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Homology modeling and insilco analysis of venom protein hyaluronidase in *Vespa Velutina* (Hymenoptera: Vespidae)

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Abstract
Vespidae is a cosmopolitan family of order Hymenoptera popularly called as wasps. Hyaluronidase is found in the venom and saliva of stinging insects. Hyaluronidase (HYases) is the major allergen in the venoms of bees, hornets, and scorpions. The Asian hornet *Vespa velutina* venom protein Hyalouronidase (HYases) sequence was selected for present *in-silico* analysis. The physicochemical analysis of the predicted Hyaluronidase (HYases) was performed using Protparam tools. The secondary structures of Hyaluronidase were predicted by using SOPMA. The 3D structures were predicted using the SWISS-MODEL server and models were validated. The results show that the most abundant amino acid was found as Asparagine and six type's motifs were observed and the highest number of the motif was ASN- Glycosylation site and Protein kinase C phosphorylation site. The secondary structure was observed that random coil was predominant (39.88%), followed by alpha helix (39.27%) and extended strand (15.11%). Ramachandran plot analysis showed that residues in most favoured regions is 90.03% residues in additional allowed regions is 9.0% indicating that the model was of reliable and good quality. The results of our study contribute to an understanding of venom protein structure in hornet species and will be scientific base for 3D modeling of Hyalouronidase (HYases) proteins in further studies.

Keywords: hymenoptera, wasp, venom, homology modelling, and Asian yellow-legged hornet

1. Introduction
Comparative modeling predicts the 3-D structure of a given protein sequence (target) based primarily on its alignment to one or more proteins of known structure (templates) [1]. Functional characterization of a protein sequence is one of the most frequent problems in biology. In the absence of an experimentally determined structure, comparative or homology modeling can sometimes provide a useful 3-D model for a protein that is related to at least one known protein structure [1-4].
Vespidae is a cosmopolitan family of order Hymenoptera (Insecta) popularly called as wasps.Hornet wasps are mainly distributed in theOriental and Palaearctic Regions of the world. Wasps of the subfamilies Vespinae and Polistinae are known as hornets, yellow jackets, and paper wasps. There are 23 valid species known from the world so far of which 16 species from Indian subcontinent and 15 species from India [5-6]. Adults are usually black or brown but are often extensively marked with yellow or white [5].
Hyaluronidase is found in the venom and saliva of several stinging or biting insects to facilitate the movement of cytotoxic or neurotoxic agents through the recipient's tissue. It is often identified as the causative allergen in patients who develop a hypersensitivity reaction to either an insect bite or sting [7]. Hyaluronidase, found in all kinds of animal venoms, hydrolyzes hyaluronic acid, one of the major components of extracellular matrix, and causes local tissue damage [8-11].
It is studied that about 75% of the world's animal species belong to arthropods a few of which have appreciable interaction with humans and is capable of causing physiological and medical problems [12]. Studies on social Hymenoptera venoms are of great medical importance, since a great number of people are sensitive and vulnerable to the venom [13]. Venomous animals deliver their toxin during stinging act [14].Venom is a form of toxin secreted by animals that aims at the rapid immobilization or inactivation of their prey or enemy. Venom components target main critical systems of targeted bitten animal, such as neuromuscular and hemostatic systems, to achieve the most efficient and rapid immobilization or death of the victim. Most of the venomous animals prey on many different species, and have a defence system against unspecified intruders, they produce various proteins and peptides both with specific molecular targets and those that are active across a wide range of animal species [15].
Hyaluronidase is the major allergen in the venoms of bees, hornets, wasps and scorpions that stimulate lethal systemic IgE-intermediated anaphylactic responses in humans[16.] There have been studies on the hyaluronidase from the venom of the honey bee [17] the funnel web-spider *Hippasa partita* [18], the social wasp *Polybia paulista* [19] and the spider *Vitalius dubius* [20].
The aim of this study was to generate a predicted 3D structure of venom protein Hyaluronidase (HAases) by using comparative homology modeling. Also, primary and secondary structure analyses were performed with various bioinformatics tools.

2. Materials and Methods
2.1 Sequences
The wasp *Vespa velutina* venom protein Hyaluronidase

sequence is retrieved from UniProtKB protein database. UniProtKB is public protein database which contains the amino acid sequences of proteins. The Hyaluronidase protein sequence were retrieved and saved in FASTA file format.

2.2 Physicochemical Analysis
The physicochemical analysis was performed by using ProtParam tool. ProtParam is a tool that 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 molecular mass and isoelectric points were computed using the Compute pI/MW tool of ExPASy Bioinformatics (http://web.expasy.org/compute_pi/). 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).

2.3 Domain Analysis
The molecular domains of Hyaluronidase of wasp species were analysed using the Pfam database. Pfam server (http://www.sanger.ac.uk/software /pfam/search.html) was used for domain analysis [21].

2.4 Secondary Structure Prediction
The secondary structures of Hyaluronidase were predicted by using SOPMA (Self- Optimized Prediction Method with Alignment). Secondary structure prediction was performed by using SOPMA [22] server (http://npsapbil.ibcp.fr/). Sub-cellular localization was predicted by using CELLO v.2.5 [23-24] 1.1 server (http://www.cbs.dtu.dk). Motif Scan [25&26] server (http://myhits.isb-sib.ch/cgibin/motif_scan) was used to identify known motifs in the sequence.

2.5 Homology Modeling and model Validation
The three-dimensional structures of Hyaluronidase were modeled using SWISS-MODEL server. The three-dimensional models were created using the SWISS-MODEL program, the automated protein homology modeling template at ExPASY (Switzerland) and a template search with the Alignment Mode program from the protein database (http://swissmodel.expasy.org/). The SWISS-MODEL is a structural bioinformatics web-server dedicated to the homology modeling of protein 3D structures. After modeling, the quality and validation of the model was evaluated by several structure assessment methods, containing Z-Score by using QMEAN [27] SAVES V 5.0 server for Ramachandran plot analysis and ERRAT [28].

3. Results and Discussion
The Asian hornet *Vespa velutina* venom protein Hyaluronidase (HYases) sequence was retrieved from the UniProtKB database and the sequence was saved in the FASTA file format. The UniProtKB ID is C0HLL5 (HUGAB_VESVE), sequence length is (331) and Enzyme Commission no is 3.2.1.35.

The physicochemical analysis of the predicted Hyaluronidase (HYases) was performed using Protparam and results were shown in Table 1.This venom protein (HYases) had 331 amino acids with a molecular weight of 39109.74 Daltons and *pI* of 9.10.

The optimum pI of Hyaluronidase is nearly 10.3. This pI value is similar to our pI value of 9.10. The theoretical pI depicts that the venom protein HYases of *Vespa velutina* is basic in nature. The most abundant amino acid was found as Asparagine (27 residues, 8.20%), whereas the lowest was Cysteine (4 residues, 1.2%). The total number of positively charged residues (Arg + Lys, 45) was found higher than the total number of negatively charged residues (Asp + Glu, 37).

Intracellular proteins have a lower number of cysteine residues, but also higher numbers of aliphatic and charged amino acid residues[29]. This data is in agreement with our finding that the highest number of amino acid residue was Asparagine while the lowest one was cysteine. It is accepted that extracellular proteins include more disulphide bridges and cysteine residues [30]. The predicting of subcellular localization of unknown proteins contributes to the understanding of their functions [31], it was performed using CELLO v.2.5 and our protein HYases was localized in cytoplasam. The instability (II) and aliphatic index revealed that this protein may be unstable and globular protein. Negative GRAVY value shows that this protein is accepted as a hydrophilic character (Table 1).

Table 1: The physicochemical properties of the predicted Hyaluronidase (HYases) venom protein of *Vespa velutina*

Parameters	Value	Explanation
pI	9.10	The protein is accepted as basic
Total number of negatively charged residues (Asp + Glu)	37	Total numbers of negatively charged residues are lesser than the total number of positively charged residues.
Total number of positively charged residues (Arg + Lys)	45	Total numbers of positively charged residues are higher than the total number of negatively charged residues.
The instability index (II)	40.05	This classifies the protein as unstable
Aliphatic index	76.19	Globular protein
Grand average of hydropathicity (GRAVY)	-0.522	the negative score indicates this protein is hydrophilic in nature

Table 2: Secondary structure analysis of wasp *Vespa velutina* Hyaluronidase (HYases) using SOPMA server

Parameters	Amino acids	Amino acids %
Alpha helix (Hh)	130	39.27%
helix (Gg)	0	0.00%
Pi helix (Ii)	0	0.00%
Beta bridge (Bb)	0	0.00%
Extended strand (Ee)	50	15.11%
Beta turn (Tt)	19	5.74%
Bend region (Ss)	0	0.00%

Random coil (Cc)	132	39.88%
Ambiguous states	0	0.00%
Other states	0	0.00%

The secondary structure of the venom protein HYases were predicted using the SOPMA server. The secondary structure elements alpha helix, beta-turn, extended strand and random coils of HYases were calculated in Table 2. It was observed that therandom coil was predominant (39.88%), followed by alpha helix (39.27%) and extended strand (15.11%). Also, beta-turn was found as5.74%. Random coils have important functions in proteins for flexibility and conformational changes such as enzymatic turnover [32]. Our findings could be related with the enzymatic function of protein. The domain analysis was conducted using the Pfam database and glycosyl hydrolase family 56 was detected. In venom, glycosaminoglycan hydrolysis by hyaluronidases is thought to participate in the degradation of the extracellular matrix, thus allowing a rapid spreading of other venom components during the envenomation process.

Glycoside hydrolases are a commonly known group of enzymes that hydrolyse the glycosidic bond between carbohydrates with more than 100 different families [33]. This data support that our protein may play a role as an enzyme in hydrolysis reactions (Figure 1).

Fig 1: Domain Structure of *Vespa velutina venom protein* Hyaluronidase (HYases) is Glycosylhydrolase family 56

Table 3: The motifs of predicted Hyaluronidase (Hyases) wasp protein by Motif Scan Tool

Motif information	No. of sites	Amino acid residues
ASN-Glycosylation site	4	79-82, 99-102,127-130,325-328
CK2-Phosphorylation site	3	274 -277, 281-284, 298- 301
Myristoylation site	1	235- 240
Protein kinase C phosphorylation site	4	150-152, 164-166, 171-173, 254-256
TYR-Phosphorylation site	1	172- 180
Glycosyl hydrolase family 56	1	1- 331

The Motif scan tool was used to determine different motifs. The six type's motifs were observed and the highest number of the motif was ASN- Glycosylation site and Protein kinase C phosphorylation site with 4 times. CK2-Phosphorylation site, Myristoylation site, TYR-Phosphorylation site, and Glycosyl hydrolase family 56 were identified as 3, 1, 1, and 1times, respectively (Table 3). The phosphorylation of a protein can affect functions and activities of proteins, including intrinsic biological activity, half-life, subcellular location, and docking with other proteins [34].

The occurrence of many phosphorylation sites in Hyaluronidase (Hyases) venom protein support that it may be regulated frequently. Myristoylation is post-translational protein modification observed in plants, animals, fungi, and viruses; it is performed by attached myristic acid in proteins. Myristoylation can affect the conformational stability of proteins by interaction with membranes or the hydrophobic domains of other proteins[35].The Asian hornet Hyaluronidase (HYases) venom protein active site is glycosyl hydrolase family 56 prove the catalytic activities of this protein.

The SWISS-MODEL homology modeling program was used for the predicting three- dimensional structure of the Hyaluronidase (HYases) (Figure 2). PDB 2atm 1.A was selected as a template with 92.75% sequence identity to query sequence. After model building, the structure was validated through energy minimization with Z-Score by using the Qmean server, ERRAT, and Ramachandran plot analysis. The Z-score is used to estimate the quality of model using structured solved proteins as references [36]. Qmean Z-Score for this protein is found as -0.32. The overall quality factor was found as 92.0 which is satisfactory. Ramachandran plot analysis showed that residues in most favoured regions are 90.03% residues in additional allowed regions is 9.0% indicating that the model was of reliable and good quality (Table 4; Figure 2 &3).

Table 4: Homology Modeling and Model Validation

Species Name	*Vespa Velutina*
Template and organism name	2atm.1.A Hyaluronoglucosaminidase Crystal structure of the recombinant allergen Ves v 2
Template identity	92.75%
Ramchandran plot (Residues in most favoured regions)	90.03%
Ramchandran plot- (Residues in additional allowed regions)	9.0%
QMEAN4 Value	-0.32
Verify 3D	>= 0.2

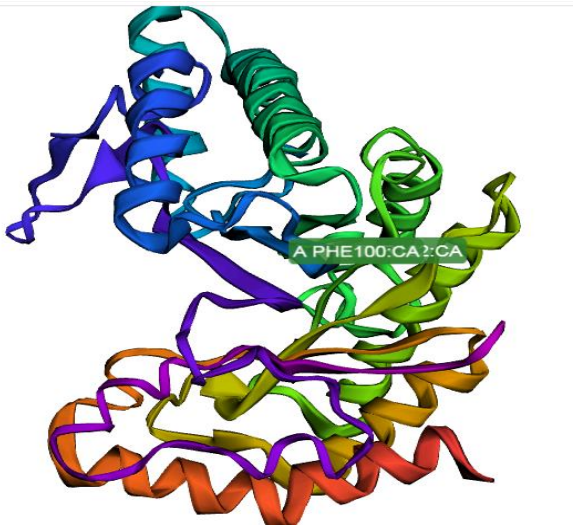

Fig 2: The three-dimensional structure of predicted Hyaluronidase (HYases) of *Vespa velutina* by modelled SWISSMODEL using PDB ID: 2atm.1.A as template and Uniprotkb Id: C0HLL5 as target

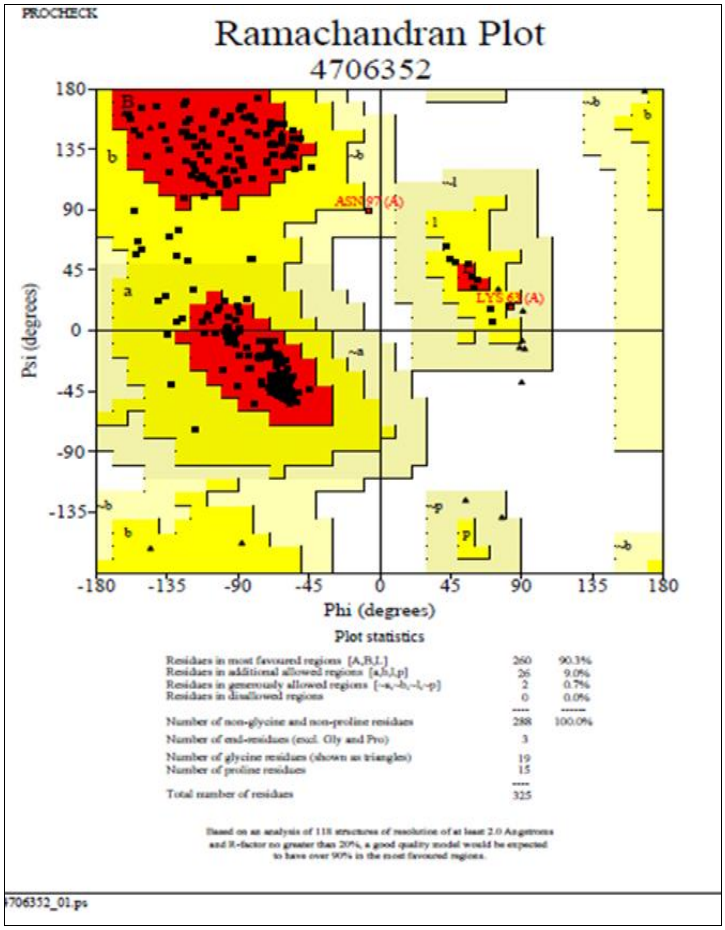

Fig 3: Stereochemical analysis of Hyalouronidase protein the red region declares the most favorable area of residues; the yellow region is additionally allowed; and generously allowed residues in the light-yellow region. RC plot declares 90.03% of residues falling in the allowed region.

4. Conclusion

The Asian hornet *Vespa velutina* venom protein Hyalouronidase (HYases) sequence was selected for present *in-silico* analysis. In the present study, sequence and structural insight of venom protein HYases highlight their basic molecular nature with understanding and composition and their biological properties. The *Vespa velutina* veom protein Hyalouronidase (HYases) sequence was retrieved from UniProtKB protein database, using those sequences their physicochemical properties were analysed from the ProtParam tool its show that the protein is glycosyl hydrolase family. Secondary structural elements were predicted from the SOPMA tool. The 3D model was built using the SWISSMODEL server. The final refined model was evaluated by using the PROCHECK, ERRAT and Z-score. The predicted 3D structure will support to the understanding of the structure of the Hyalouronidase (HYases) in wasp species.

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Conflict of interest

The authors declare that they have no conflict of interest.

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