**European Journal of Biotechnology and Bioscience**
**Online ISSN: 2321-9122**
**www.biosciencejournals.com**
**Volume 4; Issue 3; March 2016; Page No. 12-15**

# *In-silico* analysis and homology modeling of snake venom metalloproteinase

**[1] Swati S. Badagu, [2] Yogesh N.Joshi, [3] Vinod P.S.**

Department of PG studies and Research in Bioinformatics, Walchand Centre for Biotechnology, Walchand College of Arts and Science, Solapur, Maharashtra, India.

**Abstract**
Snake venoms are an extremely rich source of pharmacologically active proteins with a considerable clinical and medical potential. Snake venoms are relevant sources of toxins that have evolved towards the engineering of highly active compounds. Venom transcriptomes containing multiple toxin types including three finger toxins, cobra venom factor, cysteine-rich secretory protein, hyaluronidase, kallikrein, kunitz, lectin, matrix metalloprotease, phospholipase $A_2$, snake venom metalloproteinase, disintegrin and waprin. Snake venom metalloproteinase (SVMPs) are a set of interesting enzymes that are one of the major components of venom affecting hemostasis. A great challenge since their discovery has been to find molecular features responsible for their hemorrhagic potency and many attempts have been made without any consistent result. In the present study we describe a series of sequence and structural analysis between the snake species *Ophiophagus hannah, Bungarus fasciatus, Daboia russelii* and *Echis carinatus* applying by various *in silico* tools. These involved prediction of physical and chemical properties, motif and domain scanning and structural analysis. The physicochemical analysis was carried out by using ProtParam tool, which depicted SVMPs of *Ophiophagus hannah* and *Bungarus fasciatus* were unstable and *Daboia russelii* and *Echis carinatus* were stable. The domain analysis was performed by using Pfam domain database, the common domain found was reprolysin. The secondary structures were predicted by using SOPMA tool, the percentage of alpha helix and extended strand was more in *Daboia russelii* as compared to other two species. The 3D structures were predicted using SWISS-MODEL server and models validated using PROCHECK analysis tool.

**Keywords:** metalloproteinases, *in silico*, snake venom, 3D structures etc.

## Introduction

Snake venoms are an extremely rich source of pharmacologically active proteins with a considerable clinical and medical potential. Snake venoms are complex mixtures of a variety of substances. Predominantly, the dry weight is composed of proteins, nevertheless also organic low molecular mass compounds and metal ions account for their complexity. Among the proteins, enzymes such as acetylcholinesterases, ADPases, L-amino acid oxidases, phospholipases A2, hyaluronidases, metallo-, and serine proteases are included. Additionally, non-enzymatic proteins, like disintegrins, α-neurotoxins, C-type lectin-like proteins (CLPs) and bradykinin-potentiating peptides also contribute to toxicity [1].

Snake venom metalloproteinase (SVMPs) are a set of interesting enzymes that are one of the major components of venom affecting hemostasis. SVMPs are one of the most abundant components of viperid snake venoms. It has been assumed that more than one third of the venom's content is composed of this kind of proteinases [2]. Beside myotoxic phospholipases A2, the direct action of SVMPs is main reason for the complex and severe local pathological manifestations, including edema, blistering, dermonecrosis and myonecrosis [3]. SVMPs were categorized into four main classes (P-I, P-II, P-III, and P-IV) according to the presence or absence of different non-proteinase domains as observed in isolated proteins and their mRNA transcripts.

A great challenge since their discovery has been to find molecular features responsible for their hemorrhagic potency and many attempts have been made without any consistent result. In the present study we describe a series of comparisons between the snake species *Ophiophagus hannah, Bungarus fasciatus, Daboia russelii* and *Echis carinatus* with the help of various *in silico* tools. *Ophiophagus hannah* and *Bungarus fasciatus* are Elapid family snakes also present SVMP's [4]. The *in silico* tools involved sequence retrieval, physical and chemical properties prediction by using ProtParam tool, motif and domain scanning and structural analysis.

Detailed knowledge of the venom toxin composition and their biological properties provide the scaffold for designing new peptide based drugs for the treatment of various cancers, hemostatic and other chronic disorders and as well as new tools for clinical diagnostic and assays of hemostatic parameters. Further, the high degree of target specificity makes toxins valuable molecules for drug development viz computer aided drug designing and structural bioinformatics.

## 2. Materials and Methods

**2.1 Retrieval of Sequences**:- The SVMP's protein sequences of four snake species such as *Ophiophagus hannah* , *Bungarus fasciatus, Daboia russelii, Echis carinatus* were retrieved from UniProtKB protein database. UniProtKB is public protein database which contains the amino acid sequences of proteins. The sequence were retrieved & saved in FASTA file format with their accession IDs. [5].

**2.2 Physicochemical Analysis**:-The physicochemical analysis were performed by using ProtParam tool. ProtParam is a tool which allows the computation of various physical and chemical parameters for a given protein stored in Swiss-Prot or TrEMBL or for a user entered protein sequence. The

computed parameters include the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY). [6].

**2.3 Domain Analysis: -** The molecular domains of SVMPs of four snake species were analyzed using Pfam database. Pfam is a database of protein families that includes their annotations and multiple sequence alignments generated using hidden Markov models. [7].

**2.4 Secondary Structure Prediction**: - The secondary structures of SVMPs were predicted by using SOPMA secondary structure prediction tool. SOPMA is 'Self-Optimized Prediction Method with Alignment' is an improvement of SOPM method. This SOPMA method correctly predicts 69.5% of amino acids for a three-state description of the secondary structure (alpha-helix, beta-sheet and coil) in a whole database proteins.

**2.5 Homology Modeling and model Validation**:-The 3D structures of all four species SVMPs were modeled using SWISS-MODEL server. The SWISS-MODEL is a structural bioinformatics web-server dedicated to homology modeling of protein 3D structures. Homology (or comparative) modeling methods make use of experimental protein structures ("templates") to build models for evolutionary related proteins ("targets") [9, 10].

## 3. Results and Discussion:-

### 3.1 Retrieval of Sequences:-

The SVMP's sequences of four snake species were retrieved from UniProtKB database and sequences were saved in FASTA format. The UniProtKB IDs, sequence length and protein name were shown in Table 1.

**Table 1:** Retrieval of Sequence

| Species Names | *Ophiophagus hannah* | *Bungarus fasciatus* | *Daboia russelii* | *Echis carinatus* |
|---|---|---|---|---|
| **UniProtKB ID** | A3R0T9 | A8QL48 | B8K1W0 | Q90495 |
| **Length** | 611 | 605 | 615 | 616 |
| **Protein name** | Zinc metalloproteinase-disintegrin-like ohanin | Zinc metalloproteinase-disintegrin-like BfMP | Zinc metalloproteinase-disintegrin-like daborhagin-K | Zinc metalloproteinase-disintegrin-like ecarin |

### 3.2 Physicochemical analysis:-

The physicochemical properties were analyzed using ProtParam tool and the results were shown in Table 2. The molecular weight of all four sequences are ~70000 Daltons. The theoretical pI depicts that the SVMP of *Bungarus fasciatus* is basic in nature, while SVMPs of other three species being acidic. The Instability index indicates that the SVMPs of *Ophiophagus hannah* and *Bungarus fasciatus* are unstable and *Daboia russelii* and *Echis carinatus* are stable.

**Table 2:** Physicochemical Analysis

| Physicochemical analysis | *Ophiophagus Hannah* | *Bungarus fasciatus* | *Daboia russelii* | *Echis carinatus* |
|---|---|---|---|---|
| **Molecular weight** | 69048.5 | 68201.7 | 69554.9 | 69462.5 |
| **Theoretical pI** | 5.67 | 8.74 | 5.95 | 5.18 |
| **Instability index** | 44.17 Unstable | 42.44 Unstable | 37.66 Stable | 39.25 Stable |
| **Aliphatic index** | 69.23 | 66.38 | 73.20 | 70.28 |
| **GRAVY** | -0.473 | -0.548 | -0.438 | -0.461 |

**3.3 Domain analysis: -** The domains of four SVMP's were predicted from Pfam domain database. Domain Reprolysin was the common domain in all the four species. The domain structure of Reprolysin was shown in Figure 1. The members of this family are enzymes that cleave peptides. These proteases require zinc for catalysis. Members of this family are also known as adamalysins. Most members of this family are snake venom endopeptidases.


**Fig 1:** Domain Structure of Reprolysin (M12B) family zinc metalloprotease

**3.4 Secondary Structure Prediction:-** The secondary structures of SVMPs were predicted by SOPMA tool. The secondary structure elements alpha helix, extended strand and random coils of four snake species SVMPs were calculated in Table 3. As per the Table 3 the percentage of alpha helix and extended strand was found to be more in *Daboia russelii*, least helix percentage was found in *Bungarus fasciatus*, least strand in *Ophiophagus Hannah.*

**Table 3:** Secondary Structure Prediction

| SSE | *Ophiophagus Hannah* | *Bungarus fasciatus* | *Daboia russelii* | *Echis carinatus* |
|---|---|---|---|---|
| **Alpha helix** | 25.53% | 22.48% | 26.50% | 22.56% |
| **Extended strand** | 23.90% | 25.45% | 25.69% | 24.03% |
| **Random coil** | 50.57% | 52.07% | 47.80% | 53.41% |

**3.5 Homology Modeling and Model Validation:-**The 3D structures of SVMPs from four species were predicted by using the automated mode of SWISS Model server and predicted models were validated using PROCHECK tool. The template and organism name with its identity score and validation scores were shown in Table 4. The template identification was performed for all the sequences. The models developed were first checked for pass or fail using structure validation tool. The models were validated by PROCHECK server. The PROCHECK checks the stereochemical quality of a protein structure, producing a number of PostScript plots analyzing its overall and residue-by-residue geometry. The Ramchandran plot score was obtained for SVMPs of all four species models and was found to be above 75%. The generated models were visualized in RasMol as shown in Figures 2-5.

**Table 4:** Homology Modeling and Model Validation

| Species Name | ***Ophiophagus Hannah*** | ***Bungarus fasciatus*** | ***Daboia russelii*** | ***Echis carinatus*** |
|---|---|---|---|---|
| **Template and organism name** | 2ero.1.A<br>*Crotalus atrox* | 3k7l.1.A<br>*Naja atra* | 2dw2.1 A<br>*Crotalus atrox* | 2e3x.1A<br>*Daboia russellii siamensis* |
| **Template identity** | 51.70 | 76.90 | 62.02 | 65.16 |
| **Ramchandran plot** | 84.8% | 82.7% | 81.2% | 76.9% |

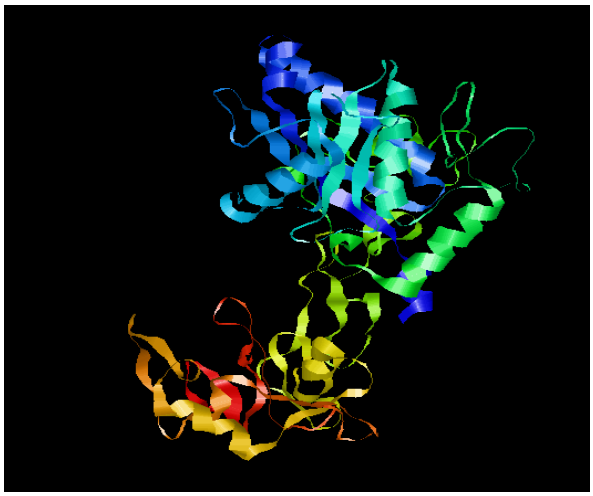

**Fig-2:-**3D model of *Ophiophagus hannah*

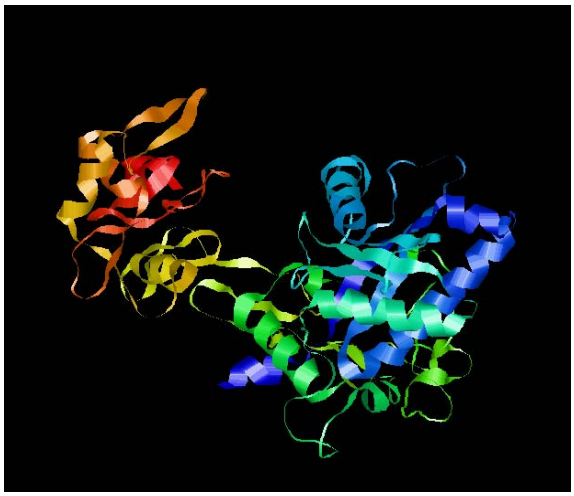

**Fig-3:-**3D model of *Bungarus fasciatus*

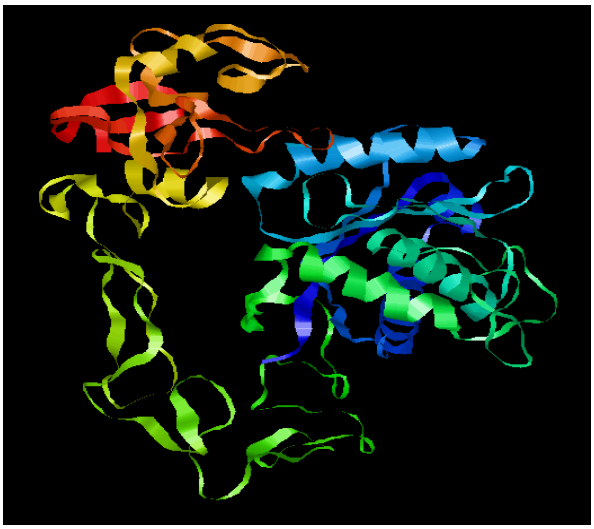

**Fig-4:-** 3D model of *Daboia russelii*

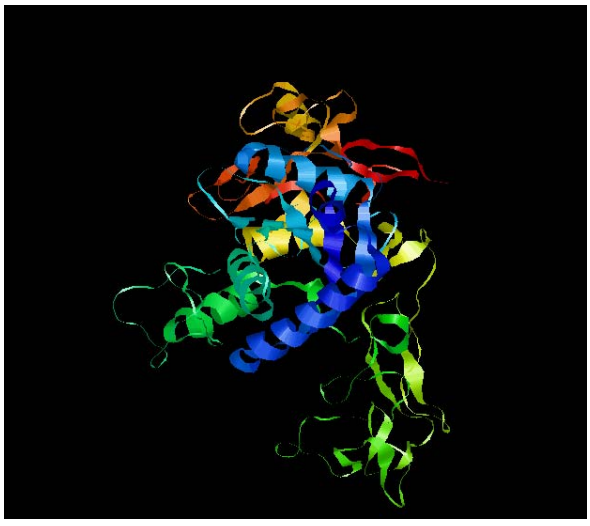

**Fig-5:-** 3D model of *Echis carinatus*

**4. Conclusion**

The SVMPs sequences of four snake species were selected for present *in-silico* analysis. In the present study, sequence and structural insight of SVMPs of four snake species emphasize their basic molecular nature with knowledge of the venom toxin composition and their biological properties for designing novel scaffold and designing new peptide based drugs for the treatment of various cancers, haemostatic and other chronic disorders and as well as new tools for clinical diagnostic and assays of hemostatic parameters. The SVMPs sequences of the snake species *Ophiophagus hannah, Bungarus fasciatus, Daboia russelii* and *Echis carinatus* were retrieved from UniProtKB protein database, using those sequences their physicochemical properties were analysed from ProtParam

tool and the domain was analyzed by Pfam database, it shows common reprolysin protein family from zinc metalloprotease secondary structural elements were predicted from SOPMA tool. The four SVMP's models were built using SWISS-MODEL server and all models were validated using PROCHECK server. Further, the high degree of target specificity makes toxins valuable molecules for drug development viz computer aided drug designing and structural bioinformatics.

**5. References**


1 Mackessy SP. Handbook of Venoms and Toxins of Reptiles, CRC Press: Boca Raton, Florida, 2009.

2 Fox JW, Serrano SMT. Timeline of key events in snake venom metalloproteinase research. *J Proteom,* 2009; 72:200-209.

3 Gutierrez JM, Rucavado A, Chaves F, Diaz C. Escalante, T. Experimental pathology of local tissue damage induced by Bothrops as per snake venom. *Toxicon*, 2009; 54:958-975.

4 Isolation and cloning of a metalloproteinase from king cobra snake venom." Guo X.-X., Zeng L., Lee W.-H., Zhang Y., Jin Y. Toxicon. 2007; 49:954-965.

5 The UniProt Consortium UniProt: a hub for protein information *Nucleic Acids Res.* 2015; 43:D204-D212.

6 Gasteiger E, Hoogland C, Gattiker A, Duvaud S, Wilkins MR, Appel RD, *et al. Protein Identification and Analysis Tools on the ExPASy Server;* (In) John M. Walker (ed): The Proteomics Protocols Handbook, Humana Press, 2005, 571-607

7 The Pfam protein families database: R.D. Finn, A. Bateman, J. Clements, P. Coggill, R.Y. Eberhardt, S.R. Eddy, A. Heger, K. Hetherington, L. Holm, J. Mistry, E.L.L. Sonnhammer, J. Tate, M. Punta *Nucleic Acids Research*, 2014; 42:D222-D230.

8 Geourjon C, Deleage G. SOPMA: significant improvements in protein secondary structure prediction by consensus prediction from multiple alignments. Computer Application Bioscience, 1995; 11(6):681-684.

9 Arnold K, Bordoli L, Kopp J, Schwede T. The SWISS-MODEL Workspace: A web-based environment for protein structure homology modeling. *Bioinformatics*, 2006; 22:195-201.

10 Laskowski RA, MacArthur MW, Moss DS, Thornton J M. PROCHECK - A program to check the stereochemical quality of protein structures. J. App. Cryst., 1993; 26:283-291.