Original Research Article

Discovery of natural-pan inhibitors by targeting snake venom secretory phospholipase A2 through computational approaches

ABSTRACT

Phytochemicals have been widely used in pharmaceuticals in recent decades due to their anti-inflammatory, cancer-preventative and cardiovascular-protective properties. These phytochemicals were tested on Crotalus durissus terrificus secretory phospholipase A2 (sPLA2), a significant protein in the release of arachidonic acid from phospholipid membranes, in this study. Circular dichroism showed that the secondary structure of the protein has been altered by treatment with phytochemicals. Another interesting finding was that, although phytochemicals reduced the enzyme activity and part of the drug-induced platelet-aggregation and myotoxicity of sPLA2 by 40 percent on average, they could do nothing to lessen the inflammatory or neurotoxic effects of this substance. These findings support the hypothesis that the protein has two separate pharmacological sites, one associated with the enzyme's active site and the other different from it. We used molecular docking to better understand how phytochemicals and sPLA2 might interact. Our docking findings indicated that additional phytochemicals with comparable structures could bind to sPLA2 via hydrogen-bonded, polar, and hydrophobic interactions. Additionally, the stability of the phytochemicals was double-checked using MD Simulation. The proposed study will help to develop better drug candidates with more therapeutic efficacy and least side effects. More research is needed to determine whether phytochemicals can be used to suppress the sPLA2 enzyme.

Keywords: *Secretory Phospholipase A2, Phytochemicals, Snake Venom, Molecular Docking, Molecular Dynamic Simulation*

1. INTRODUCTION

Secretory Phospholipases A2 (PLA2, EC 3.1.1.4 are are tiny proteins), in a Ca2+-dependent process, catalyze the hydrolysis of glycerol phospholipids at the sn-2 position, releasing lysophospho lipids and fatty acids [1]. Snake venom enzymes have long been studied because of the vast range of biological effects they have and because they are structurally similar to mammalian phospholipases. Snake venom sPLA2 toxins, on the other hand, have pharmacological effects through arachidonic acid metabolism, resulting in the generation of different lipid proinflammatory mediators such as prostaglandins, thromboxanes, and leukotrienes. Eicosanoid levels and inflammation have been lowered by inhibiting

cytosolic PLA2 (cPLA2), according to recent studies. sPLA2 inhibitors have piqued pharmaceutical interest due to their function in the inflammatory process, and phytochemicals are one of these, have been thoroughly researched. Phytochemicals are ideal target for the extraction of pharmaceuticals and chemical synthesis [2]. PLA2 can only be inhibited when it is in the inhibitory position. Although the phytochemicals inhibit the sPLA2, the specific method through which they do so remains a mystery. Even though they has been shown to have an effect on the secondary structure of sPLA2 from venom of Crotalus durissus cascavella, it had no effect on its pharmacological activity [3].

Despite the fact that studies have been done on phytochemicals as sPLA2 inhibitors, to cross check how they exert their inhibitory effect [4]. There are two main goals of this study: to learn more about how polyphenolic chemicals in snake venom behave in the mode that they do, and to evaluate the therapeutic potential against the symptoms caused by snake bites. The phytochemicals are extensively distributed in nature [5].

A time and money-saving method for creating novel medications is computational drug design [6]. In the current study, 1000 phytochemicals from various flavonoid subgroups were compared in terms of their molecular interactions. Molecular dynamic simulation was used to further validate the results. Although the results of this study were not based on experiments, we believe that our suggested medication candidates may be effective against sPLA2.

2. MATERIAL AND METHODS

2.1. Structure Retrieval

Protein three-dimensional structures was obtained from the Protein Data Bank with ID: 6G5J [7] MOE [8] with parameters MMFF94X+Solvation, force field, and 3D protonation were used to remove water molecules, minimize energy, and execute 3D protonation. Chiral Gradient: 0.05 Current geometry is a constraint [9].

2.2. Preparation of ligand library

A thorough search was conducted in order to identify effective and beneficial phytochemicals for the treatment against sPlA. 1000 phytochemicals were retrieved from medicinal plant databases like MPD3[10], CHEMBL[11], PubChem [12] and ZINC [13] for molecular docking ready to dock library was prepared. Each ligand molecule was stored into the database named as MOE in mol format after energy reduction with the following parameters: gradient: 0.05, Force Field: MMFF94X, Chiral Constraint: Current Geometry. MOE software programs for the inhibitor scan [9].

2.3. Molecular Docking

Molecular docking is a technique for determining the binding orientation of tiny molecules to their targets. As a result, techniques in medication discovery and screening of new compounds against these awful and difficult illnesses have been developed. The 3D model of the target was fetched from the PDB [7]. To improve the structure, 3D protonation and Energy minimization utilizing Molecular Operating Environment, energy minimization and other processes were conducted. Computer-assisted drug design relies heavily on molecular docking. Docking is a process for developed a ligand's binding mode with a protein whose 3D structure is known. It essentially examines the interaction of two chemicals. The main interaction residues of the target protein structure were docked with the library of 500 phytochemicals. MOE software was used to acquire several ligand conformations. Rescoring 1; London dG: retain 10, refinement: force field, rescoring 2; London dG: retain 10 were the docking parameters used.

2.4. Ligand receptor interaction analysis

Large library of compounds is virtually screened and results are checked. The receptor-ligand interactions of complexes were analyzed using the [14]. It provides a clear picture of the interactions between receptors and ligands in the best docked complexes. We looked at 2D maps of receptor-ligand interactions. It depicts the hydrogen bonding, hydrophobic interactions, electrostatic contacts, and van der wall forces that are responsible for a drug's affinity in actively docked pockets. The protein inhibitor complexes were visualized in 3D using MOE software.

2.5. Physiochemical property profile

Drug likeness properties of best dock complexes were studied using MO inspiration server (https://www.molinspiration.com). This server provides prediction based “Lipinski rule of five”. Lipinski rule of five involves the criteria of molecular properties; the value of logP should be more than 3 and molecular weight more than 100 Daltons. While hydrogen donor must be less than 5 and hydrogen acceptor less than 10.

ADMET properties were accessed using Swiss ADME (http://www.swissadme.ch/) in which Absorption, distribution, metabolism, excretion and toxicological analysis was done [15].

2.6. MD Simulation

In order to evaluate the robustness of the docked complexes, researchers performed a Molecular Dynamics simulation with a time step of 100 ns. The investigation of the complex in the explicit solvent system with the OPLS3 force field was carried out with the assistance of Schrodinger's Desmond Simulation Package. Maintain a constant temperature of 300 kelvin (K) and pressure of one bar throughout the systems. In this work, a hybrid approach to energy reduction was applied. The steepest descent method was used for 1000 steps, and then conjugate gradient approaches were used [16].

3. RESULTS

3.1. Structure Retrieval

The three-dimensional structure of sPLA2 was obtained from the protein data bank. The PDB ID was 6G5J having a resolution of 1.85 Å. The catalytic triad of sPLA2 protein was mainly involved in venom of snake.

3.2. Docking Studies

Molecular docking has grown in popularity among academic communities due to its low cost and apparent ease of use. An approach for anticipating a ligand's best orientation, affinity, and interaction at a protein's active site is termed molecular docking. Scoring algorithms can be used to evaluate the intensity of binding affinity between ligand molecules depending on the protein and ligand binding preferred orientation. The top four phytochemicals (Anthraquinone, Fenchone, Colchicine and Alpha-Methylene-gamma-butyrolactone) were chosen based on S-score and RMSD values. Phytochemicals with the lowest S-scores are at the top of the list and vice versa. With an S-score of -19.56 Kcal/mol, Anthraquinone, was ranked first, followed by Fenchone, Colchicine and Alpha-Methylene-gamma-butyrolactone as mentioned in the table 1.

Table 1: *Representation of docking score, rmsd, binding affinity and interacting residues*

Compound's I'D	Compound's Name	S-Score	Binding Affinity	Inhibition constant	Rmsd	Interacting Residues
6780	Anthraquinone	-19.56 kcal/mol	-17.67 Kj/mol	69.3 µM	1.56	Gly A30,Phe A26,Gly A28,Asp A47
14525	Fenchone	-18.91 kcal/mol	-16.32 Kj/mol	66.9 µM	2.04	Gly A28, Gly A30,Phe A26, Asp A47
6167	Colchicine	-18.05 kcal/mol	-15.67 Kj/mol	63.1 µM	0.98	Gly A30,Phe A26,Gly A28,Asp A47, Cys A27
68352	Alpha-Methylene-gamma-butyrolactone	-17.48 kcal/mol	-14.52 Kj/mol	53.8 µM	1.05	Gly A28,Tyr A20,Leu A5,Pro A17,Met A21

Top leading inhibitor Anthraquinone and Fenchone attached with the target receptor showed interactions with the amino acid residues Gly A30, Phe A26,Gly A28,Asp A47 with the docking score of -19.56 Kcal/mol to -18.91 Kcal/mol as indicated in the figure 1.

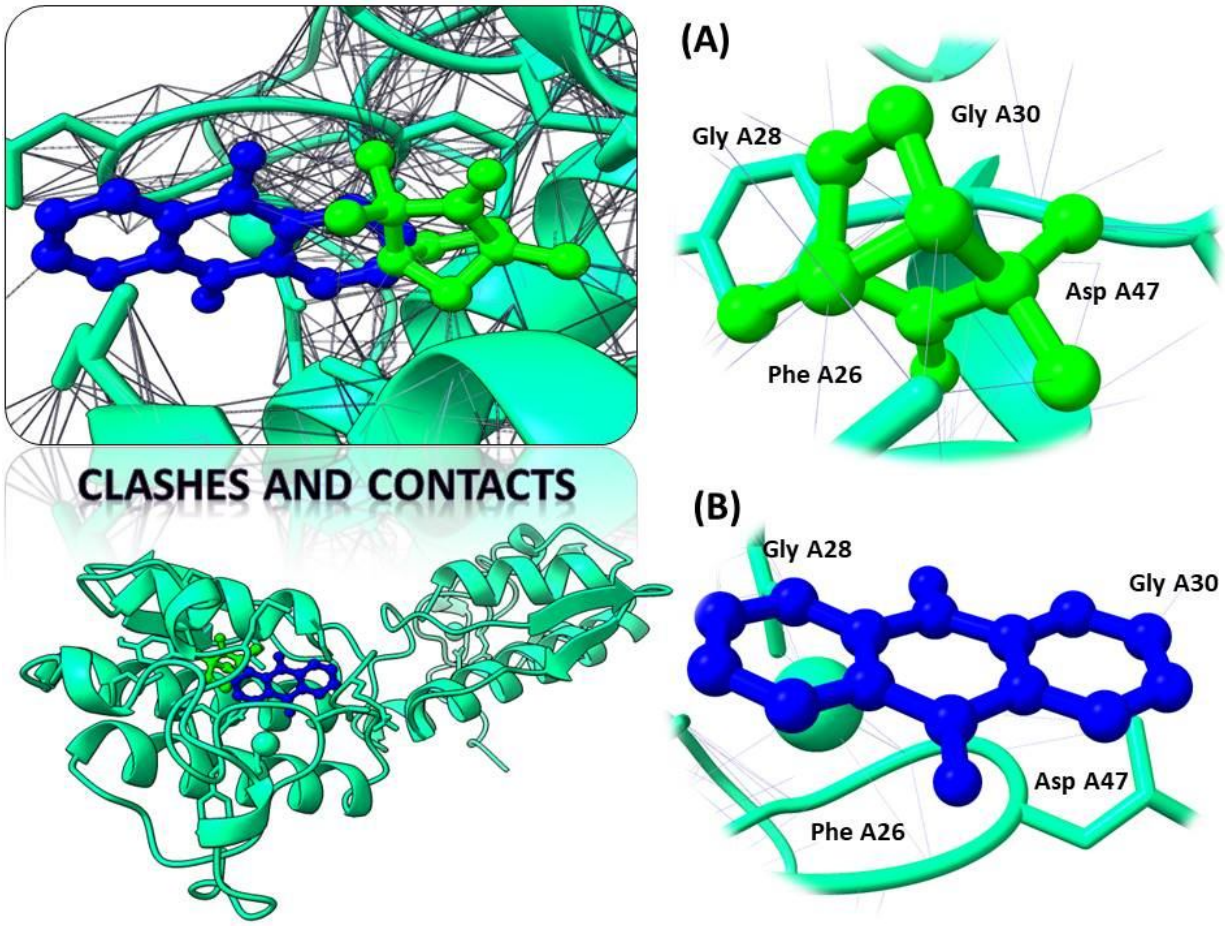

Figure 1: Inhibitors in complex with the receptor target sPLA2 along with the interacting residues **A)** Anthraquinone **B)** Fenchone

Colchicine and Alpha-Methylene-gamma-butyrolactone inhibitor attached with the target receptor sPLA2 showed interactions with the amino acid residues Gly A30,Phe A26,Gly A28,Asp A47, Cys A27, Tyr A20,Leu A5,Pro A17 and Met A21 with the docking score of -18.05 Kcal/mol and -17.48kcal/mol as mentioned in the figure 2.

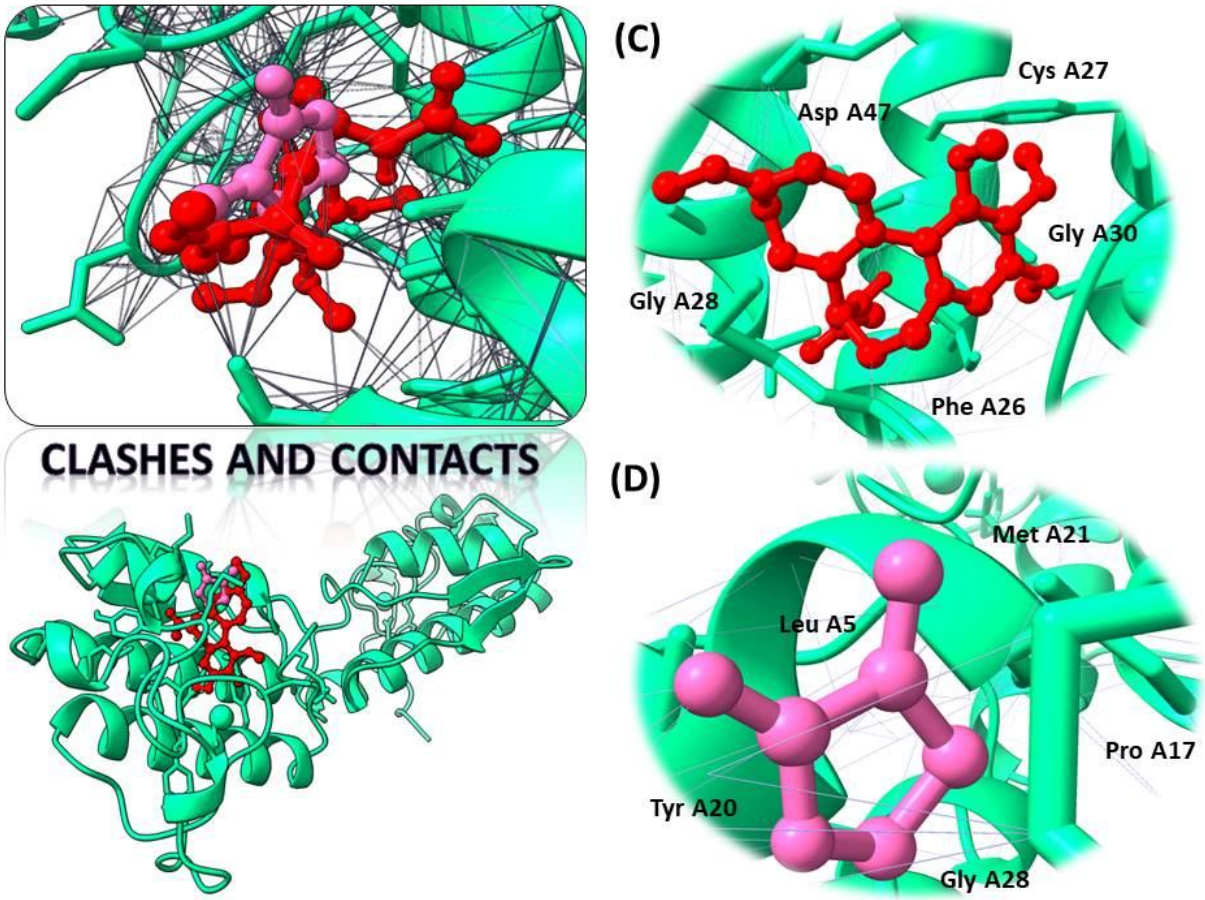

Figure 2: Inhibitors in complex with the receptor target sPLA2 along with the interacting residues ***C)*** Colchicine ***D)*** Alpha-Methylene-gamma-butyrolactone

3.3. ADMET Profiling & Druglikeness

The drug-likeness of the proposed overexpressed protein was predicted by using Molinspiration server, on the basis of Lipinski Rules of five. The selected candidate displayed zero and one violations to Lipinski's rule of five and showed adequate drug-like properties, i.e.M.W, logP, Hydrogen bond donor and hydrogen bond acceptor as indicated in table 2.

Table 2: Physiochemical properties of the compounds drug likeness according to Lipinski Rule of Five

Compounds I'Ds	Molecular Weight	Hydrogen bond acceptor	Hydrogen bond donor	LogP	Formula
6780	208.21	2	0	3.4	$C_{14}H_8O_2$
14525	152.23	0	1	2.23	$C_{10}H_{16}O$
6167	399.4	6	1	1	$C_{22}H_{25}NO_6$
68352	98.10	2	0	0.8	$C_5H_6O_2$

All the selected compounds were imperiled for evaluation of pharmacokinetic characteristics through the Swiss-ADME server in order to confirm the potential of drug likeliness as mentioned in the table 3.

Table 3: Admet profiling of the top ranked inhibitors

Compounds	6780	14525	6167	68352
Absorption				
Human Intestinal Absorption	low	high	high	low
Blood-Brain Barrier	no	no	yes	no

Caco-2 Permeable	+	_	+	+
Distribution				
P-Glycoprotein Substrate	substrate	substrate	Non-substrate	substrate
P-Glycoprotein Inhibitor I	Non-Inhibitor	Inhibitor	Inhibitor	Inhibitor
Metabolism				
CYP450 2C9 Substrate	yes	yes	yes	yes
CYP450 2D6 Substrate	yes	yes	no	yes
CYP450 3A4 Substrate	yes	yes	no	no
CYP450 1A2 Substrate	yes	yes	yes	no
CYP450 2C9 Inhibitor	no	no	no	no
CYP450 2D6 Inhibitor	yes	no	no	no
CYP450 2C19 Inhibitor	no	no	yes	no
CYP450 3A4 Inhibitor	no	no	no	yes
Toxicity				
Ames Toxicity	Non-Toxic	Non-Toxic	Non-Toxic	Non-Toxic
Carcinogenicity	Non-Toxic	Non-Toxic	Non-Toxic	Non-Toxic

3.4. MD Simulation

Computer-aided drug design requires MD simulations to comprehend the structural-functionality relationship of the target protein (CADD). MD simulations offer detailed information on the dynamical structural properties of biomolecules, a wealth of surface interactions between proteins and ligands, and energetic data. This set of data can be used to inform the creation of novel drugs, making MD simulation an important tool in today's drug discovery process.

3.4.1. RMSD

For the top four complexes and controls, MD simulations lasting 100 ns were carried out to clarify compound binding stability and extract receptor and compound structural data that is crucial for binding and that may be changed to improve binding conformation and, ultimately, compound affinity for the target biomolecule. The main chain root mean square deviations were calculated for the trajectories of sPLA2 complexes.

Anthraquinone average wavelength is at 1.55nm upto the time period of 70ns after that it showed a minor fluctuation similarly the average value for the Fenchone complex was 1.75nm it showed stability at the time period after 80 ns. Colchicine and Alpha-Methylene-gamma-butyrolactone complexes showed an average trajectories 1.25 to 2.0 nm with minor deviations as mentioned in the figure 3

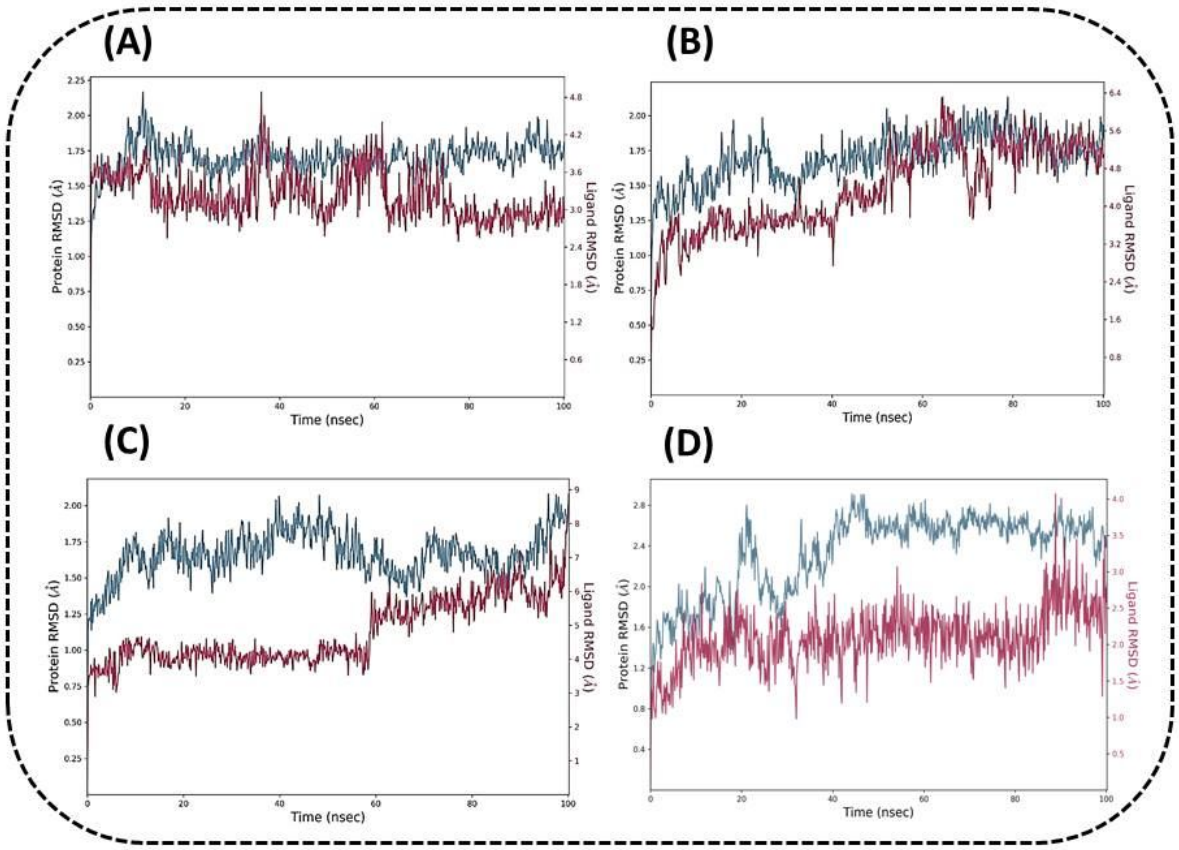

Figure 3: Root Mean Square Deviation of the top complexes **A)** Anthraquinone **B)** Fenchone **C)** Colchicine **D)** Alpha-Methylene-gamma-butyrolactone

3.4.2. RMSF

Complexes' residual flexibility and stability were also calculated by using RMSF of top four complexes. The average is very close to 1.3 nm, while the lowest and highest values are 1.2 nm and 1.6 nm, respectively. Average plot trajectories were shown in the Figure 4.

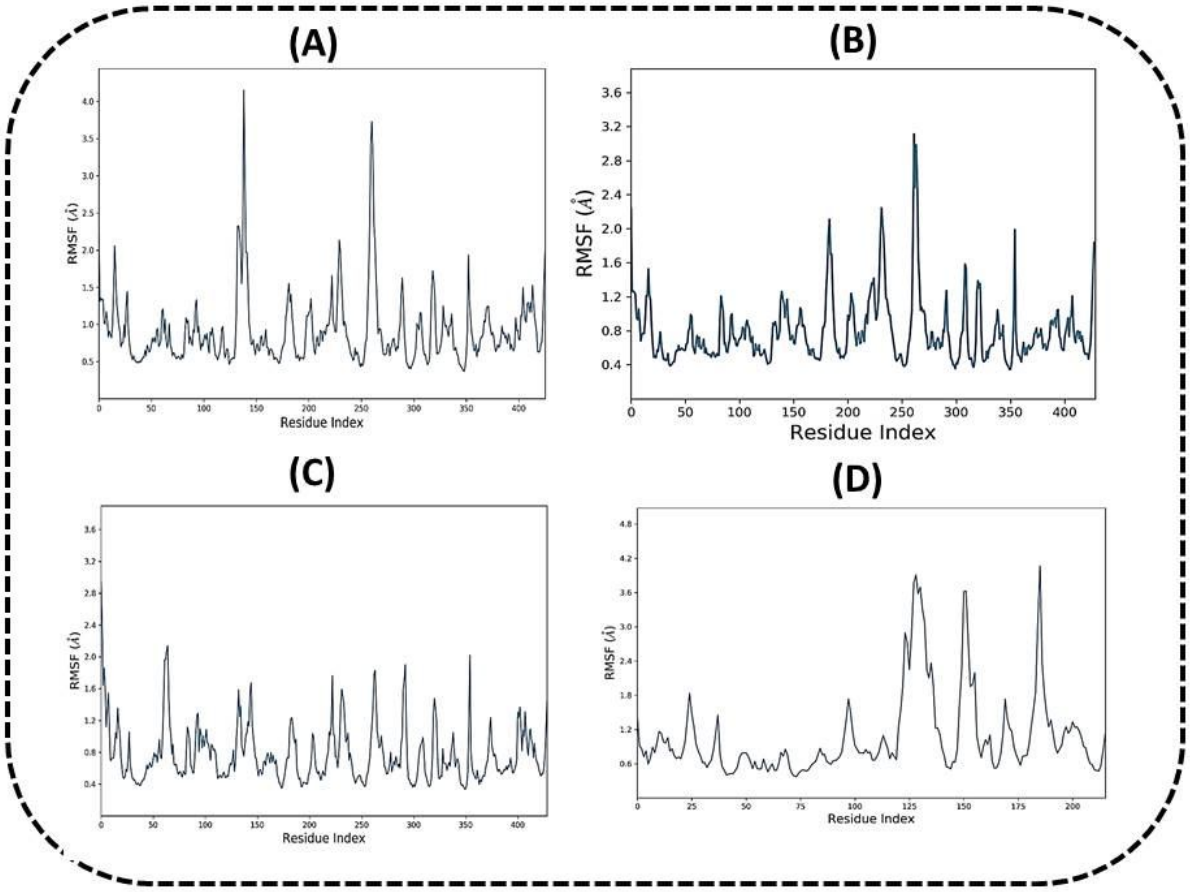

Figure 4: Root mean square fluctuation trajectories of the top inhibitors A) Anthraquinone B) Fenchone C) Colchicine D) Alpha-Methylene-gamma-butyrolactone

4. Discussion

The present study illustrated that by identifying the active sites of the target protein, we can inhibit its expression. There are some compounds those have considerable interactions with the target protein involved in sPLA2. Molecular properties and drug-likeliness of the selected complexes were estimated according to the "Lipinski Rule of Five". This rule states that, the molecular weight of the compound must less than 500 Daltons, less than 5 Hydrogen bond donors, no more than 10 Hydrogen bond acceptors, and AlogP value fewer than 5. All compounds fulfill the Lipinski's Rule of Five and show no violation. Selected compounds have low scoring values as compared to the standard drugs and have RMSD values less than 3. ADMET analysis is a challenging process in the drug discovery. This is achieved through SwissADME database and showed that selected compounds have good pharmacokinetic properties. Drug development process of many drugs do not go through the process just because of the poor pharmacokinetic properties and toxicity [17]. Identification of active lead compounds depends upon the High-performance and fast ADMET profiling assays at early drug discovery [18]. ADMET profiling shows that there is no side effect of absorption of all potential compounds. The associated ADMET properties of potential compounds for different models such as P-glycoprotein substrates, BBB penetration, and gastrointestinal absorption showed positive results that strongly support compounds' ability to function as a drug candidate. Cytochrome P450 (CYP) is a cluster of isozymes comprising fatty acids, bile acids, carcinogens,

steroids, and the metabolism of drugs. Fifty-seven CYPs are encoded by human genome, of which fifteen are participating in the xenobiotic chemicals and another drug metabolism [19]. CYP enzymes association is very important for drug metabolism almost 75 percent of the phase 1 of drug metabolism depends upon its association [20].

So it is concluded that the extracted phytochemicals can inhibit the activity of the protein involved in sPLA2 by targeting there binding pockets and hence they can be used as effective drug candidates against the disease.

Conclusion

In the current study, Anthraquinone, Fenchone, Colchicine and Alpha-Methylene-gamma-butyrolactone was evaluated as the potential sPLA2-inhibitors (with maximum binding affinity) may be used as a potential drug candidates. Our study highlights the interactive features of known synthetic and biological sPLA2 inhibitors involving interactions with the blood-brain barrier (BBB). Natural bioactive compounds have gained popularity as possible medicines in recent years, owing to their effectiveness in promoting health and having fewer side effects than synthetic bioactive compounds. Knowing inhibitors' pharmacological features, including bioactive chemicals, will help us to design and synthesize effective snake venom medicines in the future.

CONSENT (WHERE EVER APPLICABLE)

THE AUTHORS DECLARE NO CONFLICT OF INTEREST

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

NONE

REFERENCES

1. KINI, R.M., *EXCITEMENT AHEAD: STRUCTURE, FUNCTION AND MECHANISM OF SNAKE VENOM PHOSPHOLIPASE A2 ENZYMES.* TOXICON, 2003. **42**(8): P. 827-840.
2. NEVALAINEN, T.J., G.G. GRAHAM, AND K.F. SCOTT, *ANTIBACTERIAL ACTIONS OF SECRETED PHOSPHOLIPASES A2. REVIEW.* BIOCHIMICA ET BIOPHYSICA ACTA (BBA)-MOLECULAR AND CELL BIOLOGY OF LIPIDS, 2008. **1781**(1-2): P. 1-9.
3. BURKE, J.E. AND E.A. DENNIS, *PHOSPHOLIPASE A2 BIOCHEMISTRY.* CARDIOVASCULAR DRUGS AND THERAPY, 2009. **23**(1): P. 49-59.
4. SALES, T.A., S. MARCUSSI, AND T.C. RAMALHO, *CURRENT ANTI-INFLAMMATORY THERAPIES AND THE POTENTIAL OF SECRETORY PHOSPHOLIPASE A2 INHIBITORS IN THE DESIGN OF NEW ANTI-INFLAMMATORY DRUGS: A REVIEW OF 2012-2018.* CURRENT MEDICINAL CHEMISTRY, 2020. **27**(3): P. 477-497.
5. BATSIKA, C.S., ET AL., *THE DESIGN AND DISCOVERY OF PHOSPHOLIPASE A2 INHIBITORS FOR THE TREATMENT OF INFLAMMATORY DISEASES.* EXPERT OPINION ON DRUG DISCOVERY, 2021. **16**(11): P. 1287-1305.
6. WALTERS, W.P. AND M.J.N.R.D.D. NAMCHUK, *DESIGNING SCREENS: HOW TO MAKE YOUR HITS A HIT.* 2003. **2**(4): P. 259-266.
7. BURLEY, S.K., ET AL., *RCSB PROTEIN DATA BANK: POWERFUL NEW TOOLS FOR EXPLORING 3D STRUCTURES OF BIOLOGICAL MACROMOLECULES FOR BASIC AND APPLIED RESEARCH AND EDUCATION IN FUNDAMENTAL BIOLOGY, BIOMEDICINE, BIOTECHNOLOGY, BIOENGINEERING AND ENERGY SCIENCES.* NUCLEIC ACIDS RESEARCH, 2021. **49**(D1): P. D437-D451.
8. VILAR, S., G. COZZA, AND S. MORO, *MEDICINAL CHEMISTRY AND THE MOLECULAR OPERATING ENVIRONMENT (MOE): APPLICATION OF QSAR AND MOLECULAR DOCKING TO DRUG DISCOVERY.* CURRENT TOPICS IN MEDICINAL CHEMISTRY, 2008. **8**(18): P. 1555-1572.
9. REHMAN, G., ET AL., *ANTI-PROLIFERATIVE, ANTIOXIDANT AND ANTI-INFLAMMATORY POTENTIALS OF CASSIA NEMOPHILA FLOWERS.* 2021.
10. MUNEER, I., ET AL., *DISCOVERY OF NOVEL INHIBITORS FROM MEDICINAL PLANTS FOR V-DOMAIN IG SUPPRESSOR OF T-CELL ACTIVATION.* FRONTIERS IN MOLECULAR BIOSCIENCES, 2021. **8**.
11. SAID, M.A., ET AL., *IMPORTANCE OF GLUTAMINE 189 FLEXIBILITY IN SARS-COV-2 MAIN PROTEASE: LESSON LEARNED FROM IN SILICO VIRTUAL SCREENING OF CHEMBL DATABASE AND MOLECULAR DYNAMICS.* EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES, 2021. **160**: P. 105744.
12. KIM, S., ET AL., *PUBCHEM IN 2021: NEW DATA CONTENT AND IMPROVED WEB INTERFACES.* NUCLEIC ACIDS RESEARCH, 2021. **49**(D1): P. D1388-D1395.
13. BHOWMIK, R., R. NATH, AND R. ROY, *SCREENING OF ZINC DATABASE AGAINST STREPTOCOCCAL CYSTEINE PROTEASE ENZYME FOR IDENTIFICATION OF NOVEL GROUP A STREPTOCOCCUS INHIBITORS.* 2021.
14. REHMAN, A., ET AL., *THE SCREENING OF PHYTOCHEMICALS AGAINST NS5 POLYMERASE TO TREAT ZIKA VIRUS INFECTION: INTEGRATED COMPUTATIONAL BASED APPROACH.* COMBINATORIAL CHEMISTRY & HIGH THROUGHPUT SCREENING, 2021.

15. RIYADI, P., ET AL. *SWISSADME PREDICTIONS OF PHARMACOKINETICS AND DRUG-LIKENESS PROPERTIES OF SMALL MOLECULES PRESENT IN SPIRULINA PLATENSIS*. IN *IOP CONFERENCE SERIES: EARTH AND ENVIRONMENTAL SCIENCE*. 2021. IOP PUBLISHING.
16. RELEASE, S., *3: DESMOND MOLECULAR DYNAMICS SYSTEM, DE SHAW RESEARCH, NEW YORK, NY, 2017.* MAESTRO-DESMOND INTEROPERABILITY TOOLS, SCHRÖDINGER, NEW YORK, NY, 2017.
17. LIN, J.H. AND A.Y. LU, *ROLE OF PHARMACOKINETICS AND METABOLISM IN DRUG DISCOVERY AND DEVELOPMENT.* PHARMACOLOGICAL REVIEWS, 1997. **49**(4): P. 403-449.
18. TSAIOUN, K., ET AL. *ADDME–AVOIDING DRUG DEVELOPMENT MISTAKES EARLY: CENTRAL NERVOUS SYSTEM DRUG DISCOVERY PERSPECTIVE*. IN *BMC NEUROLOGY*. 2009. SPRINGER.
19. GUENGERICH, F.P., *CYTOCHROMES P450, DRUGS, AND DISEASES.* MOLECULAR INTERVENTIONS, 2003. **3**(4): P. 194.
20. BIBI, Z., *ROLE OF CYTOCHROME P450 IN DRUG INTERACTIONS.* NUTRITION & METABOLISM, 2008. **5**(1): P. 27.