# Evaluation of the efficacy of Herbal antidotes of Viper snakes through *In-Silico* docking analysis of their bioactive components targeting phospholipase A2


**Abstract:**

**Introduction**: Snake bite is common in Rural areas. Many toxic snakes produce neurotoxic, Myotoxic, and hemotoxic effects. Snake venom is rich in metalloproteinases and Phospholipases.PhospholipaseA2 accounts for most of the toxic effects of Viper snakes. Many herbal antidotes have been in practice in the Siddha system which lacks scientific evidence.

**Aim**: This study aims to rule out the Binding pose and affinity of bioactive components derived from certain herbs with the target by forming hydrogen bonds so that the function of Phospholipases A2 with PDB – 2QOG would be hindered.

**Materials and methods**: From the listed plants 6 bioactive components were selected and *In-Siico* docking analysis was done with the target2QOG. Andrographolide(49 ASP, 52 TYR), Aristolochic acid(49 ASP, 52 TYR), Genistein(49 ASP, 52 TYR), and Nimbolide (49 ASP, 52 TYR) revealed a maximum of 2 interactions (50%) with the core active amino acid residues present on the target2QOG. Piperic acid (48 HIS) and Linoleic acid(48 HIS) reveal 1 interaction with the core active amino acid residues present on the target 2QOG.

**Conclusion**: Based on the results it was concluded that the bio-active compounds Andrographolide, Aristolochic acid, and Genistein present in the herbal ingredients possess significant binding against the target enzyme phospholipases A2.



**Key Words**:

Andrographolide ,Docking, Phospholipase A2, Viper , Venom , Aristolochic acid, Genistein, Piperic acid, Linoleic acid, and Nimbolide.


## 1.INTRODUCTION

### 1.1 Snake venom:

"Snakebite envenomation is an abandoned crisis seen worldwide, especially in rural, tropical, and subtropical zones. In 2009 the World Health Organization (WHO) acknowledged snake bites as a neglected disease in tropical areas". [(1)] "Envenomation results in significant death, disability, and emotional morbidity. Snake venoms are a complex mixture of active molecules including proteins and non-protein fractions of different sizes". [(2)] "They consist of various groups of amines, nucleosides, and carbohydrates. Among all the snake members of Elapidae, hydrophile, and Viperidae have been studied much. Chemicals present in venom affect a variety of cells and

tissues. As a result, they produce definite responses such as digestion of cell membrane and even whole cell, interference of procoagulant and anticoagulant activities of blood cells, synthesis of oxidizing agents,the collapse of tissue collagen and intercellular substances , damage of nervous tissue, etc., Based on the response produced snake venoms are classified as neurotoxins, hemotoxins, cardiotoxins, cytotoxins and myotoxins. The venom of the period family constitutes as much enzymes as 90% and 25% in some elapids Over 30 enzymes have been identified snake venom so far" [(3)] .

**1.2 Phospholipase A2:**

The phospholipase A2 presents a high amount of disulfide bonds, the low molecular weight of ~13.5 k Dalton. Phospholipases are divided into five categories: 1) the secreted phospholipase A2 (sPLA2), 2) the cytosolic phospholipase A2 (cPLA2), 3) the Ca2+ independent phospholipase A2 (iPLA2), 4) the platelet-activating factor acetylhydrolase (PAF-AH), and 5) the lysosomal phospholipase A2($lPA_2$ ) The basic form of PLA 2 possess the most potent pharmacological effect causing tissue damage or toxicity (types of pla2). In a single snake venom, Phospholipase A2 is represented in many isoforms [(4)]. Phospholipase A2 is present in many cell membranes. Thus phospholipase A2 present in venoms possess both exogenous and endogenous enzyme activity. As it is cytolytic in nature It facilitates the lysis of the cell membrane of several cells including erythrocytes, which results in hemorrhage. Cell damage is caused as phospholipase A2 modifies the structure and function of the cell membrane. It forms pores on the lipid bilayer of the cell membranes as a result of oligomerization. More than 523 plant species from 122 families were reported to have a neutralizing capacity against toxic snake venom. [(5)]

**1.3 Herbal constituents:**

The secondary metabolites extracted from herbs play a vital role in neutralizing the toxic effects of venom. several studies have been done to prove the inhibitory potential of herbal plants against the enzymes of snake venom. Therefore, there is a requirement for more studies on plant metabolites and the development of an antidote for the better treatment of snakebites. In Siddha Literature, Pants such as *Indigofera tinctoria, Piper nigrum, Aristolochia bracteolate, Azadirachta indica ,Andrographis paniculata, Cyanodon dactylon, Leucas aspera*, etc., have been mentioned as antidotes against snake poisons. From the pieces of evidence available in the Siddha text we selected *Indigofera tinctoria, Piper nigrum, Aristolochia bracteolate, Azadirachta indica,Andrographis paniculata, Cyanodon dactylon*, as these plants were mentioned as prime category antidotes that were used as internal medicines. *F*rom the above-mentioned pants,6 bioactive components were taken for docking. This article focuses on the documentation of the binding pose and binding affinity of some herbal plants which were mentioned in Siddha literature as antidotes for snake bites through *In silico* Molecular docking study of some of their bioactive components with Phospholipase A2 as a common target, from the results of which the potency of those antidotes could be established further. The most

effective binding molecules are those bioactive components that can take the place of the target in binding to the membrane receptor.

## 2. AIM & OBJECTIVE:

To rule out the Binding pose and affinity of Bioactive components from the selected plants with the core amino acids (His48, Lys49, Tyr52, and Asp99) of the target by forming hydrogen bonds so that the function of Phospholipases A2 with PDB – 2QOG would be hindered.

## 3. Materials and Methods:

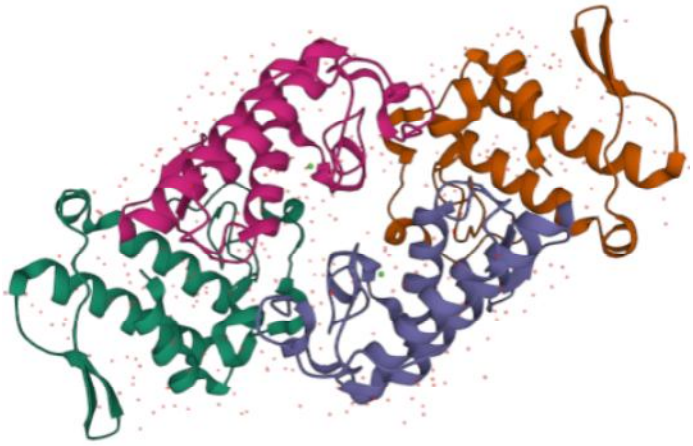
### 3.1 Preparation of Target:

The crystalline structure of the target protein phospholipase A2 was retrieved from the protein data bank and the protein clean-up process was completed(Table. 1)(Fig.1). Different orientation of the lead molecules with respect to the target protein was evaluated by Autodock program and the best dock pose was selected based on the interaction study analysis.

(Table. 1)

| PDB | Name of the Target |
|---|---|
| 2QOG[4] | Phospholipases A2 |

Fig .1 3D- Structure of Phospholipases A2 (PDB) - 2QOG



### 3.2 Preparation of Ligand:

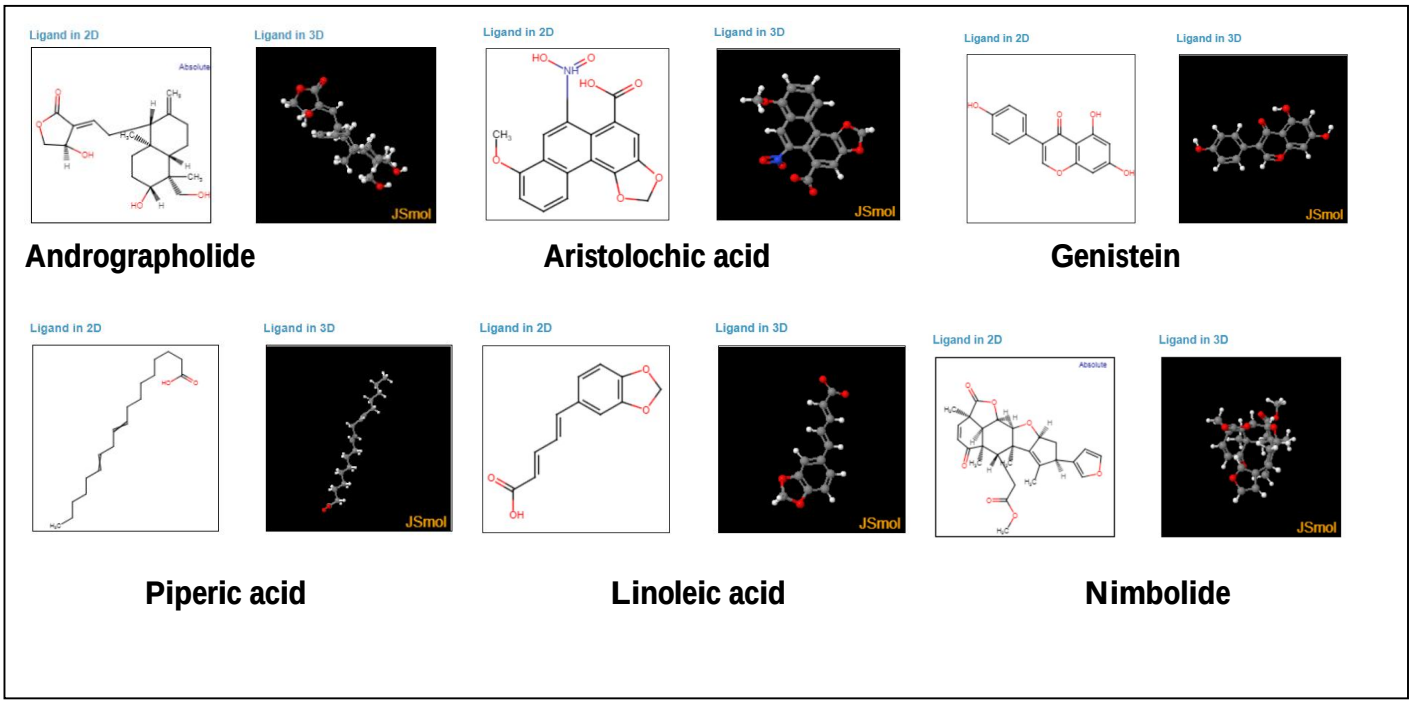
The 3 D structure of Andrographolide, Aristolochic acid , Genistein, Piperic acid, Linoleic acid and Nimbolide were downloaded. Hydrogen was added to the ligands. (Table .2)

Table .2 List of Bioactive components selected

| | |
|---|---|
| *Andrographis paniculata* | Andrographolide[6] |
| *Aristolochia bracteolata* | Aristolochic acid[7] |
| *Indigofera tinctoria* | Genistein[8] |
| *Piper nigrum* | Piperic acid[9] |
| *Cyanodon dactylon* | Linoleic acid[10] |
| *Azadirachta indica* | Nimbolide[11] |

A total of 6 bioactive lead compounds was retrieved from the herbal ingredients.

**Chart 1: 2D and 3D Structure of Selected Ligands**



### 3.3Docking:

“Docking calculations were carried out for retrieved phytocomponents against target enzyme Phospholipases A2. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools” [12]. “Affinity (grid) maps of ×× Å grid points and 0.375 Å spacing were generated using the Autogrid program. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method” [13]. The initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 2 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.

## 4. Results:

Fig.2 Docking Pose

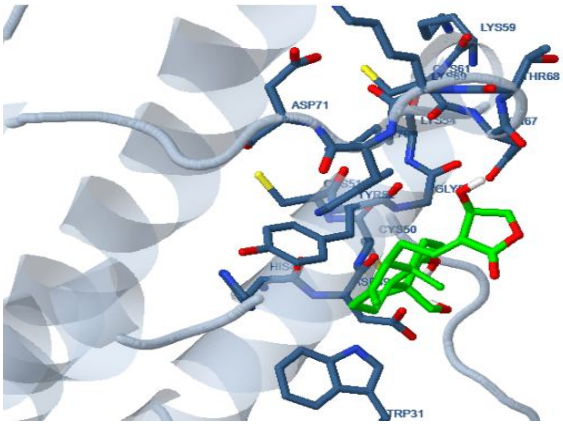



Fig 2a- Andrographolide with Phospholipases A2 (PDB) - 2QOG

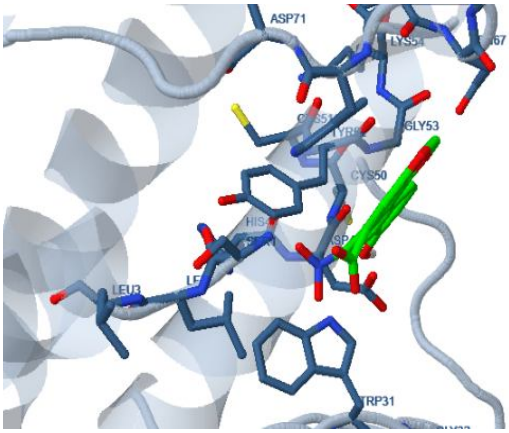



Fig 2b - Aristolochic acid with Phospholipases A2 (PDB) - 2QOG

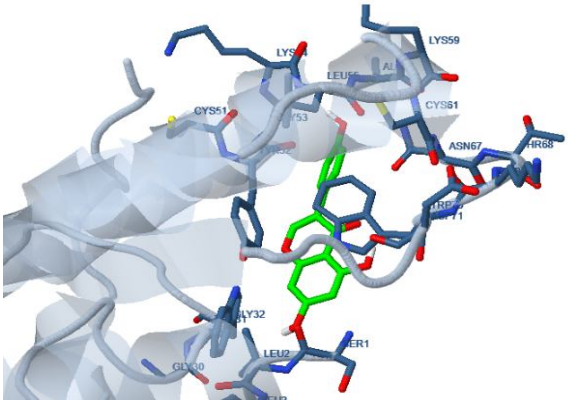



Fig 2c - Genistein with Phospholipases A2 (PDB) - 2QOG

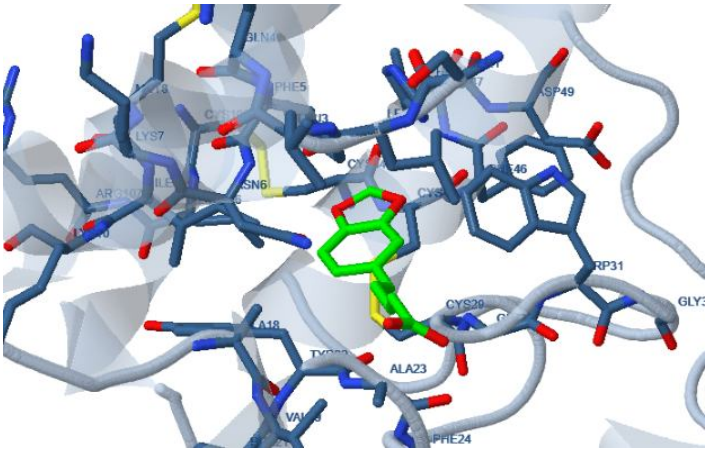


Fig 2d- Piperic acid with Phospholipases A2 (PDB) - 2QOG

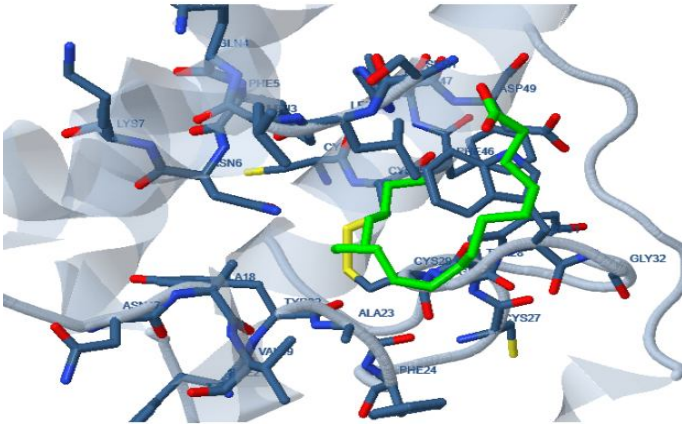



Fig 2e- Linoleic acid with Phospholipases A2 (PDB) - 2QOG

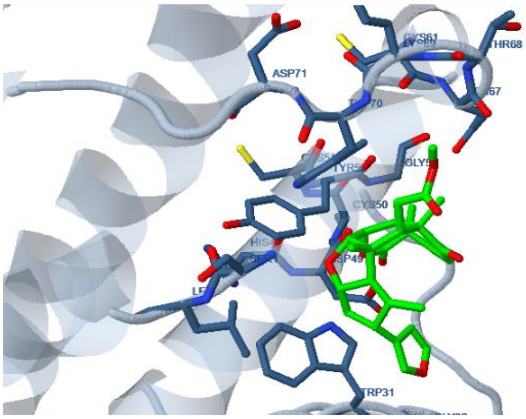



Fig 2f - Nimbolide with Phospholipases A2 (PDB) - 2QOG

| Compounds | Est.Free Energy of Binding (kcal/mol) | Est. Inhibition Constant, Ki (uM) | Electrostatic Energy (kcal/mol) | Total Intermolec. Energy (kcal/mol) | Interact. Surface | Interacting Aminoacid residues |
|---|---|---|---|---|---|---|
| Andrographolide | -6.74 | 11.55 | -0.26 | -7.48 | 537.587 | 49 ASP, 52 TYR |
| Aristolochic acid | -7.33 | 4.26 | -1.42 | -8.29 | 514.032 | 49 ASP, 52 TYR |

| | | | | | | |
|---|---|---|---|---|---|---|
| Genistein | -5.48 | 96.33 | -0.19 | -5.97 | 500.395 | 49 ASP, 52 TYR |
| Piperic acid | -5.38 | 112.96 | -0.26 | -6.27 | 520.522 | 48 HIS |
| Linoleic acid | -6.45 | 18.73 | -1.09 | -9.82 | 703.306 | 48 HIS |
| Nimbolide | -6.21 | 27.85 | -0.06 | -7.20 | 611.343 | 49 ASP, 52 TYR |

Table 3: Summary of the molecular docking studies of compounds against Phospholipases A2 (PDB) - 2QOG

Docking poses of various bioactive components of the selected herbal plants against Phospholipases A2 (PDB) - 2QOG in Fig.2. The docking results of the above-mentioned bioactive components against the core amino acids of phospholipase A2 generated negative values for free energy -6.74 kcal/mol for Andrographolide -7.33 kcal/mol Aristolochic acid -5.48 kcal/mol for Genistein -5.38 kcal/mol for Piperic acid -6.45 kcal/mol for Linoleic acid and -6.21 kcal/mol for Nimbolide in the grid box, suggestive of high affinity for the binding pocket, with Aristolochic acid with the highest affinity.(Table .3) From reported data of the herbs, Andrographolide(49 ASP, 52 TYR), Aristolochic acid(49 ASP, 52 TYR) , Genistein(49 ASP, 52 TYR), and Nimbolide (49 ASP, 52 TYR) present in the herbal ingredients reveals maximum of 2 interactions (50%) with the core active amino acid residues present on the target Phospholipases A2. Piperic acid (48 HIS) and Linoleic acid(48 HIS) reveal 1 interaction with the core active amino acid residues present on the target Phospholipases A2.

**5. Discussion**

Cytotoxins, phospholipases $A_2$, and metalloproteinases represent major classes of proteins found in snake venoms. Proteins and polypeptides make up approximately 95% of the dry weight of the venom. [14]. "Many of these proteins are responsible for producing severe pathophysiological events following envenomation and therefore represent a significant hazard for snakebite victims. Crotoxin is one such major toxic constituent present in the venom of Crotalus durissus terrificus, a viper species . It is a complex protein having the basic subunit phospholipase A2, and the acid subunit crotapotin".[15,16] phospholipase A2 (2QOG) , component of crotoxin causes neurotoxicity.[17] Hence the crystal structure of Phospholipase A2 available with the PDB ID: 2 QOG is used for the current investigation .

"As phospholipase A2 is cytolytic in nature it facilitates the lysis of cell membrane .It modifies the structure and function of cell membrane. It forms pores on the lipid bilayer of the cell membranes as a result of oligomerization that involves a His48–Asp49 pair in the catalytic site, which activates a structurally conserved water molecule, thereby initiating

the nucleophilic attack on the sn-2 position of the substrate". [18,19] . The cells with lipid bilayers made up of only phosphatidylcholine (PC) be extremely prone to lipid hydrolysis by phospholipase A2. while lipid bilayers containing more phosphatidylcholine and a less amount of acidic phospholipids were somewhat vulnerable to phospholipase A2 -mediated lipid hydrolysis. Thus it ruptures the erythrocytes.[20]

Hydrogen bonds play an important part in the determination of the specificity of protein-ligand binding.[21] Hydrogen (H) bonds enhance various cellular functions by facilitating intermolecular interactions. The affinity between ligand and protein depends on the number of hydrogen bond acceptors than donors and is also dependent on rotatable bonds. [22] Andrographolide, Aristolochic acid, and Genistein possess a total number of 8 Hydrogen bonds each, whereas, Piperic acid has 5 hydrogen bonds, Linoleic acid has 3 hydrogen bonds and Nimbolide 7 hydrogen bonds.

His48, Lys49, Tyr52, and Asp99 are the core amino acids of the protein phosphatase a2. These amino acid residues are functionally responsible for the activation of Neurotoxicity, myotoxicity, and edema. Thereby phytocomponents that inhibit the target enzyme Phospholipases A2 may occupy this active amino acid and could be able to block the hydrophobic channel and prevention of the binding of the fatty acid necessary for the toxin allosteric activation during snake envenomation and act as a potential therapeutic agent for the management of snake bite.

"Clinically, snake envenomation is very prevalent and contributes to a high mortality rate in developing countries. Moreover, it is disappointing that the lack of sufficient anti-venom supplies and clinics has not been addressed in developing countries. Hence, it is imperative that more funding and research is dedicated to finding more efficient anti-venom and alternative treatments in order to significantly decrease the number of deaths due to snakebites". [23]

Siddha system has mentioned many herbal plants and compound medicines for various types of animal poisons. A vast information is given regarding the types of snakes, the symptoms of poisoning, and their treatment plants. Many herbal plants have been mentioned as antidotes for various snake poisons which remain cost-effective and easily available. *Andrographis paniculata* is traditionally used for venomous bites in various parts of Asia[24]. In a previous study, *Andrographis paniculata* along with immunotherapy was used against Naja naja snake venom. It markedly increased the average time of survival and mortality rate when compared with control mice which received only immune therapy[25] Aqueous extract of *Aristolochia bracteolate* was found to have 80% inhibition of the Tocxic effect caused by Russell's viper and cobra venom. In the same study, it was proven that Aristolochic acid actively inhibits phospholipase A2 [26]. Extract of *Indigofera tinctoria* was found effective in treating skin lesions caused by viperine snake bites [27]. Leaf paste of *Azadirachta indica* was used against viper snake venom.(28) *Piper nigrum* was an effective antidote for cobra poisoning [29] . *Cyanodon dactylon* is being used in the treatment of snake bites in the chengalpet district [30].

This work is to repurpose those ideas of using herbal antidotes which are even safer. The need to bring equally or more potent alternatives in the form of the plant-derived drug is very essential as they are much safe. Among the chosen ligands Andrographolide, Aristolochic acid and Genistein present in the *Andrographis paniculata, Aristolochia bracteolate, and Indigofera tinctoria* respectively, reveals a maximum of 2 interactions (50%) with the core active amino acid residues present on the target Phospholipases A2. Although these Antidote properties are appreciable in silico, further studies of in vitro and clinical studies dealing with phospholipase A2 of snake venom should be considered for further studies.

**6. Conclusion:.**

Based on the results of the computational analysis it was concluded that the phytochemicals Andrographolide, Aristolochic acid, and Genistein present in the herbal ingredients possess significant binding against the target enzyme phospholipases A2. Thereby phytocomponents that inhibit the target enzyme Phospholipases A2 may occupy these active amino acids and could be able to block the hydrophobic channel and prevention of the binding of the fatty acid necessary for the toxin allosteric activation during snake envenomation and act as a potential therapeutic agent for the management of snake bite. Thus *Andrographis paniculata, Aristolochia bracteolate and Indigofera tinctoria* could be considered to possess effective antidote properties in viper snake envenomation. However further studies involving docking of all the phytochemicals in these herbs would help in establishing complete documentation. These identified bioactive components can be tested via *in vitro* and *in vivo* studies for further evaluation of the plants.

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