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ORIGINAL ARTICLE

Anti-Venom Pharmacophore Modeling and Molecular Docking Studies of *Vitis vinifera*

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ABSTRACT
Snakebite is a most important and serious medical concern and has been neglected by the World Health Organization (WHO) and other health authorities. There are between 1.2 and 5.5 million snakebites every year, leading to 25,000 to 125,000 deaths worldwide. Russell's viper or Daboia seems to be the commonest cause of mortal snakebite in Pakistan and South Asian countries. The antiserum is only a specific treatment but this antiserum does not deliver enough protection against snake venom and also has many side effects. Many medicinal plants have been used to treat envenomation by piousness snakes. The Vitis vinifera plant has been studied to be used as an antivenom for DaboiaRusselli snakebite. The purpose of the present work is to this identified the compound that will help us to design novel and potential inhibitors, as well as a good and cost-efficient drug for snake bite. Various approaches utilized including Pharmacophore modeling, virtual screening, and molecular modeling to design anti-venom drugs. Four anti-venom compounds have selected and generated a Pharmacophore model that combines their common shared features. This Pharmacophore model has used as a query against the Zinc database for screening hit compounds. As a result, we select six compounds that are most similar to the Pharmacophore model. These six compounds were docked against (1WQ9) receptor protein.
Keywords: *Pharmacophore, Snakebite, Russelli Daboia, VEGF, Virtual screening, Docking, Vitis vinifera.*

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INTRODUCTION

Snakebite is a most important and serious medical concern and has been neglected by the World Health Organization (WHO) and other health authorities [1]. Conservative data estimate that the annual death rate due to poisonous snake venom was estimated at 5.4-5.5 million, leading to 25,000 to 125,000 deaths worldwide [2, 1]. Russell's viper or *Daboia* seems to be the commonest cause of mortal snakebite in Pakistan, Burma, Southern India, Bangladesh, Sri Lanka, and Thailand [3]. Snake venoms are a compound mixture of toxic and enzymatic proteins, which contain, myotoxins, hemorrhagic metalloproteinases, proteolytic enzymes, and many other coagulant components [4]. Vascular epidermal growth factor and it's family associated proteins are vital regulators of blood vessel formation and vascular permeability. The structures and functions of tissue-type VEGFs are very much conserved in different snake species or even in the vertebrates. The venom type VEGFs of snakes are commonly distributed in several viper snake species [5].

The antiserum therapy is only a specific treatment for antivenom, which consists of immunoglobulin fragments, isolated from the plasma of several animals hyperimmunized against some specific toxins or snake venoms. Its efficiency consists in its capacity to deliver to the patient antibodies with a great affinity to snake venom and other toxins. However, the anti-venom has some side effects and limitations, such as high cost, poor capability to treat local effects, risk of immunological reactions, and challenging access in rural areas [2]. Many medicinal plants have been used for decades to treat a variety of diseases

including envenomation by piousness snakes. Glycoproteins, steroids, alkaloids, coumestin, glycosides, terpenoids, acids, pterocarpenes, tannins, and miscellaneous chemical groups present in the plant extracts have effective anti-venom against snake venom envenomation. Medicinal herbal compounds tend to neutralize toxin constituents [6]. The medicinal plant *Vitis vinifera* L. (Vitaceae) is usually known as 'wine grape'. The seed of grape was found to have potential medicinal value and showed anti-diabetic, wound healing, antiangiogenic, and antioxidant activities. The plant has been studied to be used as an antivenom for snakebite. Therefore, it is used to neutralizing the influence of the methanol extract of *Vitis vinifera* against the *Daboia Russelli* snakebite [7]. Pharmacophore modeling approaches are one of the important tools in drug discovery after the past decades' development. The Pharmacophore model is used as a query for getting likely lead compounds from different databases for designing small molecules with explicit desired attributes and for assessing the most similar and variety of molecules operation Pharmacophore fingerprints [8]. Ligand-based Pharmacophore modeling is used to develop a set of chemical compounds with essential Pharmacophore features such as hydrophobic (HyD), aromatic (Aro), hydrogen bond acceptors (HBAs) or donors (HBDs), cations, and anions. This ligand-based modeling defines the supramolecular interactions of the above-mentioned features with the desired molecular target to block its biological activity [7].

Many medicinally engrossed plant species were identified and used for several human ailments in earlier days. In each plant has 100's of bioactive compounds, and each one has its own biological and medicinal properties. The four structures of selected bioactive compounds used to treat snake bites cases are given in table 1. The main purpose of this study is an identification of potential (1WQ9) inhibitors through *in-silico* analysis, such as Pharmacophore, Virtual screening, pharmacokinetics, and molecular docking analysis.

MATERIAL AND METHODS

Selection of snake species and protein retrieval

Viper russelli is one of the four major classes of poisonous snakes that cause deaths in Pakistan and Asian countries are selected from the literature that is the commonest cause of health problems and important cause of morbidity and mortality. Vascular epidermal growth factor (VEGF-A) and its family associated proteins are vital regulators of blood vessel formation and vascular permeability and highly conserved in snake species are selected from the snake venom database (SVDB) [8].

Target Preparation and active site identification

The target protein is prepared for Pharmacophore modeling and many computational methods have been used to predict and analyze protein-ligand binding sites.

Plant selection and phytochemical screening

The medicinal plant *Vitis vinifera L*. (Vitaceae) was found to have potential medicinal value and showed anti-venom antidiabetic, wound healing, antiangiogenic, and antioxidant activities.The extracts showed complete inhibition of proteolytic and hyaluronidase activities. Analogs of Epicatichen, Kaempferol, Cianidanol, and Gallic acid were identified as potential anti-venom from the different literature review. MPD3 was used for the analysis of the phytochemical compound.

Energy Minimization and Optimization of phytochemicals

The energy of the lead compounds is minimized form Avogadro software to get minimum energy for a stable Pharmacophore model. In lead optimization, small ligand molecules ("hits") are chemically improved to increase their drug-like properties. At this stage, small molecules are modified to improve their affinity for their target.

ADMET Prediction and Toxicity Evaluation

The protocols II tool was utilized for calculating its possible toxicity of phytochemicals. ADMET properties which constitute the pharmacokinetic profile of a drug is very important in evaluating its pharmacodynamics activities. Many online tools and offline software packages are available which were utilized in predicting this behavior of the drug candidate. In the present study, we have used the PKCSM [9], SwissAdme [10], and admetSAR [11] prediction tools to check drug efficiency.

Pharmacophore Model Generation

A Pharmacophore model elucidates how various ligands can tie to a typical receptor binding site. The Pharmacophore model can be utilized to differentiate through de novo design or virtual screening novel ligands that will associate with a similar receptor. Pharmacophore features of each ligand were specified in LigandScout software and combined their shared features to design a Pharmacophore model. LigandScout permits rapidly and derive 3D Pharmacophore from basic structural data of macromolecule/ligand complexes in a fully computerized and beneficial way. LigandScout is an automatic Pharmacophore model design package [8].

Screening of phytochemicals

The Pharmacophore model was used to identify pharmacologically active lead compounds by searching a database of known 3D chemical structures. The result of this screening is a set of compounds, called hits, which best match the Pharmacophore query. ZINC database [12] is a free resource for small molecules discovery. The database contains above 20 million commercially available ligands. This database is filtered using the Pharmacophore model to specify some features and select similar compounds. Annotated compounds can be docked to the receptor and identifying the proper orientations. The procedure used for virtual screening is shown in figure 2.

Molecular Docking of screened ligands with Receptor Protein

Molecular docking is the best technique used in the structure-based drug design process. Autodock-Vina was used to find the most appropriate orientation and interactions (hydrogen bonds and hydrophobic interactions) of each lead compound at the receptor's active residues. The receptor-ligand complex was visualized in Chimera vs1.8 software.

Synthesis of Anti-Venom Compounds

Mahadeswaraswamy, *et al* [7] synthesized effective compounds whose properties inhibit hyaluronolytic, caseinolytic, and fibrinogenolytic activities of snake venom. And successfully inhibited the toxic effects, of citrated human plasma such as coagulation, myonecrosis, hemorrhage, and edema. These strategies will be considered for our future work to achieve the aim and goal of the research.

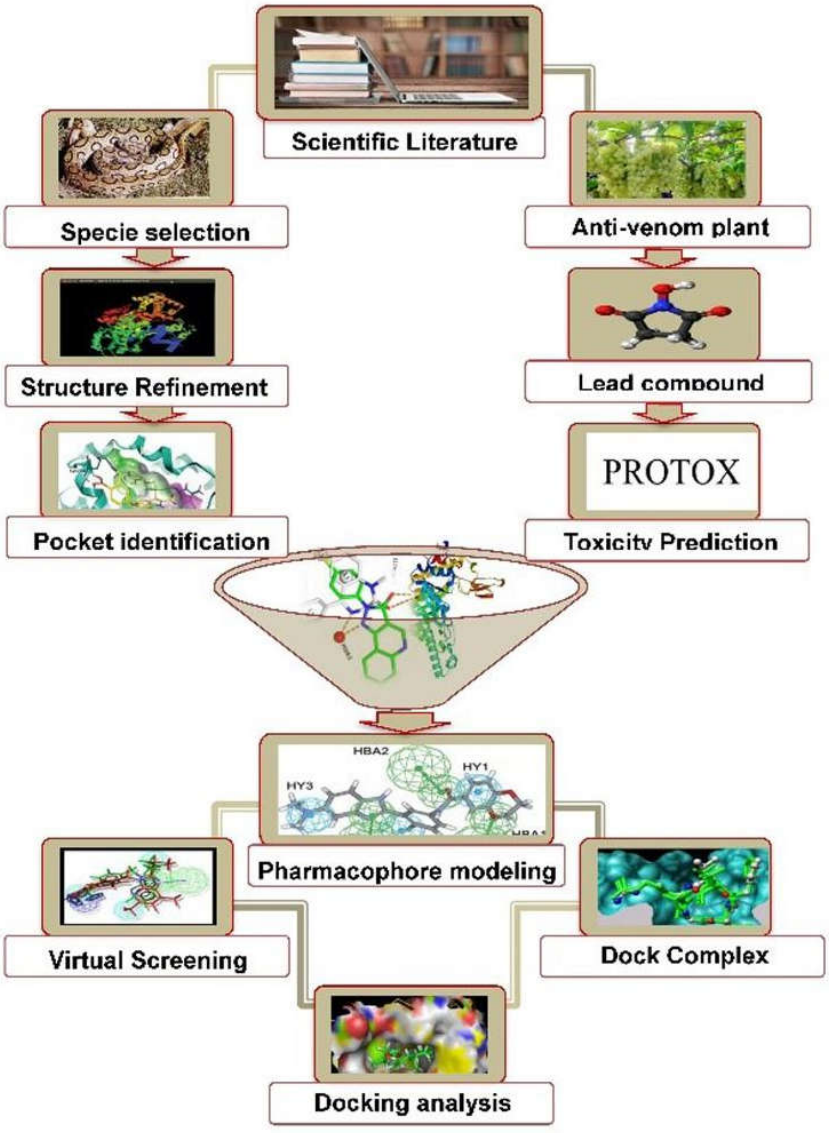

Figure 1. A schematic illustration of the overall methodology represents all Bioinformatics approaches and methods that are performed.

RESULTS AND DISCUSSION

Target Protein (1WQ9)

Vascular endothelial growth factors (VEGF-F) from snake venoms of *Daboia russelii* viper species are selected and download it from the snake venom database (SVDB) [8]. The 3D structure of VEGF-F (1WQ9) shown in (figure 3) is downloaded from the protein databank (RCSB PDB) [13] with an X-Ray diffraction resolution of 2 Å which result is best. 1WQ9 protein contains 0 mutations, with two chains Chain A Chain B, with an amino acid sequence of 144, Mass 16,278 Dalton, and with Total Structure Weight 21913.42.

Phytochemicals toxicity evaluation and Pharmacokinetic properties of compounds

Epicatechin, Kaempferol, Cianidanol, and Gallic acid are the four anti-venom compounds that have used for snakebite. We have downloaded the 3D structure in PDB format and also predict their toxicity class, LD50 value, Average similarity, and Lipinski Role of five from the Protox serverwhich lies in class 4 that is non-toxic (table 1). Here we demonstrate the use of the complete analysis system PKCSM and toxicity evaluation utilizing Protox, PKCSM, SwissAdme, and admetSAR prediction tools to allow early identification of potential problems, prioritizing of hits, and optimization of leads. The ADMET and pharmacokinetic properties are shown in table 2.

Pharmacophore generation

The Pharmacophore model was generated and used as a query for getting likely lead compounds from the Zinc database for screening small molecules with explicit desired attributes and for assessing the most similar and variety of molecules. The Pharmacophore features of every ligand were checked in LigandScout software and their shared features Pharmacophore was designed (figure 4). LigandScout is an associated automated Pharmacophore model design package. Ligand-based Pharmacophore modeling method has used to the superposition of a set of active compounds and extracting shared chemical features crucial for the bioactivity of the targeted protein molecule.

Pharmacophore screening

Pharmacophore screening approaches use a Pharmacophore model as a query to search 3D structural databases of compounds. In this search, we screened Zinc library (20 million compounds) and selected 200 topmost similar ligands based on RMSDX, RMSD-REF, and S values and make a Database. We further screened this 200 compound database and select the top 6 ligands (Table 2) and dock these 6 compounds one by one of (1WQ9) receptor protein. The RMSD value used for similarities should be minimum and the selected ligands values lie in points that show the best similarity. The S-value are lies in minus which indicate the suitable and most similar compounds to the Pharmacophore model.

Docking of Benzolyphenyl Urea with (1WQ9) Receptor protein

After the virtual screening, these six compounds have docked one by one against receptor (1WQ9) using the AutoDock Vina tool. Benzolyphenyl Urea is the most similar and best-screened compound that shares the features with the Pharmacophore model. Its chemical formula is C20H14CL2N2O2 and molecular mass is 385.243 Dalton. It is docked with (1WQ9) and visualized with Pymol and other software packages with show proper orientation and confirmation as shown in figure 5 (A). Figure 5 (A). Shows the active pocket of (1WQ9) protein and docked Urea lead compound. The 2D structure shows the interaction between Benzolyphenyl urea and interacting amino acids, which is bound with different bands. Cysteine 93 makes the arene-H bond with a distance of 4.3 along with the energy of -0.9. Threonine 50 makes the arene-H bond with a distance of 4.19 along with energy -0.5. Glycine 50 amino acid makes arene-H with a distance of 3.73 and the binding energy is -0.6. The third amino acid is threonine 16 which makes the arene-H bond with a distance of 4.71 along with the energy of -1.1 with the ligand. Cysteine 93 and threonine 50 are acidic amino acids. Glycine 50 and threonine 16 amino acids are polar which interact with ligand. All these are shown in the following figure 5 (B). In figure 5 (C). The horizontal axis shows φ values, and the vertical shows ψ values. Each dot shows the angles for a specific amino acid. The counting starts from the left-hand corner -180 and exceeds +180 for both the vertical and horizontal axes respectively. It allows a clear distinction of the characteristic sections of α-helices and β-sheets. The regions on the plot with the maximum density of dots are the so-called "allowed" regions or low-energy regions. In the following figure, most of the amino acids that interact with ligand contain β-sheets and the α-helices portion of the protein.

Synthesis of Anti-Venom Compounds

Mahadeswaraswamy, Y H, *et al* in 2008 synthesized an effective compound against *Echis carinatus* venom. For the synthesis of *Vitisvinifera* seed Methanol Extracts a protocol was followed by Mahadeswaraswamy, Y H. After the preparation of seed extracts, the Caseinolytic activity, SDS-PAGE zymogram, Hyaluronolytic activity, Haemorrhagic activity, Oedema inducing activity, Myotoxicty and myonecrosis, Coagulant activity and fibrinogenolytic activity was examined and statistical analysis was performed which shows successfully inhibited the toxic effects of citrated human plasma.

Table 1. Anti-venom compounds of *Vitis vinifera* and their toxicity prediction form different tools. All ligands lie in class 4 that is the best class with normal weight and 100 % similarity.

Name	Toxicity Class	LD 50 Value	Similarity
Epicatichen	4	1190mg/kg	100%
Kaempferol	4	1190mg/kg	100%
Cianidanol	4	1190mg/kg	100%
Gallic acid	4	1190mg/kg	100%

Table 2. Top six screened ligands from the ZINC database based on RMSD value.

S.NO	ZINC-ID	S-VALUE	RMSD_REF...	RMSDX
1	ZINC000576	-4.6970	1.3228	0.4703
2	ZINC645280	-5.5752	1.7861	0.1669
3	ZINC641031	-6.0949	1.3826	0.3999
4	ZINC146429	-5.1561	2.0136	0.1655
5	ZINC400388	-5.7975	1.6623	0.4707
6	ZINC274357	-5.7377	1.7521	0.4706

Table 3. Predicted chemical, ADMET and pharmacokinetic properties of Benzolyphenyl Urea.

BENZOLYPHENYL UREA					
STRUCTURAL PROPERTIES					
Molar Volume	Surface Area	Molar Refractivity	Num. arom. heavy atoms	ClogP	
271.9±3.0 cm3	58 Å^2	105.06	18	5.8684	
LD50	Density	Consensus Log Po/w			
1.6134 mg/kg	1.4±0.1 g/cm3	4.77			
ABSORPTION					
Solubility Water	Intestinal absorption (human)	Permeability Of Cac02	Skin Permeability	P-glycoprotein substrate	P-glycoproten I/II Inhibitor
-5.109	Yes (88.866)	1.462 (LogPapp, cm/s)	--2.765 Numeric (log Kp)	yes	Yes
DISTRIBUTION					
Blood-Brain Barrier	Fraction unbound (human)	VDss (human)	CNS permeability	Subcellular localization	Carcinogens
No (-0.089)	0 Numeric (Fu)	-0.224 Numeric (log L/kg)	-1.492 Numeric (log PS)	0.7979 Mitochondria	0.6464 Non-carcinogens
RULES					
Lipinski	Ghose	Veber	Egan	Muegge	Bioavailability	PAINS
1 violation	yes	Yes	Yes	No Violation	0.55	0 alert
TOXICITY & EXCRETION					
Total Clearance	Renal OCT2 substrate	Oral Toxicity class	Hepatotoxicity	Max.tolerated Dose	hERG I & II inhibitor	T.Pyriformis toxicity
-0.138	No	1.989	yes	0.138	No	0.457
METABOLISM						
CYP3A4 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP2C19 inhibitor	CYP3A4 substrate	CYP2D6 substrate	Lead likeliness
Yes	Yes	Yes	Yes	yes	No	No

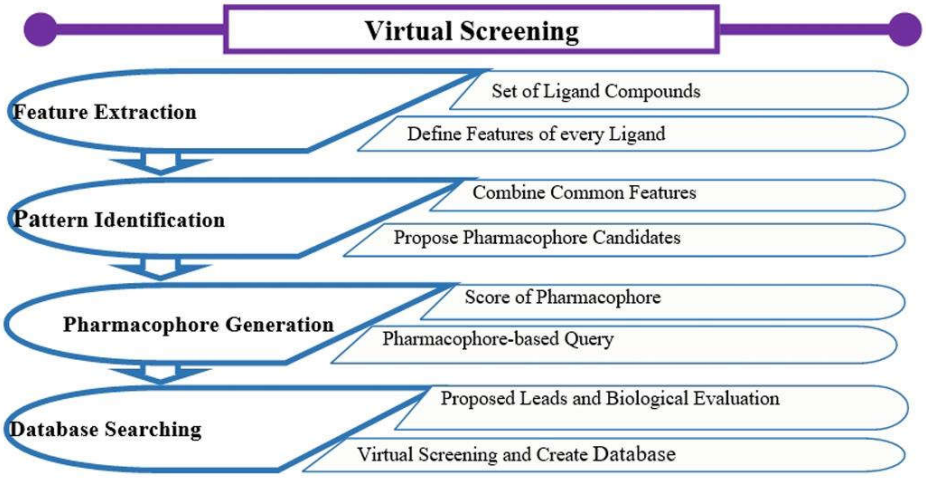

Figure 2. Flow chart of the virtual screening method using the Pharmacophore model.

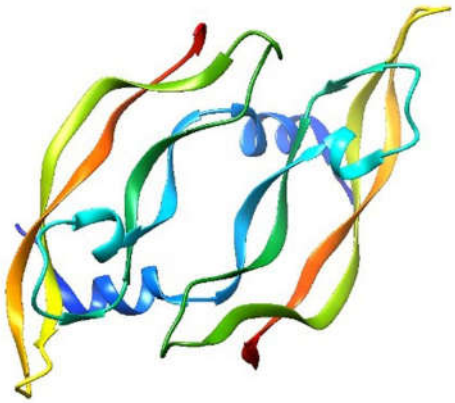

Figure 3: Purified 1WQ9 Protein

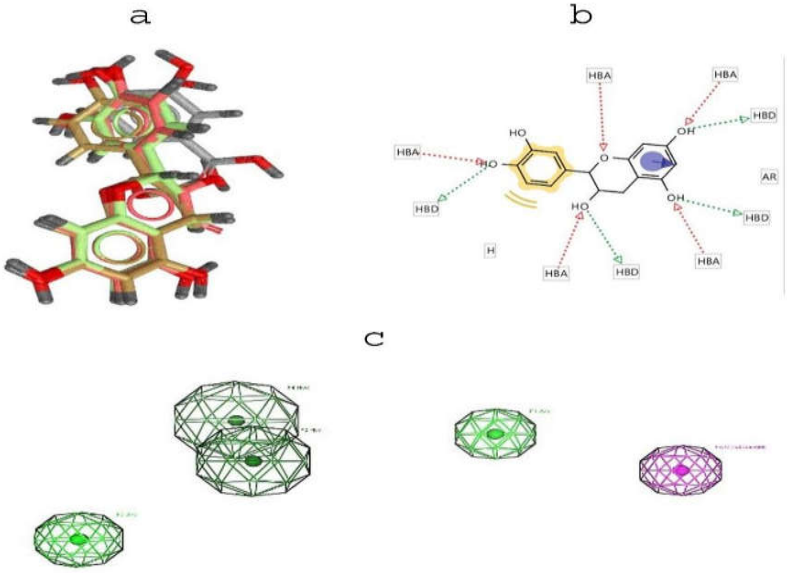

Figure 4. (A-C) A): Pharmacophore generated by Ligand Scout software. (A) Alignment of Kaempferol, Gallic acid, Cianidanol and Epicatichen to combine shared features. (B) 2D Diagram of the Pharmacophore model. Green dotted arrows show HBD and red dotted arrows show HBA. (C) The green sphere represents aromatic rings, dark green spheres represent HBD and the purple sphere represents both HBD and HBA. All thePharmacophore models generated by our method in this paper use this color scheme.

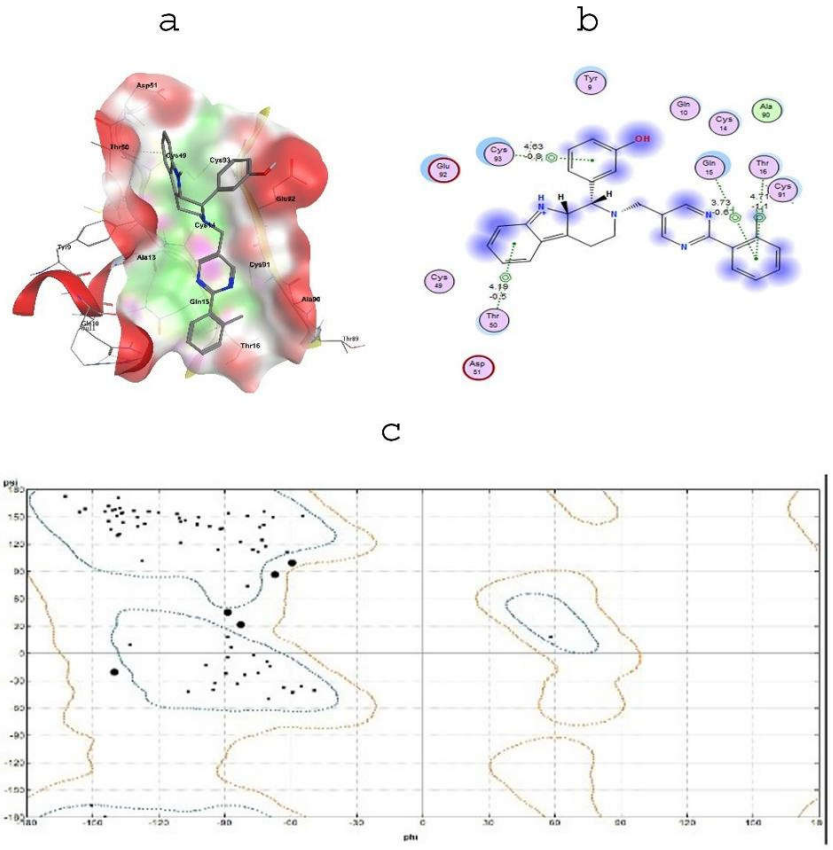

Figure 5: (A-C) Represent the active pocket in which ligand is properly docked. (B) Illustrate the interaction between (1WQ9) and Benzolyphenyl Urea compound along with distances and energy. C shows the phi Si plot of interaction. Ligand. (C): Ramachandran plot calculations for (1WQ9) protein computed with the PROCHECK program.

CONCLUSION

In the present work, we utilized various approaches, including pharmacophore modeling, virtual screening, and molecular modeling to design a cost-efficient, effective, and without causing side effects, anti-venom drug for *Russelli Daboia* snake bite from the medicinal Vitisvinifera plant. Four anti-venom compounds have selected and generated a pharmacophore model that combines their common shared features. This pharmacophore model has used as a query against the Zinc database that contains over 20 million compounds for screened hit compounds. As a result, we get 200 similar compounds that share features with the pharmacophore model. Further screening was performed and finally, six compounds that are most similar to the pharmacophore model have selected. These six compounds were docked against (1WQ9) receptor protein of russelli snake that causes abnormalities after a snake bite. After analyzing docking results we have selected the maximum interacting compound (Benzolyphenyl Urea) and validate the results by different software. For further experimental validation, the above-mentioned protocol for the synthesis of the compound will follow. Hence, this identified compound will help us to design novel and potential inhibitors, which are considered to be an alternative of anti-serum as well as a good and cost-efficient drug for snake bite.

ETHICAL STATEMENT

The manuscript has no ethical concerns in the context of publishing, financial ties, and other ethical adherence.

CONFLICT OF INTEREST

None

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REFERENCES

1. Samkumar, Amos, et al. (2019). "Strategy for Early Callus Induction and Identification of Anti-Snake Venom Triterpenoids from Plant Extracts and Suspension Culture of Euphorbia Hirta L." *3 Biotech*, vol. 9, no. 7, 201-219.
2. Juliana, Félix- Silva, et al. (2017). "Medicinal Plants for the Treatment of Local Tissue Damage Induced by Snake Venoms: An Overview from Traditional Use to Pharmacological Evidence." *Evidence-Based Complementary and Alternative Medicine*, vol. 20 17: 20-29.
3. Anas ahmad, et al. (2010). "Anti-Snake Venom Activity Of Different Extracts Of Pouzolzia Indica Against Russel Viper Venom." *International Journal of ChemTech Research*, vol. Vol.2, 129-134.
4. Makhija, Inder Kumar, and Devang Khamar.(2010). "Anti-Snake Venom Properties of Medicinal Plants." *Der Pharmacia Lettre*, 2(5), 11-19.
5. Yamazaki Yasuo, et al. (2009)."Snake Venom Vascular Endothelial Growth Factors (VEGF-Fs) Exclusively Vary Their Structures and Functions among Species." *Journal of Biological Chemistry*, vol. 284, 289-309.
6. Brindha Durairaj, et al. (2014). "*In Vitro* Antivenom and Antioxidant Potential of Vitex Negundo Leaves (Green and Blue) against Russell's Viper (Daboia Russelli) and Indian Cobra (Naja Naja) Venom." *European Journal of Experimental Biology*, 4(4):207-219
7. Mahadeswaraswamy, Y H, et al. (2008). "Local Tissue Destruction and Procoagulation Properties of Echis Carinatus Venom: Inhibition by *Vitis vinifera* Seed Methanol Extract: *Vitis vinifera* Extract and Echis Carinatus Venom." *Wiley InterScience*, 22(7)..963-969
8. Munir, Anum, et al. (2016)."Structure-Based Pharmacophore Modeling, Virtual Screening and Molecular Docking for the Treatment of ESR1 Mutations in Breast Cancer." *Drug Designing: Open Access*, vol. 05, no. 03:12-19, doi:10.4172/2169-0138.1000137.
9. Hossain, Md. Monir, et al. (2018). "Snake Venom Database (SVDB)." *Proceedings of the 2018 2nd International Conference on Computational Biology and Bioinformatics - ICCBB 2018*, doi:10.1145/3290818.3290832.
10. Pires, Douglas E. V., et al. (2015). "PkCSM: Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures." *Journal of Medicinal Chemistry*, vol. 58, no. 9 pp. 4066–4072., doi:10.1021/acs.jmedchem.5b00104
11. Daina, Antoine, et al. (2017). "SwissADME: a Free Web Tool to Evaluate Pharmacokinetics, Drug-Likeness and Medicinal Chemistry Friendliness of Small Molecules." *Scientific Reports*, vol. 7, no. 1, 1-13. doi:10.1038/srep42717.
12. Cheng, Feixiong, et al.(2012). "AdmetSAR: A Comprehensive Source and Free Tool for Assessment of Chemical ADMET Properties." *Journal of Chemical Information and Modeling*, vol. 52, no. 11, pp. 3099–3105, doi:10.1021/ci300367a
13. Irwin, John J., and Brian K. Shoichet. (2015)."ZINC ? A Free Database of Commercially Available Compounds for Virtual Screening." *ChemInform*, vol. 36, no. 16, 1621-1629. doi:10.1002/chin.200516215.
14. Ullivan, Kevin P., et al. (2017). "Economic Impacts of the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank." *RCSB Protein Data Bank*, pp18. doi:10.2210/rcsb_pdb/pdb-econ-imp-2017.