Original Article

# Screening of Phytochemicals Against Snake Venom Metalloproteinase: Molecular Docking and Simulation Based Computational Approaches

**Ahmad Mohajja Alshammari[1]***

[1]Department of Biology, College of Science, University of Hail, Kingdom of Saudi Arabia.

## Abstract

Echis coloratus (Carpet viper), which is a snake, produces an enzyme called snake venom metalloproteinase (SVMP), which has multiple functions. The venomous viper Echis coloratus is endemic to the Middle East and Egypt. Its symptoms include severe local bleeding, nervous system impacts and tissue necrosis. The target proteins' three-dimensional (3D) structures were predicted using the I-TASSER server because the 3D structure of SVMP is unknown. Using a molecular docking technique, the molecular operating environment (MOE) application was used to screen a library of 1000 phytochemicals against the interaction residues of the target protein. Additionally, molecular dynamics simulations and the widely used MM-GBSA and MM-PBSA binding free energy techniques were used to assess the molecular docking experiments. The results showed that in the SVMP active region, the selected lead compounds were remarkably stable. Promising potential drug candidates (Rutamarin, Enterodiol, Butyl butyrate, Colchicine, Sanggenon A, Quercetin, Campesterol, and Cholesterol) for novel target against SVMP has been discovered in the conducted studies.

**Keywords:** SVMP, Phytochemicals, Molecular docking, Drug targets, Molecular dynamic simulation

## Introduction

Venoms are essential in the defense of venomous animals. Venom components activate cells, receptors, and signalling pathways, resulting in discomfort and inflammation [1]. Envenomation by snakes has a huge health and economic impact around the world. Snakebite is a deadly environmental and occupational disease, especially in rural areas of tropical countries, where around 40,000 to 50,000 snakebites are reported each year [2]. The poisonous viper Echis coloratus is indigenous to the Gulf Region and Egypt. It reaches a maximum total length of 75 cm. From sea level to 2,500 meters (8,200 ft) above sea level, it can be found in stony deserts [3]. Sand deserts are devoid of it.

Notable envenoming snakes include Echiscoloratus and Naja n. Nigricollis, whose bites cause both systemic and local pathology [4]. However, when it comes to their pathophysiological activities, the venoms of the two species react differently [5]. The bite of an Echis coloratus (Carpet viper) causes inflammation (swelling, blistering, and necrosis) as well as haemorrhages [6]. Snake venom is a complex mixture of proteins and peptides that serve a number of biological purposes. Regularly used to assess snake envenomation are horses and sheep. Snake venom metalloproteinase (SVMP) is a zinc metalloproteinase that belongs to the extracellular matrix (ECM) protein family [7]. SVMP has been implicated as a mediator for edoema, local tissue injury, inflammation, and bleeding in recent research [8]. Because of the limited haemorrhage and damage to endothelial cells, SVMP is an enzymatic toxin [9].

As a result of the hemorrhaging brought on by SVMP, tissue necrosis, shock, hypotension, hypovolemia, inflammation, and a diminished ability for muscle tissue to regenerate were all caused. The toxin SVMP is considered to be the most significant in the pathology brought on by snake venom. The majority of the time, SVMP disrupts the junction between the basement membrane and the endothelium, which directly affects capillary blood vessels and damages them, causing hemorrhage [10, 11]. It is obvious that SVMP-targeted medication therapy would have a positive consequence by lowering patient mortality rates [12].

**Address for correspondence:** Ahmad Mohajja Alshammari, Department of Biology, College of Science, University of Hail, Kingdom of Saudi Arabia.
dr.mohajja@gmail.com



**How to cite this article:** Alshammari AM. Screening of Phytochemicals Against Snake Venom Metalloproteinase: Molecular Docking and Simulation Based Computational Approaches. Arch Pharm Pract. 2022;13(3):76-84. https://doi.org/10.51847/HIrDcdPCGL

Antivenin is the only specific treatment for snakebite envenoming. Investigations have shown that antivenin is ineffective against venom-induced local tissue damage and frequently causes clinical symptoms like anaphylactic shock. As a result, alternative, synthetic or natural snake venom inhibitors are required to help get around antivenin limitations. The overall local tissue damage after envenomation may be significantly reduced by inhibition of SVMPs and other components. Finding SVMP inhibitors is now a top research priority as a result. Studies have revealed that snake venom enzymes are inhibited by plant secondary metabolites.

The interaction between the receptor protein SVMP and the ligand is demonstrated using a molecular docking approach. Ligands are small molecules that bind to the active pockets of protein. These pockets are the specific sites where the ligand can interact with the receptor protein (SVMP). There are numerous unique pocket active sites on which binding can occur in receptor atoms. There are different binding pockets, and binding modes. Docking also provides information on the strength of the binding, the ligand-receptor complex energy, and the complex docking score calculation [13]. The most advantageous aspect of docking is its use in medicine. The algorithm and energy scoring function are used to anticipate different possess when ligand binds with the receptor protein.

The search for investigation of inhibitors is a crucial phase in medication development. By discovering inhibitors against the target protein (SVMP), this research aids in evaluating the inhibitors blocking the effect of SVMP and are useful for drug designing to overcome antivenin restrictions. Furthermore, the current study predicts the inhibitors that showed strong binding affinity during docking and good stability with receptor protein in the process of molecular dynamic simulation.

## Materials and Methods

### Protein Preparation

Amino acid sequence of SVMP was retrieved from Uniprot [14]. Using computer algorithms, the amino acid sequence can be used to create a three-dimensional model of a protein. I-TASSER was used to model the 3D structure of SVMP [15]. The above server uses multiple-threading alignments and iterative prototype fragment assembly simulations to create 3D protein structures. I-TASSER provides confidence scores to determine the accuracy of the model. The predicted tertiary structure was checked for errors using the ProSA-web, verify 3D, ERRAT server, and Ramachandran plot analysis [16-19]. The average quality score of the structure is described by ProSA-web. Furthermore, non-bonded atom-atom interactions were evaluated using the ERRAT server. The energetically permitted and prohibited dihedral angles of amino acid residues, phi (φ) and psi (ψ), were evaluated using the Ramachandran plot. Protein structure preparation for docking was carried out using the Molecular Operating Environment (MOE) [20].

### Ligand Database Preparation

A thorough literature search was done to find phytochemicals that have been noted to be effective against snake bites. Chemical structures of phytochemicals were obtained from the MPD3 database, the Pubchem database, the MAPS database, and the Zinc database in a variety of ligand file formats, including mol, sdf, and mol2 [21-23]. The Protonate3D module was used in MOE to modify these ligand structures by adding partial charges. The MMFF94X force field was used to lower the energy of each ligand. The ligands were then added one by one to the MOE ligand database for docking.

### Active Sites Prediction

CPORT, a tool included in the Haddock interphase, was used to predict active sites of the target protein (SVMP) [24]. These active site pockets will bind to the target protein, at which point the ligand will bind to inhibit the protein's activity. Donor, acceptor, hydrophobic, hydrophilic, and metal-binding domains make up the sites. For molecular docking, the most precise predicted binding pocket was chosen.

### Molecular Docking

A ligand database of over 2500 phytochemicals was docked using the MOE Dock tool at the SVMP protein's designated docking sites. The triangular matcher algorithm was used to find 1,000 best poses for docked molecules using the default ligand placement method. The London DG scoring function was used to redo the scoring of simulated poses [25]. The London dG algorithm, which determines final binding energy using the Generalized Born Solvation Model while maintaining rigid receptor residues, was used to further reduce the top ten scored poses per molecule. In addition, the compounds' binding affinity, S-score, and Root-Mean-Square Deviation (RMSD) were used to rank them. Only substances with a high docking score that bind to the SVMP protein's active residues were to be chosen from the top-ranked poses.

### Ligand-Protein Interaction Analysis

To better comprehend the most intricately docked complex protein-ligand interactions, 2D plots of ligand-protein interactions were examined using the MOE LigX tool [26]. In the SVMP active site, it generates a two-dimensional graph of hydrogen bonds, electrostatic interactions, hydrophobic interactions, and all of these factors contribute to the increased affinity of drug-like molecules. 3D images of SVMP protein inhibitor complexes were created using UCSF Chimera [27].

### Drug scan/ADME Toxicity

Using the drug scanning tool on the Molinspiration server, a computational approximation of the drug probability of docked phytochemicals was discovered within the parameters

established by Lipinski's Rule [28, 29]. The ADMETSAR server was used to simulate the absorption, deposition, metabolism, excretion, and toxicity profiles of these hits [30]. Additionally evaluated were AMES toxicity and the inhibitors' potential carcinogenicity.

### MMGBSA/MMPBSA Analysis

Using the Prime module of the Schrodinger suite and the OPLS-2005 force field, it was possible to calculate the binding free energies of protein-ligand complexes [31, 32]. Binding free energies were assessed using the MM-GBSA method and the MM-PBSA.py program, with 250 snapshots taken throughout the final 20 ns of simulation at regular intervals. The complex free energy was subtracted from the total of the ligand and protein free energies to determine the binding free energy.

$$\Delta G(\text{binding}) = \Delta G(\text{complex}) - [\Delta G(\text{ligand}) + \Delta G(\text{protein})] \quad (1)$$

Where ΔG(binding) denotes the binding free energy and ΔG(complex), ΔG(ligand), and ΔG(protein) denote the complex, ligand, and protein free energies, respectively.

### MD Simulations

MD simulations were conducted using the Desmond software [33]. The OPLS-2005 force field and the TIP4P water model were used to analyze the protein-ligand interactions in this system [34]. A 10 Å buffer region was made between the orthorhombic water box's sides and the protein atoms using this method [35]. The systems were defused by adding Na+ ions after the overlapping water molecules were removed. The OPLS-2005 force field was used to calculate energy. A 2.0 fs value was obtained by maintaining the temperature at 300 K throughout the integration step. The consistency of the SVMP protein in its natural motion was tracked using the root mean square fluctuations (RMSF), root mean square deviation (RMSD), radius of gyration (RoG), and solvent accessible surface area (SASA). After saving the synchronized file at intervals of 5000 ps for up to 100 ns, the results were examined using the Nagasundaram *et al*. method [36].

## Results and Discussion

### Protein Preparation

The SVMP protein's amino acid sequence was obtained from Uniprot (UNIPROT ID: E9JGE0) in fasta format. The I-TASSER server was employed to predict SVMP's 3D structure. I-TASSER was used to develop the top five models, from which the best model was chosen, with a C-score of around -5.04, indicating high quality. The model's quality was confirmed by Ramachandran plot analysis, which revealed that 90.5 % and 7.1 % of overall amino acids are found in the most favorable and allowed regions, respectively, while only 0.4 percent are found in the disallowed region. Further analysis revealed that the model was of good quality, with a Z-score of -6.86, a compatibility score of 81.62 % (as determined by Verify 3D), and a quality factor of 80.8 (as determined by ERRAT).

The structure was prepared for docking using MOE. MOE was used to perform 3D protonation, water molecule removal, and energy minimization.

### Active Site Prediction

Cport was used to select binding pockets against the target protein. The reported active residues for the receptor SVMP were discovered to be Pro 1, Tyr3, Tyr 40, Arg 41, Asn 44, His 46, Glu 209, Leu 210, Leu 211, Gln 127, Asp 220, Pro 221, Thr 223, Ser 232.

### Molecular Docking

With the aid of a database of phytochemical ligands, the SVMP protein was docked. An S-score function was used to calculate and represent the hydrogen bonding strength, maximum binding pocket occupancy, lowest Gibbs free energy, and potential non-covalent interactions in order to rank the docked compounds. Out of 25, 000 docked molecules, the best docking molecules were chosen. According to threshold-based criteria, the ligand had to bind to the target SVMP protein using all of the hotspot conserved residues in the binding pocket and achieve the necessary S-score values (the lower the score, the greater the interactions and affinities).

The active sites of the SVMP were found to bind Rutamarin, Enterodiol, Butyl butyrate, Colchicine, Sanggenon A, Quercetin, Campesterol, and Cholesterol with high affinity **(Figures 3 and 4)**. The minimum binding energy of these selected phytochemicals ranged from -19.87 kcal/mol to -13.85 kcal/mol. The scoring function and minimum binding energy of each docked ligand are shown in **Table 1**.

**Table 1.** Docking Statistics of SVMP against Plant Compound

| Snake venom metalloproteinase (SVMP) | | | | |
|---|---|---|---|---|
| **Compounds I'D** | **Compounds name** | **Binding Affinity** | **RMSD** | **Interacting Residues** |
| 26948 | Rutamarin | -19.87 kcal/mol | 1.23 | Asn 44, Arg 41, His 46, Thr 223, Tyr 3, Glu 209, Pro 1 |
| 115089 | Enterodiol | -18.76 kcal/mol | 0.94 | Asn 44, Pro 1, Arg 41,Asp 220, Pro 221, Ser 232, Tyr 3,His 46, Tyr 40 |

| | | | | |
|---|---|---|---|---|
| 7983 | Butyl butyrate | -16.01 kcal/mol | 1.76 | Asn 44, Pro 1, Arg 41, Tyr 3, Thr 223, Pro 221 |
| 6167 | Colchicine | -15.67 kcal/mol | 2.09 | Arg 41, Asn 44, Pro 1, Thr 223, His 46, Glu 209, Leu 210, Leu 211, Tyr 3 |
| 156707 | Sanggenon A | -15.34 kcal/mol | 1.56 | Glu 209, Gln 197, Cys 218, Tyr 3, Glu 209, Arg 41, His 46 |
| 5280343 | Quercetin | -14.87 kcal/mol | 1.99 | Leu 211, Cys 219, Val 222, His 46, Thr 223, Tyr 3, Arg 41 |
| 173183 | Campesterol | -14.39 kcal/mol | 1.02 | Asn 44, Pro 221, Glu 209,Pro 1, Arg 41,Thr223 |
| 5997 | Cholesterol | -13.85 kcal/mol | 2.04 | Tyr 3, Arg 41, Pro 1, Glu 209,His 46, Thr 223, Gln 197 |

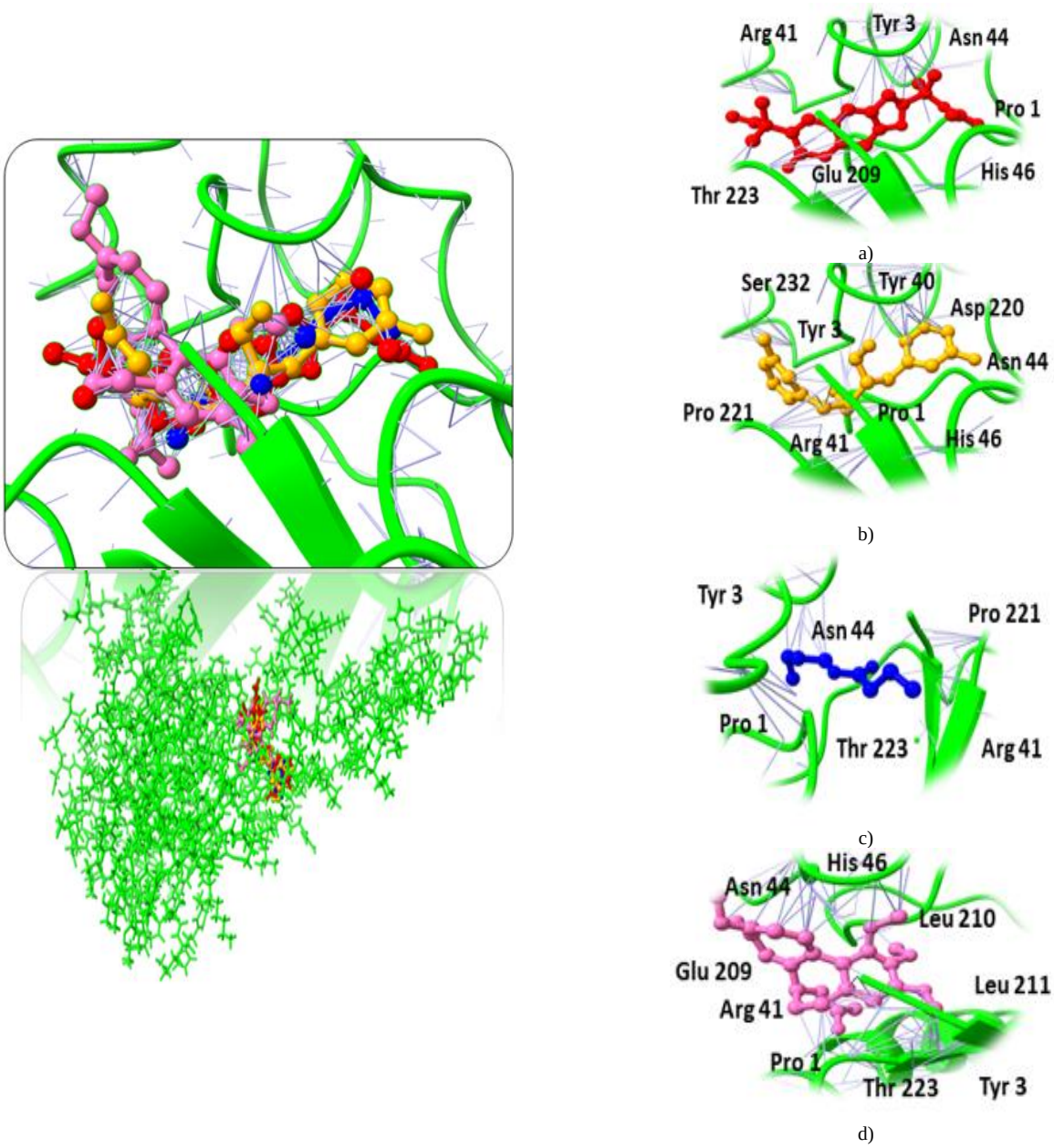



**Figure 1.** Interaction mechanisms and binding modes of SVMP protein inhibitors. Interaction analysis of Rutamarin (a), Enterodiol (b), Butyl butyrate (c), Colchicine (d)

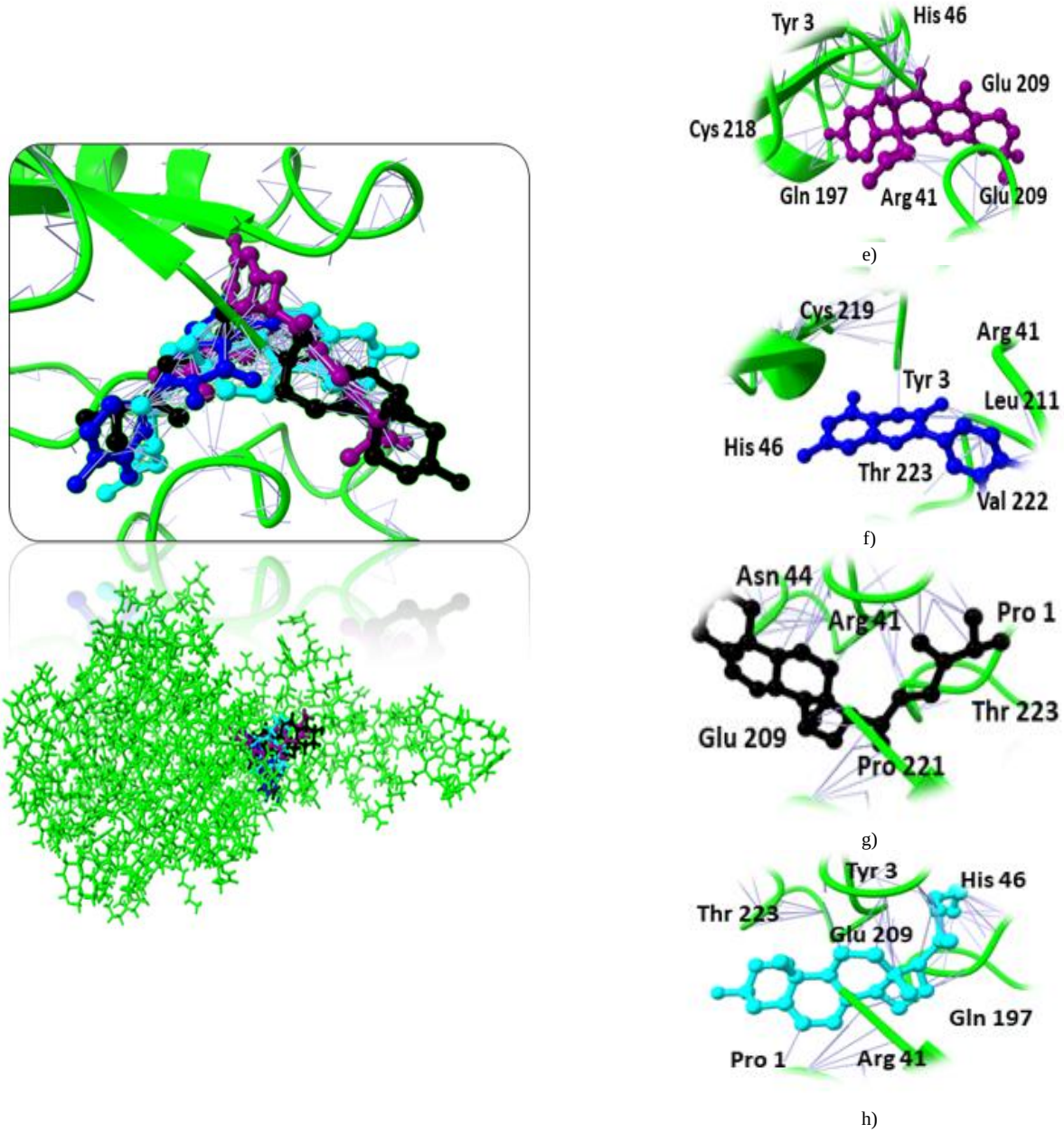



**Figure 2.** SVMP protein inhibitors' mechanisms of interaction and modes of binding. Rutamarin (e), Enterodiol (f), Butyl Butyrate (g), and Colchicine (h) Interaction Analysis

Rutamarin, on the other hand, was ranked first among the revealed SVMP binding residues due to its high affinity and binding score. Most phytochemicals seem to have a strong affinity for the amino acids Arg 41 and Thr 223, implying that these are the most active residues.

### *Drug Scan/ADMET Results*

The Lipinski Rules of Five-based Molinspiration server was used to predict the drug-likeness of screened compounds. The chosen candidates exhibited drug-like properties and did not violate the "rule of five" **(Table 2)**.

**Table 2.** Drug-likeliness properties of selected compounds

| Compound I'D | Molecular Weight (g/mol) | Hydrogen Bond Acceptor | Hydrogen Bond Donor | miLogP |
|---|---|---|---|---|
| 26948 | 356.4 | 0 | 5 | 4.4 |
| 115089 | 302.37 | 4 | 4 | 2.39 |
| 7983 | 144.21 | 0 | 2 | 2.2 |

| | | | | |
|---|---|---|---|---|
| 6167 | 399.44 | 7 | 1 | 1.10 |
| 156707 | 436.46 | 7 | 3 | 4.60 |
| 5280343 | 302.23 | 7 | 5 | 1.5 |
| 173183 | 400.7 | 1 | 5 | 4.2 |
| 5997 | 386.7 | 1 | 1 | 4.7 |

The pharmacokinetic properties of selected compounds were analyzed using the admetSAR server to further verify the potential of drug likeliness, and the results are presented in **Table 3**.

**Table 3.** ADMET profiling of the compounds

| Compound's I'Ds | 26948 | 115089 | 7983 | 6167 | 156707 | 5280343 | 173183 | 5997 |
|---|---|---|---|---|---|---|---|---|
| **Absorption/Distribution** | | | | | | | | |
| Blood Brain Barrier | No | No | No | No | No | No | No | No |
| Caco-2 Permeability | Low | High | High | Low | Low | Low | Low | High |
| Pgp-inhibitor | No | No | No | No | No | Yes | No | No |
| Pgp-substrate | No | No | No | No | No | No | Yes | No |
| **Metabolism** | | | | | | | | |
| CYP1A2 inhibitor | No | No | No | No | Yes | Yes | Yes | No |
| CYP1A2 substrate | No | No | No | Yes | No | No | Yes | No |
| CYP2C19 inhibitor | No | No | No | No | No | No | No | No |
| CYP2C19 substrate | No | Yes | No | Yes | No | No | No | No |
| CYP2C9 inhibitor | No | No | Yes | No | No | yes | No | No |
| CYP2C9 substrate | No | No | No | No | No | No | No | Yes |
| CYP3A4 inhibitor | No | No | No | No | No | No | No | No |
| CYP3A4 substrate | No | Yes | No | No | No | No | No | No |
| **Toxicity** | | | | | | | | |
| Rat Oral Acute Toxicity | No | No | No | No | No | No | No | No |
| AMES Toxicity | Non Toxic | Non Toxic | Non Toxic | Non Toxic | Non Toxic | Non Toxic | Non Toxic | Non Toxic |

## *MMGBSA/MMPBSA Analysis*

To better comprehend how well the complexes bind to the SVMP protein, the binding free energies were calculated using the MMGBSA/MMPBSA techniques. Stable complexes are produced because all of the binding interactions are advantageous in terms of energy. In all of the complexes, gas-phase energy outweighs van der Waals energy and electrostatic energy in terms of system energy. More details about the complexes' binding energies can be found to be −27.37 kcal/mol-1 for complex (26948), -29.74 kcal/mol-1 for complex (115089), −24.58 kcal/mol-1 for complex (7983), −27.04 kcal/mol-1 for (6167), −24.58 kcal/mol-1 for complex (156707) while the remaining complexes having delta total enegy of -23.12 kcal/mol-1(5280343), −30.45 kcal/mol-1 (173183) and −30.45 kcal/mol-1 (5997).

## *Molecular Dynamic Simulation*

The docking analysis was used to select the optimum possible pose in which the ligand might make a strong binding with the receptor. In addition, an MD simulation was carried out in order to determine the interaction patterns of ligands (Rutamarin and Enterodiol) with the target protein SVMP. Desmond was the one who carried out the real time MD simulation. The system builder for Desmond made use of an overt aqueous medium to bring the complex to its lowest possible energy level, which was then immediately followed by the complex minimization stage. The complete simulation procedure, which includes three runs at a real-time rate of 100 ns each. Each of these simulations provided an explanation of the interaction patterns and the stability of the complexes in terms of the RMSD and RMSF

## *Root Mean Square Deviation (RMSD)*

Despite this, RMSD was determined for both Rutamarin and Enterodiol compound after 100 ns of simulation, taking into account both the ligand and the protein. The relative mean standard deviation (RMSD) for the ligands name with the SVMP is depicted in **Figures 3a and 3b** respectively. The findings of the RMSD analysis showed that the MD simulation had reached equilibrium, and the conformational changes that occurred ranged from 5 to 6 degrees for first complex Rutamarin/SVMP, while second complex Enterodiol/SVMP showed a minor deviation between the time periods of 40 to 60 ns both of which are considered acceptable for small globular proteins. Based on these RMSD

measurements, it can be concluded that the SVMP has not undergone significant structural changes.

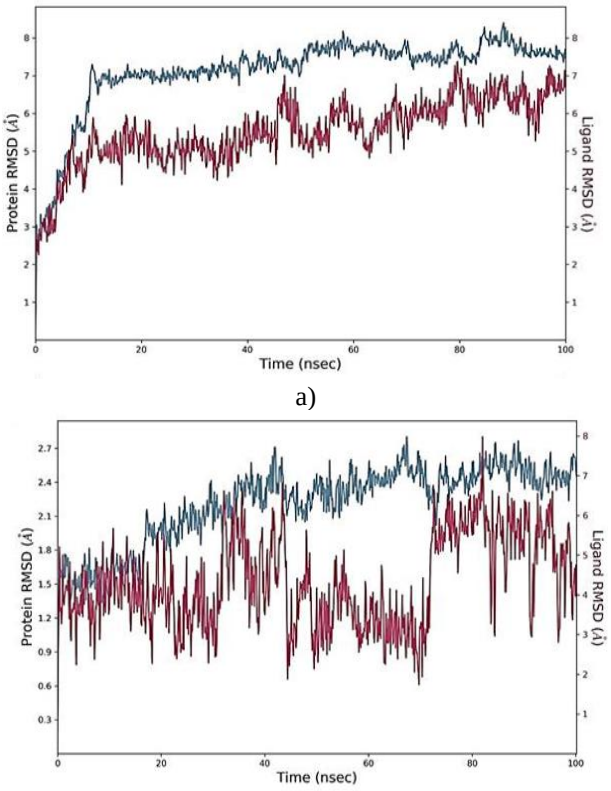
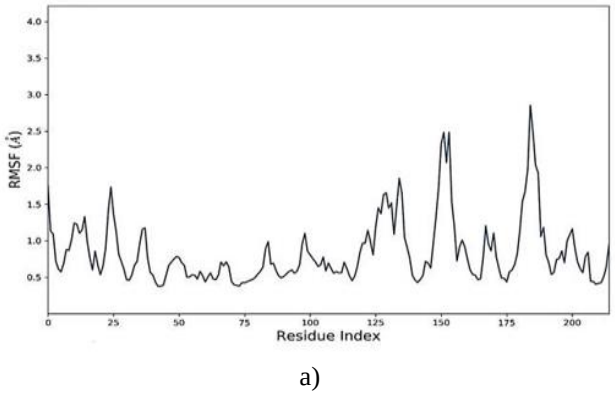



**Figure 3.** Root mean square Deviation of both complexes a) Rutamarin/SVMP, b) Enterodiol/SVMP

## *Root Mean Square Fluctuation (RMSF)*

The root mean square flacuation (RMSF) plot of the ligands (Rutamarin and Enterodiol) positions along the right side of the Y axis indicated that all of these ligands remained stable during the simulation with regard to the protein binding pocket as indicated in the **Figure 4**.



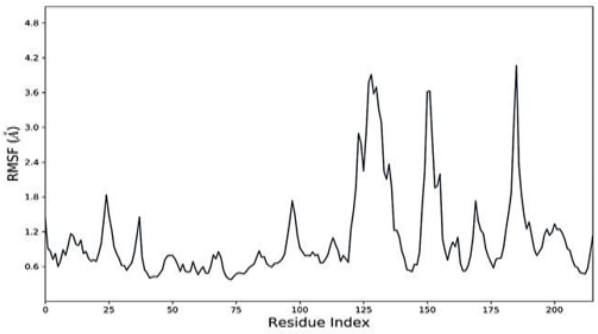




**Figure 4.** Graphical representation of RMSF plots a) Rutamarin/SVMP, b) Enterodiol/SVMP

Snakebites are a major health concern, affecting 1.8–2.2 million people each year and causing 81,000–138,000 deaths [37]. These envenomations, which occur in severe and moderate cases, are characterized by local pathological changes like dermonecrosis, myonecrosis, edema, hemorrhage, and blistering as well as systemic changes like cardiovascular shock, coagulopathies, and acute renal failure [38]. Various enzymes, such as snake venom metalloproteinases (SVMPs), are primarily responsible for this particular set of systemic and local changes [39].

Intravenous administration of anti-venoms derived from animals (mostly sheep or horses) seems to be the only effective remedy for snakebite envenomation. Clinical studies show that anti-venoms are efficacious in inactivating toxins that cause systemic effects like hemodynamic disturbances and coagulopathy [40]. The inability of antivenom to neutralize local tissue damage caused by snake venoms limits its effectiveness. Antivenom's effectiveness is constrained by its inability to counteract the localized tissue damage brought on by snake venoms. This problem is caused by the incredibly rapid local pathology development, which makes it difficult for inactivating antibodies to reach the area before irreversible damage occurs, rather than a deficiency in neutralizing antibodies in antivenoms [41, 42]. As a result, it is necessary to develop synthetic and natural venom inhibitors to supplement the effects of antivenoms, particularly in the prevention of local tissue damage.

Plants have been used for centuries to treat various diseases, and there has been a growing emphasis in recent years on recognizing and employing plant-derived compounds that can act as effective anti-venom agents [43]. Different flavonoids have also been shown to inhibit the hemorrhagic activity of isolated SVMPs or whole snake venoms [44, 45]. Hence, we chose 2500 plant-derived natural compounds and flavonoids to test for inhibitory properties in our research.

Molecular docking is a well-known technique to investigate the interactions of plant compounds with target protein active sites. In this study, SVMP was docked against the

phytochemical ligand database. The process of molecular docking makes predictions about how substances will bind to their intended protein targets. Information on compound activity and protein binding affinity is provided by these computational techniques [46, 47].

Rutamarin, Enterodiol, Butyl butyrate, Colchicine, Sanggenon A, Quercetin, Campesterol, and Cholesterol were discovered to bind to the active sites of the SVMP protein with a high affinity. The new substances may work together to counteract SVMP in an additive or synergistic manner.

To gain a deeper understanding of their binding modes, molecular interaction mechanisms, and ADMET evaluation, these eight compounds were chosen for additional computational studies. Further research is being done on compounds that have the potential to be drugs' ADMET characteristics. Determining the ADMET properties of compounds is a major challenge in the drug development phase. The majority of drugs that fail to pass the drug approval process fail due to toxicity and poor pharmacokinetic properties. ADMET profiling evaluations have facilitated in the early detection of active compounds during the process of drug discovery [48, 49].

Drug distribution and absorption are influenced by both blood-brain barrier (BBB) permeation and gastrointestinal (GI) absorption. Another indication of the compounds' absorption was Caco-2 permeability. Numerous cytochromes (CYPs) regulate drug metabolism, with the biotransformation of drug molecules requiring the presence of CYP2C9, CYP2C19, CYP3A4, CYP1A2, and CYP2D6 . Additionally, drugs that are known to be transported by p-glycoprotein have decreased bioavailability. The safety profile of the compounds was then assessed using a toxicity prediction study . It was found that none of the selected compounds were toxic or carcinogenic. These findings point to the possibility of using particular compounds to make snake-bite drugs.

MD simulations are thought to be an effective tool for investigating the underlying dynamics of protein-ligand interactions. Because these ligands demonstrated strong affinity, as indicated by a high dock score and an excellent molecular interaction network, MD simulations and MMGBSA/MMPBSA analysis were performed on the best docked complexes with the inhibitors rutamarin and enterodiol. According to MD simulation and MMGBSA/MMPBSA studies, these compounds were stable as strong inhibitors within the protein binding pocket. By successfully inhibiting and concentrating on the catalytic function of the SVMP protein, these inhibitors may result in a single therapeutic approach. Therefore, further research for structure-based lead optimization is necessary in light of our findings regarding the bioactivity of rutamarin, enterodiol, butyl butyrate, colchicine, sanggenon A, quercetin, campesterol, and cholesterol.

## Conclusion

The current study sought to design therapeutic interventions by performing molecular docking and MD simulation studies on natural compounds targeting SVMP protein. Although computational validations were covered in this article, future research should focus on in vivo studies or experimental evaluations.

ACKNOWLEDGMENTS: None
CONFLICT OF INTEREST: None
FINANCIAL SUPPORT: None
ETHICS STATEMENT: This material is my own original work, which has not been previously published elsewhere.

## References

1. Fry BG, Roelants K, Champagne DE, Scheib H, Tyndall JD, King GF, et al. The toxicogenomic multiverse: convergent recruitment of proteins into animal venoms. Annu Rev Genomics Hum Genet. 2009;10(1):483-511.
2. Yang S, Liu Z, Xiao Y, Li Y, Rong M, Liang S, et al. Chemical punch packed in venoms makes centipedes excellent predators. Mol Cell Proteomics. 2012;11(9):640-50.
3. Petrilla V, Polláková M, Bekešová B, Andrejčáková Z, Vlčková R, Marcinčáková D, et al. A Comprehensive Study Monitoring the Venom Composition and the Effects of the Venom of the Rare Ethiopian Endemic Snake Species Bitis parviocula. Toxins. 2021;13(5):299.
4. Mallow D, Ludwig D, Nilson G. True vipers: natural history and toxinology of old world vipers. Krieger Publishing Company; 2003.
5. Aoki-Shioi N, Koh CY, Kini RM. Natural inhibitors of snake venom metalloproteinases. Aust J Chem. 2020;73(4):277-86.
6. Al Masroori S, Al Balushi F, Al Abri S. Evaluation of risk factors of snake envenomation and associated complications presenting to two emergency departments in Oman. Oman Med J. 2022;37(2):e349.
7. Gutiérrez JM, Albulescu LO, Clare RH, Casewell NR, Abd El-Aziz TM, Escalante T, et al. The search for natural and synthetic inhibitors that would complement antivenoms as therapeutics for snakebite envenoming. Toxins. 2021;13(7):451.
8. Kalita B, Saviola AJ, Samuel SP, Mukherjee AK. State-of-the-art review-A review on snake venom-derived antithrombotics: Potential therapeutics for COVID-19-associated thrombosis?. Int J Biol Macromol. 2021;192:1040-57.
9. Tasoulis T, Pukala TL, Isbister GK. Investigating toxin diversity and abundance in snake venom proteomes. Front Pharmacol. 2021;12.
10. Oyama E, Takahashi H. Structures and functions of snake venom metalloproteinases (SVMP) from Protobothrops venom collected in Japan. Molecules. 2017;22(8):1305.
11. Adeyi AO, Mustapha KK, Ajisebiola BS, Adeyi OE, Metibemu DS, Okonji RE. Inhibition of Echis ocellatus venom metalloprotease by flavonoid-rich ethyl acetate sub-fraction of Moringa oleifera (Lam.) leaves: in vitro and in silico approaches. Toxin Rev. 2022;41(2):476-86.
12. Wolfsberg TG, Primakoff P, Myles DG, White JM. ADAM, a novel family of membrane proteins containing A Disintegrin And Metalloprotease domain: multipotential functions in cell-cell and cell-matrix interactions. J Cell Biol. 1995;131(2):275-8.
13. Pagadala NS, Syed K, Tuszynski J. Software for molecular docking: a review. Biophys Rev. 2017;9(2):91-102.
14. Boutet E, Lieberherr D, Tognolli M, Schneider M, Bairoch A. Uniprotkb/swiss-prot. InPlant bioinformatics 2007 (pp. 89-112). Humana Press.
15. Zhang Y. I-TASSER server for protein 3D structure prediction. BMC Bioinform. 2008;9(1):1-8.
16. Wiederstein M, Sippl MJ. ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. Nucleic Acids Res. 2007;35(suppl_2):W407-10.
17. Xia TH, Bushweller JH, Sodano P, Billeter M, Björnberg O, Holmgren A, et al. NMR structure of oxidized Escherichia coli

glutaredoxin: comparison with reduced E. coli glutaredoxin and functionally related proteins. Protein Sci. 1992;1(3):310-21.
18. Lovell SC, Davis IW, Arendall III WB, De Bakker PI, Word JM, Prisant MG, et al. Structure validation by Cα geometry: ϕ, ψ and Cβ deviation. Proteins. 2003;50(3):437-50.
19. Eisenberg D, Lüthy R, Bowie JU. [20] VERIFY3D: assessment of protein models with three-dimensional profiles. InMethods in enzymology 1997 Jan 1 (Vol. 277, pp. 396-404). Academic Press.
20. Vilar S, Cozza G, Moro S. Medicinal chemistry and the molecular operating environment (MOE): application of QSAR and molecular docking to drug discovery. Curr Top Med Chem. 2008;8(18):1555-72.
21. Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, et al. PubChem substance and compound databases. Nucleic Acids Res. 2016;44(D1):D1202-13.
22. Mumtaz A, Ashfaq UA, Ul Qamar MT, Anwar F, Gulzar F, Ali MA, et al. MPD3: a useful medicinal plants database for drug designing. Nat Prod Res. 2017;31(11):1228-36.
23. Irwin JJ, Shoichet BK. ZINC− a free database of commercially available compounds for virtual screening. J Chem Inf Model. 2005;45(1):177-82.
24. de Vries SJ, Bonvin AM. CPORT: a consensus interface predictor and its performance in prediction-driven docking with HADDOCK. PloS One. 2011;6(3):e17695.
25. Podvinec M, Lim SP, Schmidt T, Scarsi M, Wen D, Sonntag LS, et al. Novel inhibitors of dengue virus methyltransferase: discovery by in vitro-driven virtual screening on a desktop computer grid. J Med Chem. 2010;53(4):1483-95.
26. Khalifa I, Zhu W, Mohammed HH, Dutta K, Li C. Tannins inhibit SARS-CoV-2 through binding with catalytic dyad residues of 3CLpro: An in silico approach with 19 structural different hydrolysable tannins. J Food Biochem. 2020;44(10):e13432.
27. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, et al. UCSF Chimera—a visualization system for exploratory research and analysis. J Comput Chem. 2004;25(13):1605-12.
28. Panda M, Purohit P, Meher BR. Structure-based virtual screening, ADMET profiling, and molecular dynamics simulation studies on HIV-1 protease for identification of active phytocompounds as potential anti-HIV agents. Mol Simul. 2022:1-9.
29. Jeysiha C, Abilasha D, Thusnavis GR, Kumaresan S, Palanisamy P. A combined study on the Molecular Docking, ADMET Profiling and Anti-tuberculosis Activity of Phytocompounds Obtained from the Barks of Cassia auriculata as Potential Inhibitors of Mycobacterium tuberculosis (H37Rv) Protein (5HKF).
30. Abdul-Hammed M, Adedotun IO, Olajide M, Irabor CO, Afolabi TI, Gbadebo IO, et al. Virtual screening, ADMET profiling, PASS prediction, and bioactivity studies of potential inhibitory roles of alkaloids, phytosterols, and flavonoids against COVID-19 main protease (Mpro). Nat Prod Res. 2022;36(12):3110-6.
31. Chandrasekaran J, Elumalai S, Murugesan V, Kunjiappan S, Pavadai P, Theivendren P. Computational design of PD-L1 small molecule inhibitors for cancer therapy. Mol Divers. 2022:1-2.
32. Raghi KR, Sherin DR, Archana TM, Saumya MJ, Sajesha KP, Manojkumar TK. Identification of Potent ABL Inhibitors from Coumestrol: An Integrative In Silico Approach. J Comput Biophys Chem. 2022:1-3.
33. Release S. 3: Desmond molecular dynamics system, DE Shaw research, New York, NY, 2017. Maestro-Desmond Interoperability Tools, Schrödinger, New York, NY. 2017.
34. Shivakumar D, Williams J, Wu Y, Damm W, Shelley J, Sherman W. Prediction of absolute solvation free energies using molecular dynamics free energy perturbation and the OPLS force field. J Chem Theory Comput. 2010;6(5):1509-19.
35. Jorgensen WL, Chandrasekhar J, Madura JD, Impey RW, Klein ML. Comparison of simple potential functions for simulating liquid water. J Chem Phys. 1983;79(2):926-35.
36. Zhu H, Liu J, Chakraborty C, Chen L. Analysing the effect of mutation on protein function and discovering potential inhibitors of CDK4: molecular modelling and dynamics studies. PLoS One. 2015;10(8):e0133969.
37. Gutiérrez JM, Calvete JJ, Habib AG, Harrison RA, Williams DJ, Warrell DA. Snakebite envenoming. Nat Rev Dis Primers. 2017;3(1):1-21.
38. Otero-Patiño R. Epidemiological, clinical and therapeutic aspects of Bothrops asper bites. Toxicon. 2009;54(7):998-1011.
39. Gutiérrez JM, Rucavado A, Chaves F, Díaz C, Escalante T. Experimental pathology of local tissue damage induced by Bothrops asper snake venom. Toxicon. 2009;54(7):958-75.
40. Gutiérrez JM, Theakston RD, Warrell DA. Confronting the neglected problem of snake bite envenoming: the need for a global partnership. PLoS Med. 2006;3(6):e150.
41. Sandesha VD, Darshan B, Tejas C, Girish KS, Kempaiah K. A comparative cross-reactivity and paraspecific neutralization study on Hypnale hypnale, Echis carinatus, and Daboia russelii monovalent and therapeutic polyvalent anti-venoms. PLoS Negl Trop Dis. 2022;16(3):e0010292.
42. Menzies SK, Dawson CA, Crittenden E, Edge RJ, Hall SR, Alsolaiss J, et al. Virus-like particles displaying conserved toxin epitopes stimulate polyspecific, murine antibody responses capable of snake venom recognition. Sci Rep. 2022;12(1):1-5.
43. Barua A, Kesavan K, Jayanthi S. Molecular Docking Studies of Plant Compounds to Identify Efficient Inhibitors for Ovarian Cancer. Res J Pharm Technol. 2018;11(9):3811-5.
44. Vale HF, M Mendes M, S Fernandes R, R Costa T, IS Hage-Melim L, A Sousa M, et al. Protective effect of Schizolobium parahyba flavonoids against snake venoms and isolated toxins. Curr Top Med Chem. 2011;11(20):2566-77.
45. Preciado LM, Comer J, Núñez V, Rey-Súarez P, Pereañez JA. Inhibition of a snake venom metalloproteinase by the flavonoid myricetin. Molecules. 2018;23(10):2662.
46. Lengauer T, Rarey M. Computational methods for biomolecular docking. Curr Opin Struct Biol. 1996;6(3):402-6.
47. ul Qamar MT, Kiran S, Ashfaq UA, Javed MR, Anwar F, Ali MA, et al. Discovery of novel dengue NS2B/NS3 protease inhibitors using pharmacophore modeling and molecular docking based virtual screening of the zinc database. Int J Pharmacol. 2016;12(6):621-32.
48. Lin B, He S, Yim HJ, Liang TJ, Hu Z. Evaluation of antiviral drug synergy in an infectious HCV system. Antivir Ther. 2016;21(7):595-603.
49. Tsaioun K, Bottlaender M, Mabondzo A. ADDME–Avoiding Drug Development Mistakes Early: central nervous system drug discovery perspective. BMC Neurol. 2009;9(1):1-1.