In silico Evaluation of Phytochemicals as Inhibitors of Snake Venom Toxins

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Abstract

Ophitoxaemia, known as snake bite envenomation, is a significant global health challenge in tropical and subtropical regions. Leading causes for snake bite mortality and morbidity include inadequate healthcare facilities in rural areas, transportation, cost of anti-venom, insufficient availability and delayed administration of antivenom in rural areas. This study aims to perform in silico molecular docking and toxicity evaluations as preliminary investigations to formulate an emergency drug that could extend the lifespan of snake bite victims by serving as a safeguard until further treatment. Snake venom is a cocktail of enzymes classified as toxins responsible for causing pathological conditions from tissue damage to paralysis. Molecular docking studies were performed by targeting snake venom toxins including phospholipase A2 (PDB ID: 1SXK), snake venom metalloprotease (PDB ID: 2E3X), snake venom serine protease (PDB ID: 1OP2), three-finger toxin (PDB ID: 3VTS) and L-amino acid oxidase (PDB ID: 5TS5), using major phytoconstituents from Piper longum through Schrödinger software and to estimate phytoconstituents pharmacokinetic profile.

The docking scores ranged from -1.73 to -6.51 indicating a significant binding towards the toxins. India's rich heritage in medicinal plants and traditional knowledge, combined with the limitations of current anti-snake venom treatments, underscores the potential of isolated phytoconstituents as inhibitors. Computational tools offer a promising approach to discovering lead molecules derived from traditional remedies, bridging folklore.

Keywords: Snake envenomation, *Piper longum*, phytoconstituents, docking and pharmacokinetics.

Introduction

Snakebite envenomation is a significant global health challenge, particularly in tropical and subtropical regions[10]. Snakebite mortality and morbidity are encountered majorly in India by venomous snakes such as the Indian spectacled cobra (*Naja naja*), common krait (*Bungarus caeruleus*), Russell's viper (*Daboia russelii*) and saw-scaled viper (*Echis carinatus*)[11]. Delayed approaches to healthcare, insufficient antivenom supplies and reliance on traditional remedies exacerbate the high mortality and morbidity associated with snakebites, particularly in rural areas[2,7,8].

Snake venom is a highly complex and potent biological mixture primarily of proteins and peptides which can be divided into different toxin families each causing different and multi-pathological effects on the human body[13]. Predominantly the toxin families in snake venom are phospholipase A_2 (PLA_2), snake venom metalloproteinase (SVMP), snake venom serine protease (SVSP) and three-finger toxins (3FTx) which cause pathological effects by rupturing cell membranes, blocking nerve signal transmission, affecting the intrinsic and extrinsic factors of coagulation cascade, causing oxidative stress and disrupting the cellular signaling pathways[12]. Interestingly, along with the complexity, venom composition significantly varies due to factors like age, gender, geographic location, diet and seasonal changes. This variability paves the way for more therapeutic innovation[3].

The traditional system of medicine and folklore remedies have been the primary treatment followed by people in rural areas. Studies have long been employed to manage snakebite envenomation, with many plants demonstrating potential venom-neutralizing properties. Among these, *Piper longum* (long pepper) has been selected for the current study because of its rich phytoconstituents including alkaloids, terpenoids, flavonoids and polyphenol composition which exhibit anti-inflammatory, antioxidant and enzyme-inhibitory activities[4]. Preliminary studies suggest that these compounds can mitigate venom-induced pathophysiological effects including tissue damage and coagulation disturbances, highlighting their potential as natural antidotes. However, the molecular mechanisms underlying these effects and their efficacy against specific venom toxins remain inadequately explored[1,6].

In this study, we aim to use molecular docking to investigate the interactions of phytoconstituents from *Piper longum* and

major snake venom toxins including PLA_2, SVMP, SVSP, $3FT_X$ and LAAO. Targeting the major components of snake venom and exploring the pharmacokinetic profile will pave the way for developing plant-based therapeutic agents for snakebite envenomation.

Material and Methods

Protein and ligand retrieval: A total of 73 phytoconstituents were retrieved from the IMPPAT database and downloaded in SDF file format. Toxins such as phospholipase A_2 (PDB ID: 1SXK), snake venom metalloprotease (PDB ID: 2E3X), snake venom serine protease (PDB ID: 1OP2) and three-finger toxins (PDB ID: 3VTS) were retrieved from RSCB-Protein Data Bank.

Molecular docking: The binding modes of phytoconstituents in the catalytic pocket of a target protein were studied using molecular docking methodology.

Ligand preparation: 2D structures of 73 phytoconstituents were downloaded from the IMPPAT database and converted to 3D by using the LigPrep module of Schrodinger Suite 2024-1 and optimized to generate possible tautomeric states and low-energy conformers. OPLS4 (Optimized Potentials for Liquid Simulations) force field was utilized for energy minimization.

Protein preparation: The protein structures were prepared using the Protein Preparation Wizard tool of Schrodinger Suite 2024-1. The 3D crystallographic structure of proteins was prepared by removing water molecules, refining bond orders and addition of hydrogens by using protein preparation wizard tools. Prime (v4.3) module was used for adding missing side chains and loops followed by the generation of protonation and tautomeric states for acidic and basic residues at pH 7.0[9]. To optimize and minimize the protein structure, OPLS4 force field was used. with RMSD of crystallographic heavy atoms kept at 0.30 Å[14]. For proteins containing the co-crystals, a grid box was generated at the centroid of active sites keeping the Van der Waals scaling of 0.8 for the receptor with 0.15 as the partial charge cut-off. For apoproteins, a site map was used to generate active sites and a grid.

Ligand Docking: The generated low energy poses of phytoconstituents were docked using the Glide module of Schrodinger Suite 2024-1 into the active site of phospholipase A_2 (PDB ID: 1SXK), snake venom metalloprotease (PDB ID: 2E3X), snake venom serine protease (PDB ID: 1OP2), three finger toxin (PDB ID: 3VTS) and L-anino acid oxidase (PDB ID: 5TS5) using extra precision mode (XP)[5] keeping other parameters default. The best docking pose was selected based on glide g-score and glide energy values.

***In silico* pharmacokinetic evaluation:** The ADME properties for the selected compounds showing maximum glide score were investigated by the *in-silico* method by using SwissADME software.

Results

Molecular docking: From the molecular docking results top binding affinities of phytochemicals against five key snake venom toxin targets: phospholipase A_2 (PLA_2, PDB ID: 5TS5), snake venom metalloproteinase (SVMP, PDB ID: 3VTS), snake venom serine proteinase (SVSP, PDB ID: 2E3X), three-finger toxin (3FTX, PDB ID: 1SXK) and L-amino acid oxidase (LAAO, PDB ID: 1OP2) were given in the table 1. The docking results (binding affinities in kcal/mol) revealed varying degrees of interaction strength, with lower values indicating higher binding affinities. Pluviatilol consistently demonstrated the strongest binding across most toxin targets, with binding affinities of -6.38 kcal/mol (5TS5), -6.51 kcal/mol (2E3X) and -3.85 kcal/mol (1OP2).

Similarly, p-Cymene exhibited strong interactions with 5TS5 (-6.18 kcal/mol) and 2E3X (-5.16 kcal/mol). In contrast, compounds like (E)-beta-ocimene exhibited relatively weaker binding affinities, with values ranging from -1.74 kcal/mol (3VTS) to -3.13 kcal/mol (5TS5), indicating lower efficacy against venom toxins.

Toxicity studies: The ADMET analysis of seven phytochemicals identified from *Piper longum* revealed their potential as drug-like molecules. Key parameters such as molecular weight, lipophilicity (Log P), topological polar surface area (TPSA) and drug-likeness according to Lipinski's Rule of Five were evaluated. All compounds exhibited molecular weights below 500 Da, indicating their suitability for oral bioavailability.

Table 1
Binding Scores of Selected Phytoconstituents from *Piper longum*

S.N.	Phytochemical	5TS5	3VTS	2E3X	1SXK	1OP2
1	p-Cymene	-6.18237	-3.31933	-5.16211	-3.36301	-4.68735
2	Pluviatilol	-6.38832	-3.85561	-6.51104	-2.5721	-3.85208
3	Terpinolene	-5.55852	-3.25483	-5.18374	-2.86194	-4.4584
4	4'-Methoxyacetophenone	-4.09314	-4.63777	-6.31694	-3.50519	-4.89893
5	(+)-beta-Phellandrene	-5.10686	-3.47859	-5.5477	-3.19614	-4.80311
6	Alpha-thujene	-4.83887	-3.4406	-5.47022	-3.18134	-4.60166
7	(E)-beta-ocimene	-3.1315	-1.73929	-2.69801	-2.0498	-2.33494

Log P values were in the range of -1.2 to 2.3, reflecting balanced hydrophilic and lipophilic properties conducive to membrane permeability. The TPSA values (ranging from 15.47 Å² to 26.30 Å²) further supported their favorable absorption profiles. Notably, none of the compounds inhibited CYP450 enzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4), which reduces the likelihood of drug-drug interactions. Furthermore, the phytoconstituents except pluviatilol were not substrates for P-glycoprotein, suggesting efficient intracellular retention. All compounds demonstrated high bioavailability scores (0.55), minimal PAINS alerts and moderate synthetic accessibility, further highlighting their potential for therapeutic development.

2-D Interactions: The molecular docking analysis of pluviatilol and p-cymene against five venom toxin targets: 3FTx, SVMP, PLA_2, LAAO and SVSP revealed binding interactions mediated through hydrogen bonds, hydrophobic interactions and Pi-Pi stacking. A summary of these interactions is mentioned in tables 3 and 4 and the 2-D interaction of pluviatilol and p-cymene with toxins is demonstrated in figures 1 and 2.

Discussion

The results of this study demonstrate the potential of phytochemicals from *Piper longum* as inhibitors of key snake venom toxins. Among the tested compounds, pluviatilol exhibited the strongest binding affinities across the toxin targets, particularly with PLA_2 (-6.38 kcal/mol) and SVSP (-6.51 kcal/mol). PLA_2 and SVSP are critical components of snake venom, contributing to hemotoxicity, neurotoxicity and tissue necrosis. The high binding affinities of Pluviatilol suggest its potential to neutralize these effects effectively. p-Cymene also demonstrated strong interactions with PLA_2 and SVSP, highlighting its potential as a secondary lead compound. Its favorable ADMET profile, including high bioavailability and lack of CYP enzyme inhibition, further supports the selection of these phytoconstituents for drug development.

In contrast, (E)-beta-ocimene showed relatively weaker binding affinities across all toxin targets, which, coupled with its lower ADMET favorability (e.g. low permeability), suggested its limited potential as a therapeutic agent against snake venom. The absence of CYP inhibition and P-gp substrate activity across all compounds reduce the likelihood of adverse pharmacokinetic interactions and fewer toxicity. This feature is particularly advantageous in the context of snakebite treatment, where polypharmacy is often required to address complex systemic effects of envenomation.

Furthermore, the low PAINS alerts and moderate synthetic accessibility scores enhance the feasibility of advancing these phytochemicals to preclinical development. The docking and ADMET results together suggest that pluviatilol and p-Cymene are promising candidates for further investigation, by *in vitro* and *in vivo* studies, to validate their venom-neutralizing activity and safety.

Conclusion

This study validates the therapeutic potential of phytochemicals from *Piper longum* as inhibitors of snake venom toxins such as PLA_2, SVMP, SVSP, 3FTX and LAAO. The evaluation of molecular docking and ADMET profiling identified pluviatilol and p-cymene as the most promising candidates due to their strong binding affinities and favorable pharmacokinetic properties.

Table 2
ADME properties of phytoconstituents

Parameter	p-Cymene	Pluviatilol	Terpinolene	4'-Methoxyacetophenone	(+)-beta-Phellandrene	Alpha-thujene	(E)-beta-ocimene
Molecular weight (MW)	134.22	356.37	136.23	150.17	136.23	136.23	136.23
Log P	2.51	3.02	2.71	1.93	2.65	2.67	2.8
TPSA	0	66.38	0	26.3	0	0	0
Rotatable bonds	1	3	0	2	1	1	3
Lipinski Rule Violations	1	0	0	0	0	1	0
Bioavailability score	0.55	0.55	0.55	0.55	0.55	0.55	0.55
CYP Inhibition (All enzymes)	No	No	No	No	No	No	No
P-gp Substrate	No	Yes	No	No	No	No	No
PAINS Alerts	0	0	0	0	0	0	0
Brenk Alerts	0	0	1	0	0	1	2
Log Kp (cm/s)	-4.21	-6.71	-3.96	-5.98	-4.69	-5.11	-4.11
Synthetic accessibility score	1	4.19	2.98	1	3.73	3.99	3.63

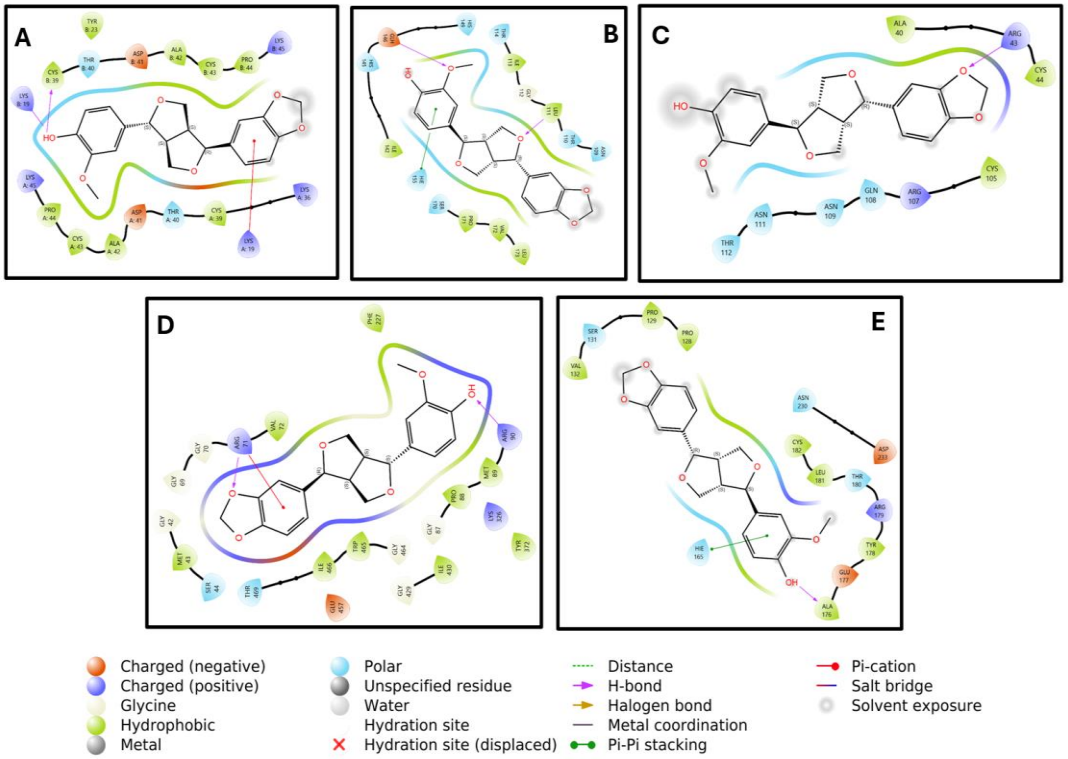

Figure 1: Two-dimensional interaction diagrams of Pluviatilol with different snake venom toxins.
The interactions of Pluviatilol with key amino acid residues of five toxin targets are illustrated. (A) Interaction with a 3-finger toxin (3FTx); (B) interaction with snake venom metalloproteinase (SVMP); (C) interaction with phospholipase A_2 (PLA_2); (D) interaction with L-amino acid oxidase (LAAO); and (E) interaction with snake venom serine proteinase (SVSP). The diagrams depict hydrogen bonds, hydrophobic interactions, salt bridges, Pi-cation interactions and solvent-exposed residues, highlighting the binding modes and key stabilizing forces.

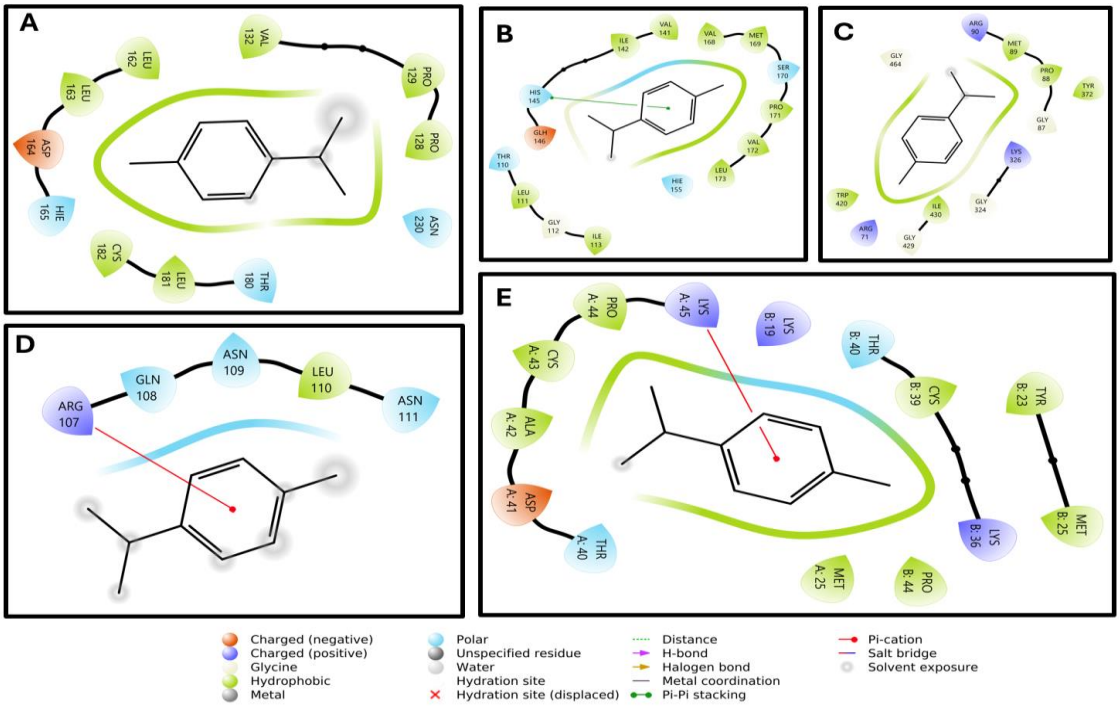

Figure 2: Two-dimensional interaction diagrams of p-cymene with different snake venom toxins.
The interactions of p-cymene with key amino acid residues of five toxin targets are illustrated. (A) interaction with snake venom serine proteinase (SVSP); (B) interaction with snake venom metalloproteinase (SVMP); (C) interaction with L-amino acid oxidase (LAAO); (D) Interaction with PLA_2; and (E) Interaction with 3-finger toxin (3FTx) The diagrams depict hydrogen bonds, hydrophobic interactions, salt bridges, Pi-cation interactions and solvent-exposed residues, highlighting the binding modes and key stabilizing forces

Table 3
Interaction of ligand Pluviatilol within the catalytic pocket of toxins

Toxin Target	Hydrogen Bonds	Pi-Pi stacking	Hydrophobic interactions
3-Finger Toxin (3FTx)	CYS B:39, LYS B:19	-	PRO A:44, CYS A:43, ALA A:42, CYS A:39, CYS B:39, ALA B:42, CYS B:43, PRO B:44, TYR B:23
Snake Venom Metalloproteinase (SVMP)	GUH 146, LEU 111	HIE 155	ILE 142, PRO 171, VAL 172, LEU 173, ILE 173, LEU 111
Phospholipase A_2 (PLA_2)	ARG 43	-	CYS 105, CYS 44, ALA 40
L-Amino Acid Oxidase (LAAO)	ARG 71, ARG 90	-	VAL 72, MET 43, ILE 466, TRP 465, ILE 430, TYR 372, PRO 88, MET 89, PHE 227,
Snake Venom Serine Protease (SVSP)	ALA 176	HIE 165	VAL 132, PRO 129, PRO 128, CYS 182, LEU 181, TYR 178,

Table 4
Number of hydrogen bonds and specific amino acid residues involved in p-cymene within the catalytic pocket of toxins

Toxin Target	Hydrogen Bonds	Pi-Pi stacking	Hydrophobic interactions
3-Finger Toxin (3FTx)	-	-	ALA A:42, CYS A:43, PRO A:44, CYS B:39, PRO B:44, MET A:25, TYR B:23, MET B:25
Snake Venom Metalloproteinase (SVMP)	-	HIS 145	ILE 142, VAL 141, VAL 168, MET 169, PRO 171, VAL 172, LEU 173, ILE 113, LEU 111
Phospholipase A_2 (PLA_2)	-	-	LEU 110
L-Amino Acid Oxidase (LAAO)	-	-	MET 89, PRO 88, TYR 372, ILE 430, TRP 420
Snake Venom Serine Protease (SVSP)	-	-	LEU 162, LEU 163, CYS 182, LEU 181, PRO 128, PRO 129, VAL 132

From the results, it is evident that these phytochemicals after screening through molecular docking studies and toxicity screening can be further tested *in vitro* and *in vivo* studies and can be converted into an emergency dosage form which can prolong the life of snake bite victims until they receive the antivenom.

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