected *Pongamia pinnata* phytochemicals against the KPC-2 β -lactamase enzyme. A total of ten phytochemicals were evaluated alongside the reference inhibitor avibactam (CID: 9835049). Binding scores, predicted inhibition constants (pK_i), ligand efficiency values, and torsional energies were calculated for each compound (Table 1). Among the tested ligands, Ponganone V (-9.0 kcal/mol, pK_i 6.6) exhibited the strongest binding affinity, outperforming all other phytochemicals and the control drug. Ovalichromene B and Isopongachromene followed closely with binding energies of -8.8 kcal/mol (pK_i 6.45). These top three candidates also displayed favorable ligand efficiencies (>0.31 kcal/mol/non-H atom), suggesting balanced interactions relative to molecular size. Notably, Friedelin, despite having no rotatable bonds (torsional energy = 0), showed a competitive binding energy of -8.7 kcal/mol. In contrast, Avibactam, the standard KPC inhibitor, yielded a significantly weaker docking score (-6.3 kcal/mol, pK_i 4.62), reinforcing the superior binding performance of several *P. pinnata*-derived compounds. Overall, the docking analysis highlights Ponganone V, Ovalichromene B, and Isopongachromene as leading candidates, combining strong predicted binding, structural stability, and efficiency. These compounds were

shortlisted for further structural interaction analysis and molecular dynamics simulation to validate their potential as novel KPC inhibitors.

Table 1. Molecular docking results of *Pongamia pinnata* phytochemicals and control (Avibactam) against KPC-2 β -lactamase.

Ligand Code	Ligand Name	Binding Free Energy (kcal/mol)	pKi	Ligand Efficiency (kcal/mol/non-H atom)	Torsional Energy
IMPHY003033	Ponganone V	−9	6.6	0.3214	1.5565
IMPHY000058	Ovalichromene B	−8.8	6.45	0.3385	0.3113
IMPHY004719	Isopongachromene	−8.8	6.45	0.3143	0.6226
IMPHY011688	Friedelin	−8.7	6.38	0.2806	0
IMPHY006225	Glabrachromene II	−8.6	6.31	0.3308	1.2452
IMPHY001489	Ovalifolin	−8.4	6.16	0.3231	1.2452
IMPHY014969	kaempferol 7-O-glucoside	−8.4	6.16	0.2625	3.4243
IMPHY012756	Quercimeritrin	−8.4	6.16	0.2545	3.7356
IMPHY011471	Lupenone	−8.3	6.09	0.2677	0.3113
IMPHY008838	Gamatin	−8.3	6.09	0.332	0.6226
CID 9835049	Avibactam (Control)	−6.3	4.62	0.3706	1.2452

3.1.1. Binding Interaction Analysis

To gain further insight into the ligand–receptor interactions, the top five docked complexes were analyzed through 2D interaction diagrams (Figure 1). These visualizations revealed key hydrogen bonds and non-covalent contacts stabilizing ligand binding within the KPC-2 β -lactamase active site. Ponganone V exhibited a strong binding profile primarily stabilized by a single conventional hydrogen bond with Ser70, a key catalytic residue of KPC-2. Additionally, a π – π stacked interaction with Trp105 and several hydrophobic contacts contributed to its favorable binding energy (−9.0 kcal/mol), indicating effective positioning within the active site pocket. Ovalichromene B formed a conventional hydrogen bond with Asn170, reinforcing its anchoring in the binding pocket. A prominent π – π stacking interaction with Trp105 further enhanced binding stability. These interactions, located near catalytically relevant residues, support its strong docking performance. Isopongachromene, although lacking conventional hydrogen bonding, demonstrated strong aromatic stacking through π – π interaction with Trp105 and π –sigma interaction with Pro107. These non-polar interactions helped maintain the ligand’s orientation in the hydrophobic core of the active site, reflecting its competitive binding affinity. Friedelin, a non-polar triterpenoid, also lacked any conventional hydrogen bonds. However, it engaged in π –sigma interactions with Trp105, supported by van der Waals and hydrophobic contacts, particularly with residues like Pro107 and Tyr129. Despite the absence of polar interactions, its binding was stabilized through shape complementarity and hydrophobic fit. Glabrachromene II demonstrated the most extensive interaction network among all candidates. It formed multiple conventional hydrogen bonds with Ser70, Asn132, Asn170, and Thr237, establishing a robust polar interaction profile. Additionally, a π – π T-shaped interaction with Trp105 contributed to aromatic stacking, confirming its structurally stable and tightly bound conformation. Collectively, these results highlight Glabrachromene II as the most interaction-rich ligand, followed closely by Ponganone V and Ovalichromene B, which demonstrated key contacts with essential catalytic residues. These interaction features provide strong structural justification for their prioritization in further simulations and validation studies.

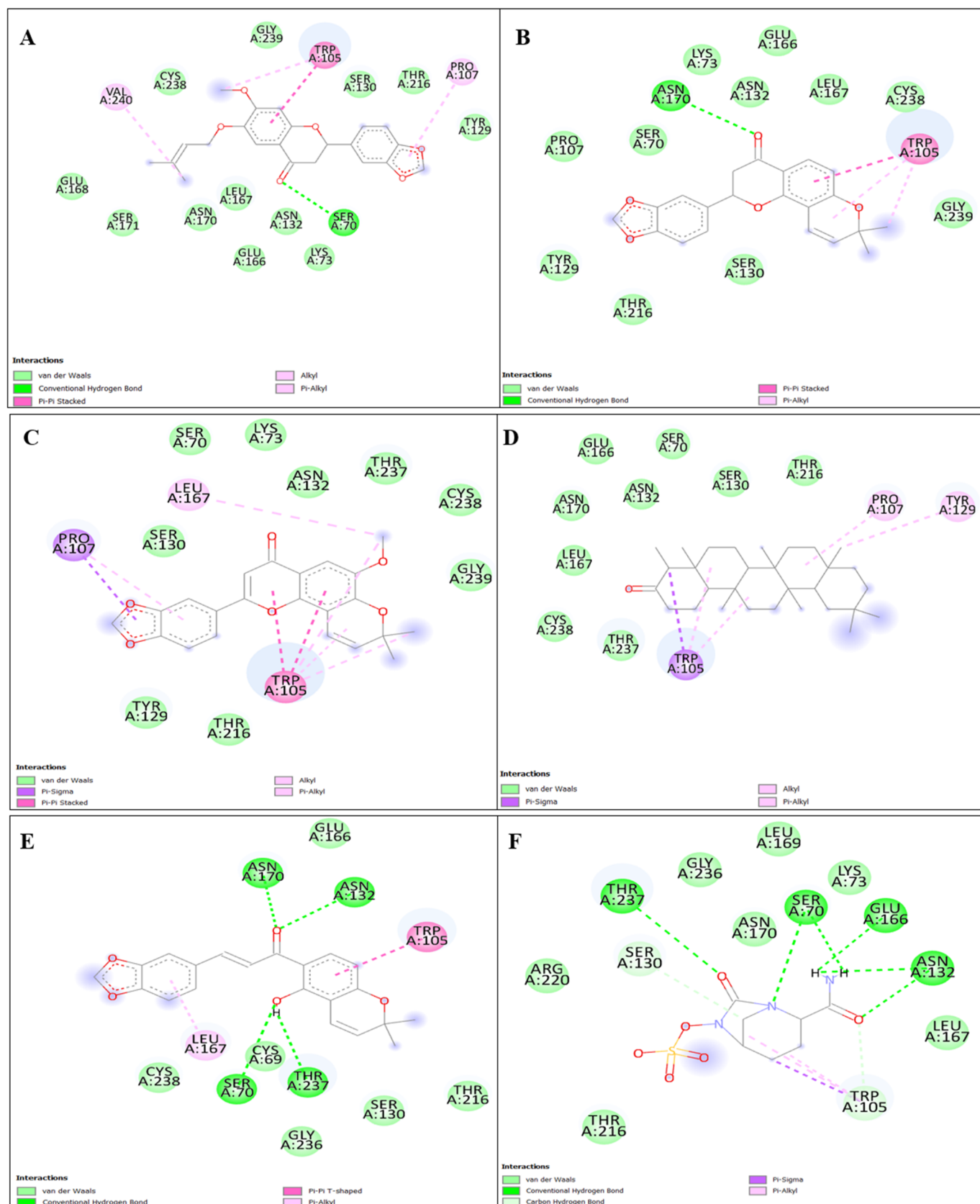


Figure 1. Molecular Interaction KPC with the Ligand 1 (A), Ligand 2 (B), Ligand 3 (C), Ligand 4 (D), Ligand 5 (E) and control Avibactam (F).

3.1.2. Reference Interaction Analysis (Avibactam)

The binding interaction profile of avibactam (CID: 9835049), a clinically approved β -lactamase inhibitor, was analyzed for comparison (Figure 1). Avibactam formed conventional hydrogen bonds with four key residues: Ser70, Asn132, Thr237, and Glu166, which are critical to the catalytic mechanism of KPC-2. Additionally, carbon hydrogen bonds were observed with Ser130 and Trp105, while π -sigma interaction with Trp105 added further stabilization through aromatic stacking. Although avibactam forms a well-distributed hydrogen bond network with catalytically important residues, its overall binding free energy (-6.3 kcal/mol) was significantly lower than that of several *Pongamia pinnata* phytochemicals. In particular, Glabrachromene II and Ponganone V not only achieved stronger binding energies but also replicated or exceeded the key residue interactions exhibited by avibactam suggesting their potential to act as natural KPC inhibitors with comparable or enhanced binding behavior.

3.2. ADMET Prediction

The pharmacokinetic and toxicity profiles of five phytochemicals from *Pongamia pinnata* which are Ponganone V, Ovalichromene B, Isopongachromene, Friedelin, and Glabrachromene II were evaluated using the pkCSM platform (Table 2). All compounds exhibited high predicted intestinal absorption ($>94\%$), with Friedelin showing the highest absorption rate (98.7%). Water solubility was low across all molecules, as expected for lipophilic phytochemicals, with values ranging from -4.42 to -5.73 log mol/L. Caco-2 permeability was acceptable in most cases, particularly for Ovalichromene B (1.485 log Papp), suggesting good passive diffusion potential. None of the compounds were predicted to be P-glycoprotein substrates, although all acted as P-glycoprotein I inhibitors. Three compounds were predicted to inhibit both P-gp I and II (Ponganone V, Isopongachromene, and Friedelin), which could influence efflux and drug–drug interaction profiles. Distribution predictions revealed moderate volume of distribution values (VDss), with Isopongachromene showing the highest (0.166 log L/kg). Notably, Friedelin was the only compound predicted to cross the blood–brain barrier (0.72 log BB), while CNS permeability values remained low for all candidates, limiting potential central nervous system exposure. In terms of metabolism, all compounds were predicted to be substrates of CYP3A4 but not of CYP2D6. Most compounds showed broad CYP inhibition potential, especially against CYP1A2, CYP2C19, CYP2C9, and CYP3A4, with Friedelin being the least inhibitory. Excretion profiles indicated low to moderate total clearance, and only Isopongachromene was identified as a renal OCT2 substrate. All five compounds were non-mutagenic (AMES test negative), non-hepatotoxic, and non-sensitizing to the skin. None were predicted to inhibit hERG I channels, while Ponganone V, Friedelin, and Glabrachromene II showed potential hERG II inhibition. The estimated acute oral toxicity (LD_{50}) values were within a close range (2.45 – 2.77 mol/kg), with Ponganone V showing the highest safety margin. Chronic toxicity (LOAEL) predictions were lowest for Friedelin (0.909 log mg/kg/day), suggesting potential long-term toxicity concerns. Overall, Ovalichromene B and Isopongachromene emerged as favorable candidates due to their high absorption, minimal CYP inhibition, and clean toxicity profiles, supporting their prioritization for further molecular dynamics simulations and toxicity validation.

Table 2. Predicted ADMET and toxicity profiles of selected *Pongamia pinnata* phytochemicals using the pkCSM servers.

Property	Model Name	Unit	Ponganone V	Ovalichromene B	Isopongachromene	Friedelin	Glabrachromene II
			Predicted Value				
Absorption	Water solubility	Numeric (log mol/L)	−5.733	−4.823	−4.429	−5.514	−4.578
	Caco2 permeability	Numeric (log Papp in 10 ^{−6} cm/s)	1.3	1.485	0.592	1.266	0.78
	Intestinal absorption (human)	Numeric (% Absorbed)	95.538	96.351	96.828	98.736	94.204
	Skin Permeability	Numeric (log Kp)	−2.742	−2.798	−2.655	−2.605	−2.978
	P-glycoprotein substrate	Categorical (Yes/No)	No	No	No	No	No
	P-glycoprotein I inhibitor	Categorical (Yes/No)	Yes	Yes	Yes	Yes	Yes
	P-glycoprotein II inhibitor	Categorical (Yes/No)	Yes	No	Yes	Yes	Yes
Distribution	VDss (human)	Numeric (log L/kg)	−0.165	0.058	0.166	−0.272	0.071
	Fraction unbound (human)	Numeric (Fu)	0	0.025	0.087	0	0
	BBB permeability	Numeric (log BB)	−0.751	−0.454	−0.61	0.72	−0.448
	CNS permeability	Numeric (log PS)	−2.792	−1.721	−1.926	−1.555	−1.891
Metabolism	CYP2D6 substrate	Categorical (Yes/No)	No	No	No	No	No
	CYP3A4 substrate	Categorical (Yes/No)	Yes	Yes	Yes	Yes	Yes
	CYP1A2 inhibitor	Categorical (Yes/No)	Yes	Yes	Yes	No	Yes
	CYP2C19 inhibitor	Categorical (Yes/No)	Yes	Yes	Yes	No	Yes
	CYP2C9 inhibitor	Categorical (Yes/No)	Yes	Yes	Yes	No	Yes
	CYP2D6 inhibitor	Categorical (Yes/No)	No	No	No	No	No
	CYP3A4 inhibitor	Categorical (Yes/No)	Yes	Yes	Yes	No	Yes
Excretion	Total Clearance	Numeric (log ml/min/kg)	0.021	−0.196	0.394	−0.04	−0.141
	Renal OCT2 substrate	Categorical (Yes/No)	No	No	Yes	No	No
Toxicity	AMES toxicity	Categorical (Yes/No)	No	No	No	No	No
	Max. tolerated dose (human)	Numeric (log mg/kg/day)	0.663	0.193	−0.074	−0.213	−0.405
	hERG I inhibitor	Categorical (Yes/No)	No	No	No	No	No
	hERG II inhibitor	Categorical (Yes/No)	Yes	No	No	Yes	Yes
	Oral Rat Acute Toxicity (LD50)	Numeric (mol/kg)	2.775	2.722	2.668	2.64	2.455
	Oral Rat Chronic Toxicity (LOAEL)	Numeric (log mg/kg_bw/day)	1.596	1.79	1.236	0.909	1.598
	Hepatotoxicity	Categorical (Yes/No)	No	No	No	No	No
	Skin Sensitisation	Categorical (Yes/No)	No	No	No	No	No
	<i>T.Pyriformis</i> toxicity	Numeric (log ug/L)	0.396	0.457	0.349	0.3	0.643
	Minnow toxicity	Numeric (log mM)	0.193	0.831	0.029	−2.384	−0.081

3.3. Molecular Dynamics Simulation

To evaluate the dynamic stability and interaction persistence of the top hit compounds, molecular dynamics (MD) simulations were carried out for Glabrachromene II and Ponganone V, both selected based on superior docking scores and rich interaction

profiles with the KPC-2 enzyme. The clinically validated inhibitor avibactam was used as a reference control for comparative analysis. Each protein–ligand complex was simulated for 100 nanoseconds under physiological conditions to assess structural stability, flexibility, and binding retention throughout the simulation period. The resulting trajectories were analyzed in terms of RMSD, RMSF, radius of gyration (Rg), solvent accessible surface area (SASA), hydrogen bonding, and free energy landscapes (FEL).

3.3.1. Root Mean Square Deviation (RMSD)

Root mean square deviation (RMSD) analysis was conducted to assess the global structural stability of the KPC-2 enzyme in complex with the top two ligands Glabrachromene II and Ponganone V compared to the reference inhibitor avibactam over a 100 ns simulation period (Figure 2). All three systems showed low and stable RMSD values suggesting the lack of large conformational changes and preserving the overall structural integrity throughout the trajectory. The KPC–Glabrachromene II complex (green) showed the lowest and most stable RMSD profile with a narrow fluctuation around ~0.11–0.14 nm, hence, indicating excellent structural stability and tight binding within the active site. The KPC–Ponganone V complex (red) displayed slightly higher fluctuations of RMSD (~0.12–0.17 nm), mostly in the terminal phase of the simulation (90–100 ns), although still in an acceptable and stable range. This implies moderate level of dynamic flexibility but no structural destabilization. For comparison, the KPC–Avibactam complex (black) was also stable with RMSD values showing close overlapping with Glabrachromene II (~0.12–0.15 nm) reaffirming its reliability in binding as a control. Overall, the RMSD analysis shows that both the phytocompounds were able to form stable and long-lasting complexes with KPC-2, with Glabrachromene II showing the best dynamic stability compared to both Ponganone V and avibactam.

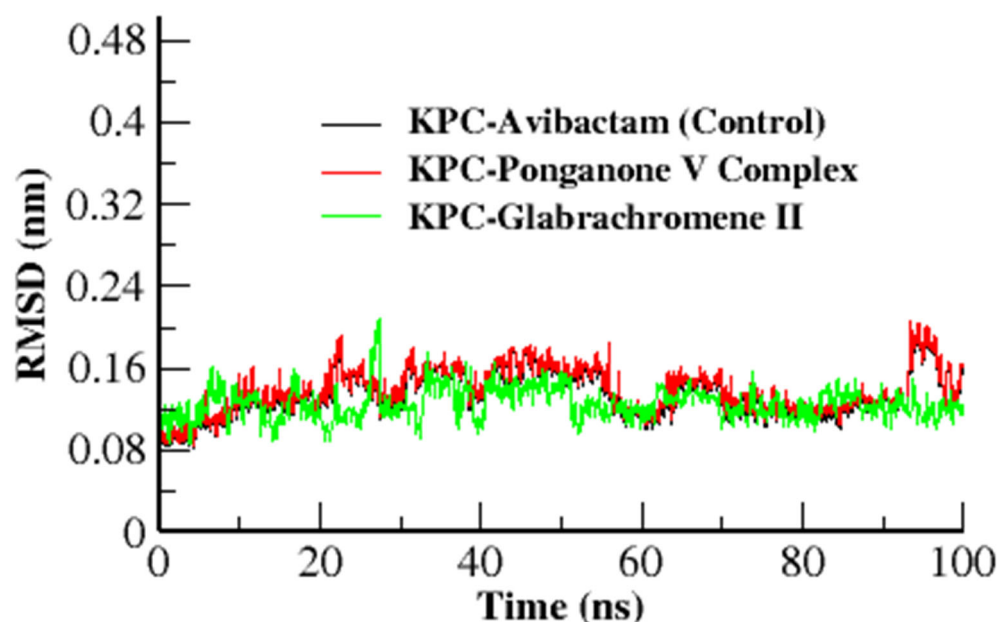


Figure 2. Root mean square deviation (RMSD) profiles of KPC-2 β -lactamase backbone atoms bound to avibactam (black), Ponganone V (red), and Glabrachromene II (green) over a 100 ns molecular dynamics simulation. All complexes exhibit low and consistent RMSD values, indicative of structural stability. The Glabrachromene II complex shows minor elevation in terminal fluctuations, suggesting modest dynamic deviation compared to the avibactam and Ponganone V complexes.

3.3.2. Root Mean Square Fluctuation (RMSF)

Over the 100 ns simulation time, the RMSF analysis was done to understand how flexible the KPC-2 protein is when bound with Glabrachromene II, Ponganone V and avibactam

(Figure 3). The measure of RMSF tells us how each amino acid fluctuates, providing clues about flexibility in the nearby structure upon binding with a ligand. Total RMSF stayed under 0.3 nm in all three structures, meaning that the proteins did not change their structures significantly. The flexible top zones of the protein could still adjust to the influence of all the ligand molecules. There were elevated fluctuations in several loop regions of the KPC complex with Ponganone V (red), especially at residues ~95–110 and ~260–270. These regions likely correspond to flexible surface loops distant from the catalytic core, and the elevated mobility may be attributed to allosteric breathing or localized conformational accommodation. The KPC–Glabrachromene II complex (green) showed moderate fluctuations around similar flexible regions but maintained consistently low RMSF values across catalytically important residues, indicating strong localized stability at the active site. This aligns with the strong hydrogen bonding observed in docking. The KPC–Avibactam complex (black) demonstrated the most uniform and restrained fluctuation profile, as expected for a known, clinically validated inhibitor. However, its flexibility profile was comparable to that of Glabrachromene II, further reinforcing the latter's dynamic compatibility with the active site. In summary, Glabrachromene II maintained optimal rigidity at key functional regions, while Ponganone V introduced marginally higher flexibility in non-essential loops. These findings are consistent with RMSD results and support the dynamic suitability of Glabrachromene II as a stable KPC-2 inhibitor candidate.

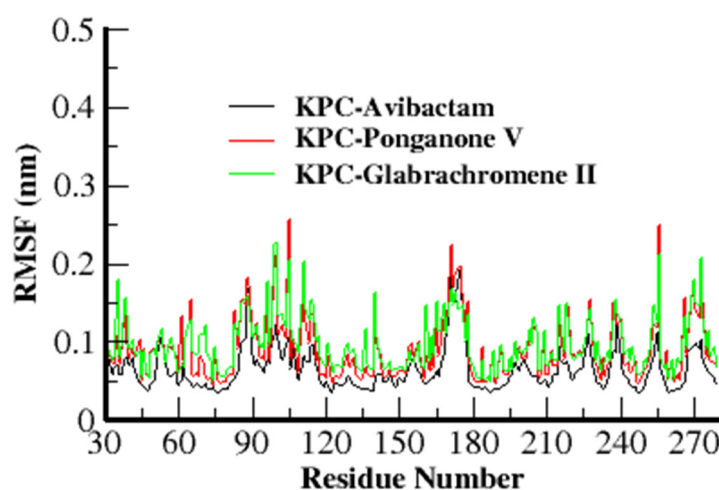


Figure 3. Root mean square fluctuation (RMSF) analysis of residue-wise flexibility for KPC-2 complexes over the 100 ns simulation. The reference complex with avibactam (black) and the Ponganone V complex (red) display low and consistent fluctuations, particularly across catalytically important residues. The Glabrachromene II complex (green) exhibits higher variability, notably in loop and terminal regions, suggesting increased dynamic motion and potential flexibility within these domains.

3.3.3. Radius of Gyration

The radius of gyration (R_g) was calculated to assess the overall compactness and folding stability of the KPC-2 protein in complex with Glabrachromene II, Ponganone V, and avibactam across a 100 ns molecular dynamics simulation (Figure 4). For the majority of the simulation period (0–80 ns), all three systems exhibited tightly clustered R_g values between 1.75 and 1.85 nm, indicating the protein maintained a compact and well-folded structure regardless of the ligand bound. The KPC–Avibactam complex (black) demonstrated the lowest and most consistent R_g values, reflecting a highly stable conformational state. The KPC–Ponganone V complex (red) remained largely unchanged, just like the control and proving the structure was strong during the simulation. However, the KPC–Glabrachromene II complex (green) remained stable with a small R_g for the first few nanoseconds, then swerved greatly to values over 2.2 nm. R_g increasing now implies the

protein is becoming less tightly packed, perhaps due to conformational changes caused by the ligand. From the findings, Glabrachromene II is able to help the protein fold properly but also becomes more flexible after some time, while Ponganone V and avibactam maintain the firm structure all through the simulation.

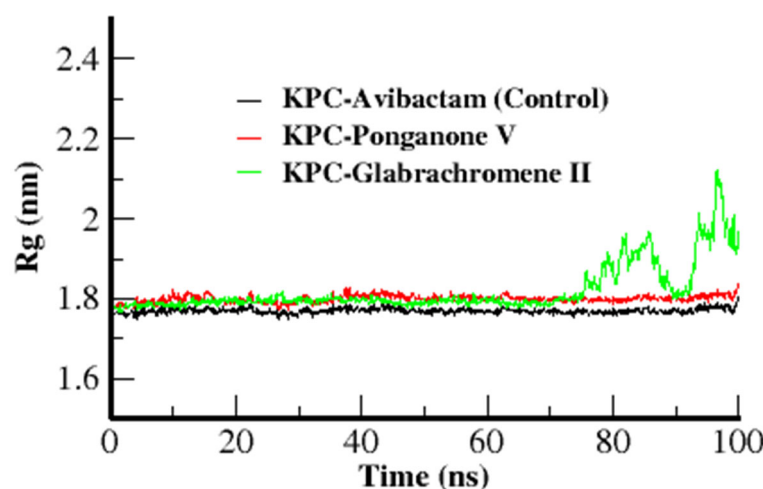


Figure 4. Radius of gyration (Rg) profiles of KPC-2 complexes over 100 ns of molecular dynamics simulation. The complexes with avibactam (black) and Ponganone V (red) exhibit minimal fluctuation in Rg, indicating sustained global compactness and structural stability. In contrast, the Glabrachromene II complex (green) shows a marked increase in Rg beyond 80 ns, suggesting partial unfolding or loosening of the protein-ligand architecture.

3.3.4. Solvent Accessible Surface Area (SASA)

The solvent accessible surface area (SASA) has been analyzed over the 100 ns simulation to observe the change in protein surface exposure and structural breathing during the ligand binding (Figure 5). The KPC-Avibactam complex (black) had the largest and most stable SASA values over the simulation with values around ~ 152 – 155 nm², indicating little solvent exposure change and a densely packed structure. In contrast, both phytochemical-bound systems KPC-Ponganone V (red) and KPC-Glabrachromene II (green) showed a lower baseline SASA value (~ 115 – 125 nm²), indicating a decreased surface exposure and potentially a more compaction locally. Notably, a progressive increase in SASA was observed in the Glabrachromene II complex after ~ 70 ns, peaking at >130 nm² at the end of the simulation, which agrees with the Rg data and points to some partial unfolding/increased surface exposure. Ponganone V showed a more uniform trend with limited fluctuation indicating greater surface stability under dynamic conditions. Overall, the data suggest that avibactam promotes the most stable surface exposure, while Glabrachromene II induces slight solvent-accessible expansion at later simulation stages.

To complement the time-dependent analysis, residue-wise SASA was calculated to identify specific regions of the KPC-2 protein exhibiting differential solvent exposure upon ligand binding (Figure 6). The control complex with avibactam (black) consistently had high SASA values in most of the residue positions, suggesting a more solvent-exposed surface throughout the structure of the enzyme. By contrast, both phytochemical-bound complexes Ponganone V (red) and Glabrachromene II (green) were found to have significantly reduced SASA values in nearly all the residues, especially in the core catalytic region. This implies a more compact, tighter binding (or shielding effects) which possibly stabilizes the active site conformation. Interestingly, Glabrachromene II revealed a significant rise in solvent exposure at the C-terminal region (residues ~ 280 – 290), which is linked to late stage Rg and total SASA peaks detected before. These localized changes in exposure could be indicative of flexible loop regions dynamically adapting to binding by the ligand. Overall,

residue-wise SASA profiling reinforces a common theme that both phytochemicals are less solvent-accessible in the active core than avibactam, and that Glabrachromene II is more terminally flexible, consistent with its more dynamic binding profile.

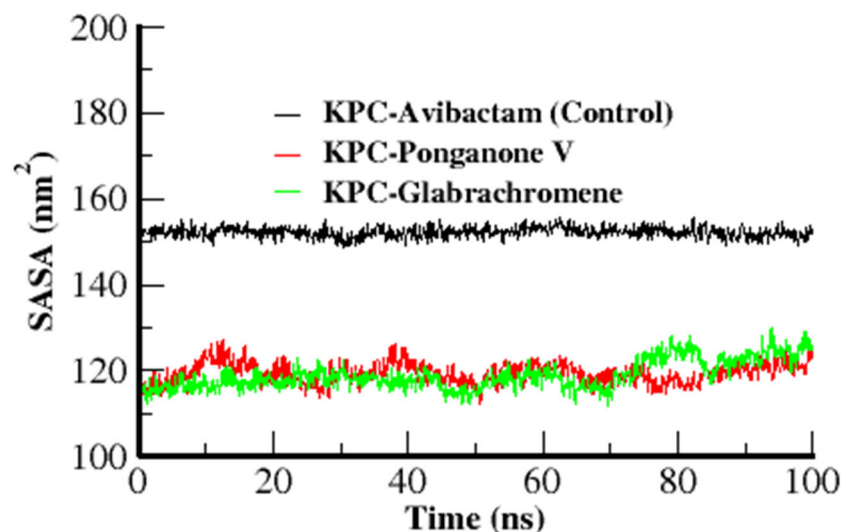


Figure 5. Time-dependent solvent accessible surface area (SASA) profiles of KPC-2 complexes over 100 ns simulation. The avibactam-bound complex (black) displays the highest and most stable solvent exposure. Ponganone V (red) shows consistently lower SASA values, reflecting reduced surface dynamics. Glabrachromene II (green) exhibits a noticeable increase in SASA after 70 ns, indicating late-stage structural relaxation or partial unfolding, consistent with its elevated radius of gyration.

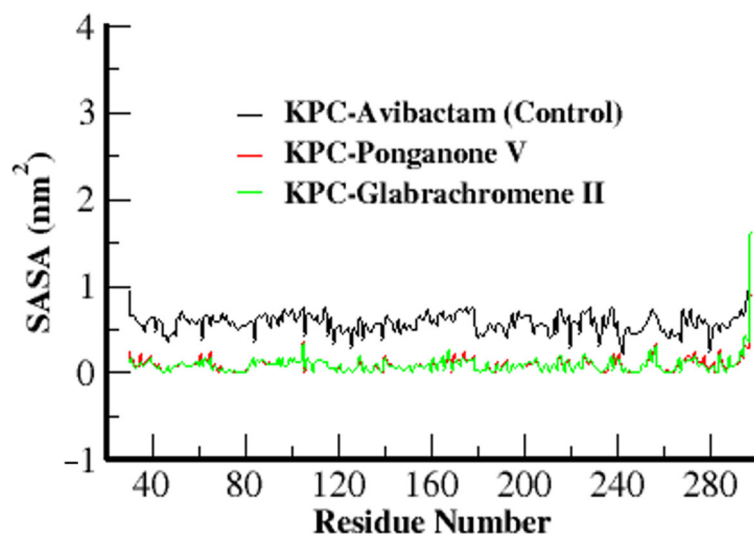


Figure 6. Per-residue SASA profile of KPC-2 complexes with avibactam (black), Ponganone V (red), and Glabrachromene II (green) over 100 ns simulation. The control complex (avibactam) exhibits higher solvent exposure across most residues. Both phytochemical-bound complexes reduce solvent accessibility at the catalytic core, while Glabrachromene II shows a marked increase in SASA at the C-terminal region, indicating localized flexibility or structural opening.

3.3.5. Hydrogen Bond

Hydrogen bond analysis gave us insight about the strength and persistence of polar interactions between the ligands and the KPC-2 protein during the 100 ns simulation (Figure 7). The KPC-Glabrachromene II complex exhibited a high number of hydrogen bonds at the start of the simulation, up to 8 H-bonds in the first 5 ns of the simulation. However, this number steadily decreased over time, with few to no hydrogen bonds being observed beyond ~70 ns, which is consistent with a slow weakening of the polar contacts as

the ligand destabilized or increased its distance from the active site. Despite this decline, a significant number of interaction pairs within 0.35 nm (red traces) was maintained between 20–70 ns, indicative that, even though formal hydrogen bonds were lost, non-bonded polar contacts were intermittently maintained. In contrast, the KPC–Ponganone V complex had a lower but more evenly distributed hydrogen bond number, usually between 0 and 3 bonds during the simulation. Of note, there was a slight re-emergence of hydrogen bonding at ~80–100 ns with the consistent black and red bar over-lap corresponding to some re-establishment of stabilizing contacts. This suggests that while Ponganone V did not create as many hydrogen bonds as Glabrachromene II, the interactions it did create were more sustained after a period of time, reflecting a more dynamically stable binding pattern with less fluctuation in polar interaction behaviour.

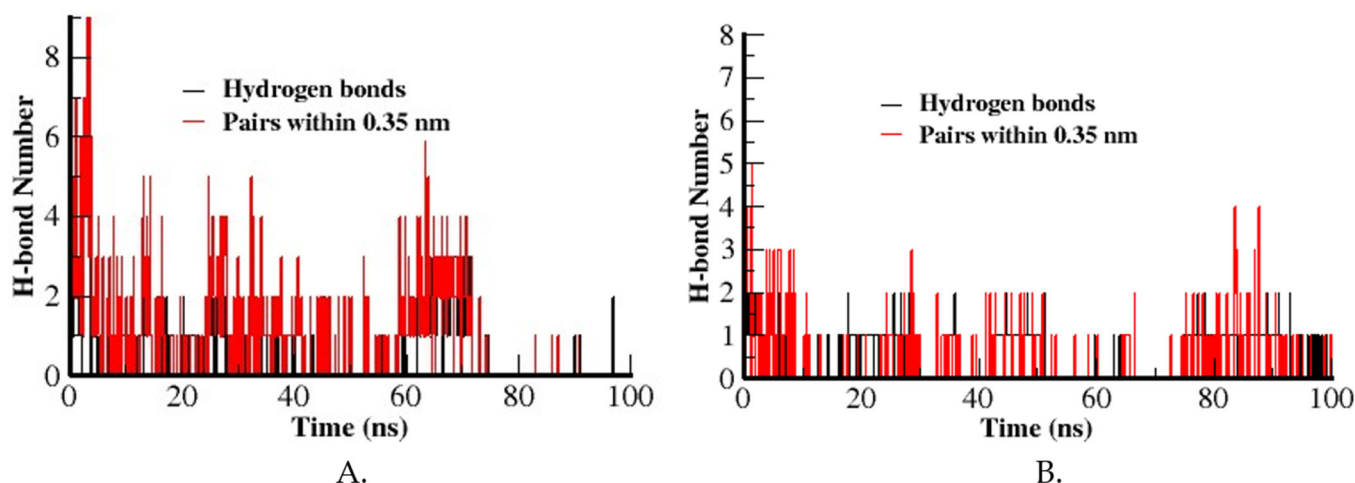


Figure 7. Hydrogen bond analysis of KPC-2 complexes during 100 ns molecular dynamics simulation. (A) Glabrachromene II complex showing a high initial number of hydrogen bonds (up to 8) which decline significantly after 60 ns, indicating transient interaction behavior and potential loss of binding stability over time. (B) Ponganone V complex with fewer hydrogen bonds overall (0–3 range) but greater continuity and resurgence of bonding after 80 ns, supporting more stable and persistent binding. Black bars represent formal hydrogen bonds; red bars indicate interacting atom pairs within 0.35 nm.

3.3.6. Principal Component Analysis (PCA) and Free Energy Landscape (FEL)

To capture the global motions and conformational transitions of the KPC-2–ligand complexes, Principal Component Analysis (PCA) was performed on the C α atomic trajectories of the 100 ns simulations. The PCA projections along the first two principal components (PC1 and PC2) reveal the essential dynamics and dominant motion patterns of the protein in each ligand-bound state (Figure 8). The KPC–Avibactam complex exhibited a broad and scattered distribution in the phase space, indicating higher conformational flexibility and sampling of multiple conformational substates. In contrast, the KPC–Ponganone V complex displayed a relatively compact and confined motion space, suggesting a more restricted and stable conformational ensemble. Similarly, the KPC–Glabrachromene II complex showed moderate dispersion, reflecting intermediate flexibility compared to the control and Ponganone V. To further probe the conformational free energy distribution, Free Energy Landscape (FEL) plots were constructed based on the first two principal components (Figure 7). The FEL of the KPC–Ponganone V complex revealed a deep and well-defined global energy minimum, representing a highly stable conformational basin. The control complex (KPC–Avibactam) displayed multiple shallow minima, indicative of a more dynamic conformational sampling with frequent transitions between metastable states. Interestingly, the KPC–Glabrachromene II complex exhibited two partially separated energy basins, implying possible conformational switching and reduced stability over the simulation period. Together, PCA and FEL analyses

reinforce the superior structural stability of the KPC–Ponganone V complex, in agreement with RMSD, Rg, SASA, and hydrogen bonding data. The compact energy well and limited essential motion highlight Ponganone V's ability to anchor the KPC-2 structure into a stable dynamic state, whereas Glabrachromene II appears to induce a more flexible and partially unstable conformational regime.

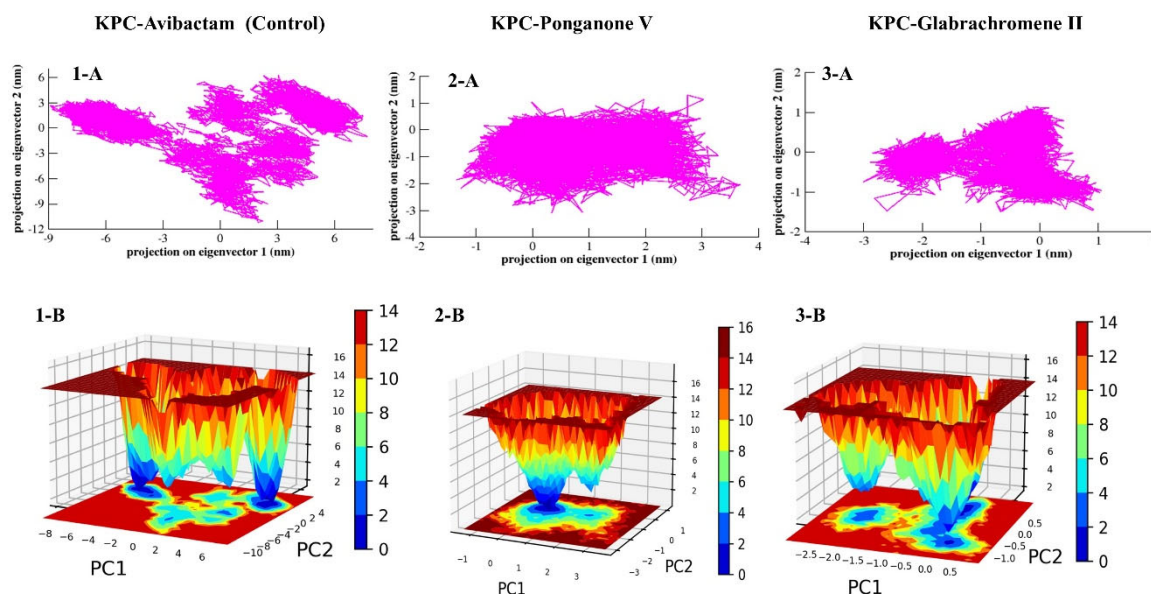


Figure 8. Principal Component Analysis (PCA) and Free Energy Landscape (FEL) of KPC-2 complexes over a 100 ns molecular dynamics simulation. (A) Two-dimensional PCA plots showing projections along the first and second eigenvectors (PC1 vs. PC2) for KPC-2 in complex with avibactam (1-A), Ponganone V (2-A), and Glabrachromene II (3-A). The avibactam complex displays broader conformational sampling, while Ponganone V shows restricted and stable motion space. (B) Corresponding FEL plots based on PC1 and PC2, depicting conformational free energy basins for avibactam (1-B), Ponganone V (2-B), and Glabrachromene II (3-B). Ponganone V exhibits a single deep and stable energy minimum, indicative of high conformational stability. Avibactam shows multiple shallow minima, while Glabrachromene II displays bifurcated wells, suggesting conformational heterogeneity.

4. Discussion

The current study employed an integrated computational pipeline to screen phytochemicals from *Pongamia pinnata* as potential inhibitors of *Klebsiella pneumoniae* carbapenemase-2 (KPC-2), a serine β -lactamase enzyme that confers resistance to nearly all β -lactam antibiotics. With the rise of carbapenem-resistant Enterobacterales (CREs) and the emergence of resistant variants even to advanced β -lactamase inhibitor combinations like ceftazidime-avibactam and meropenem-vaborbactam [32], identifying new, nature-derived inhibitors remains a critical need [33]. From molecular docking, Ponganone V and Glabrachromene II emerged as top candidates based on binding affinity, surpassing the clinically used inhibitor avibactam. Ponganone V exhibited a docking score of -9.0 kcal/mol with a key hydrogen bond to Ser70 and π - π stacking with Trp105, mimicking the binding geometry critical for β -lactam inhibition [34]. Glabrachromene II formed more extensive polar interactions including Ser70, Asn132, and Thr237, yet its dynamic profile showed some destabilization over time. Pharmacokinetic evaluation through pkCSM confirmed both compounds possess favorable ADMET properties, including high intestinal absorption, non-substrate behavior toward P-glycoprotein, and absence of mutagenicity or hepatotoxicity. While Glabrachromene II exhibited slight liabilities in the inhibition of CYP enzymes, Ponganone V had a much cleaner ADMET profile which increases its drug-likeness while reducing the concern for toxicity associated with metabolism. Molecular dynamics simulations also helped to strengthen

the case for Ponganone V. It maintained compactness and backbone stability with RMSD and Rg values closely overlapped with the avibactam-KPC reference system. In contrast, Glabrachromene II, while stable at the outset, displayed an increasing value of RMSD, radius of gyration and SASA after 80 ns, indicating a partial conformational relaxation or destabilization of the ligand. Hydrogen bonding analysis was consistent with these observations. Glabrachromene II formed numerous H-bonds that were short-lived initially in the simulation giving way to significant diminishing patterns over time which indicated transient interaction patterns. Ponganone V although forming fewer bonds, had more consistent and sustained hydrogen bonding contributing to stable occupancy within the binding pocket over the course of the full 100 ns. These results are important when viewed within the wider context of KPC inhibition in the landscape. While there are advanced inhibitors such as meropenem-vaborbactam and ceftazidime-avibactam that are two paradigms of the front-line therapy, resistance by KPC variants (e.g., KPC-33, KPC-14) and porin loss is becoming increasingly reported and push the field to consider alternative scaffolds including natural products [33]. Studies have highlighted that broad-spectrum β -lactamase inhibitors derived from bacterial proteins like BLIPs can bind across multiple serine β -lactamases, including KPC-2, which supports the plausibility of phytochemical inhibitors designed through rational docking and dynamics evaluation [35].

Despite its strength, the study is by no means without limitations. The computational findings, while predictive, need experimental confirmation by enzymatic inhibition assays, MIC determination and cytotoxicity screening in relevant bacterial and mammalian systems. Moreover, compound bioavailability and synthetic accessibility have to be assessed before any kind of translational advancement. In summary, the present work identifies Ponganone V as a very promising candidate of KPC-2 inhibitors, which not only shows consistent binding in the structure, but also has better pharmacokinetics and molecular stability than the standard avibactam. Glabrachromene II, although it has a strong initial binding, showed signs of late stage instability. These insights support further preclinical development of phytochemicals from *Pongamia pinnata* as potential next-generation agents to combat resistant pathogens against beta-lactams. While Ponganone V showed great computational promise, there are still several translational considerations that can be studied with specific experimental investigations. These include its low predicted aqueous solubility, the currently unknown serum protein binding profile, which could affect free drug availability, and the potential for structural or medicinal chemistry optimisation to balance potency with favourable pharmacokinetics. In addition, it remains to be established whether Ponganone V has its own antibacterial activity such as interaction with penicillin-binding proteins, and how this works in combination with the beta-lactam antibiotics, which would be at the centre of its therapeutic use as beta-lactamase inhibitor. Practical aspects such as synthetic accessibility and feasibility of the preparation of preparative quantities will also need evaluation. Addressing these elements will be the key to turning the present in silico lead identification results into meaningful in vitro and in vivo validation.

5. Conclusions

This study describes an exhaustive in silico screening of phytochemicals of *Pongamia pinnata* as a potential beta-lactamase inhibitor of KPC-2. Through molecular docking, ADMET and toxicity profiling, molecular dynamics simulations, and principal component analysis, Ponganone V was identified as the best candidate with the best binding affinity, active site interaction stability, favorable pharmacokinetic properties, and lowest toxicity compared to the reference inhibitor, avibactam. PCA and free energy landscape analysis complemented the dynamic stability providing restricted conformational motion and one deep energy basin. While Glabrachromene II had a good initial binding and interaction

richness, it had a less stable structure during the long simulation. These findings give a sound scientific foundation to promote the further development of Ponganone V as a lead compound for further in vitro and in vivo validation that is crucial in the development of novel strategies against the development of Beta-lactam resistant pathogens. This study is constrained by its reliance on preliminary computational analyses without accompanying experimental validation, which may limit the accuracy and immediate applicability of the findings. Computational predictions provide only indicative trends and cannot fully capture the complexity of biological interactions. Future research should incorporate rigorous in vitro assays, followed by in vivo evaluation, to substantiate the proposed mechanisms and confirm the therapeutic potential of the identified compounds. Although some of these tools were developed earlier, AutoDock Vina-based docking, pkCSM ADMET prediction, and GROMACS molecular dynamics remain standard and widely used approaches in 2025, as they continue to be applied and validated across numerous recent published studies and provide reliable early-stage insights before experimental testing.

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