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HOMOLOGY MODELLING, SECONDARY STRUCTURE PREDICTION AND PHYLOGENETIC COMPARATIVE ANALYSIS OF PHOSPHOLIPASE A2FROM DIFFERENT ORGANISMS

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ABSTRACT: Phospholipase A2 is the enzyme that catalyzes the hydrolysis of the *sn*-2 fatty acyl bond of phospholipids, liberating free fatty acids and lysophospholipids. These enzymes are found in mammalian tissues, insects, reptiles, urochordates, invertebrates and echinoderms. Some of the PLA2 are toxic in nature. Venom from snakes and insects is largely composed of melittin, which is a stimulant of PLA2. In this study, Phylogenetic relationship of Phospholipase A2 were compared, tertiary &secondary structures of PLA2 in different species like *Apismellifera*, *Najaatra*, *Varanusscalaris*, *Asterinapectinifera*, *Mesobuthustumulus*, *Rophilemanomadica* were predicted.

INTRODUCTION: Phospholipase A2 is commonly found in mammalian tissues as well as in insects, reptiles, urochordates, invertebrates and echinoderms. Venom from both snakes and insects is largely composed of melittin, which is a stimulant of PLA2. Due to the increased presence and activity of PLA2 resulting from a snake or insect bite, arachidonic acid is released from the phospholipid membrane disproportionately. As a result, inflammation and pain occur at the site.

The venom of the bee has a very complex mixture of active peptides, enzymes and amines. The Major components of the bee venom are melittin, histamine and phospholipase A2. The effects of bee venom on humoral and cellular immune responses have been tested in different reports.

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Hadjipetrou-Kourounakis *et al* (1984) [1] showed that bee venom treatment significantly reduces the number of plaque-forming cells found in the spleens of rats immunized with sheep red blood cells (SRBC's) as well as the splenocytes responses to T cell mitogens.Both suppression and enhancement of immune reactivity was seen using bee venom when assayed on the splenic lymphocytes [2].Varanid venom was revealedby toxicological analyses to be as complex. Liquid chromatography/mass spectrometry showed that the varanid secretions to be rich in proteins. The varanid PLA2 inhibits the platelet aggregation. Snake venom is highly modified saliva that is produced by special glands of snakes. Snake venom contains many enzymes, proteins and substances with cytotoxic, neurotoxic effects.

More than 20 enzymes are found in snake venom and 12 enzymes are common to all venom. Enzyme levels of Viperid and Crotalid venom fall in the range 80 to95% of the dry matter, whereas for

Elapid venoms the range lies between 25 to 70%. The enzyme content of hydrophid venoms is at the lower end of the Elapid range. All snake venom consists of: L-Amino acid oxidase, Phosphodiesterase, Phospho monoestrase, Deoxyribonuclease, Ribonuclease, Adenosine triphosphatase, Hyaluronidase, NAD-Nucleosidase, and Peptidase. In addition to this Crotalid and Viperid venoms consists of Endopeptidase, Arginine ester hydrolase, Kinninogenase, Thrombin like enzyme, factor X activator prothrombin activator, and Elapid venom includes Acetylcholinesterase, Phospholipase B, Phospholipase A2, Glycerophosphatase.

Phospholipase A2 of the starfish *Asterinapectinifera* has the molecular weight of 20,000 Daltons. These phospholipase A2 are present in pyloric ceca [3]. Starfish phospholipase A2 hydrolyze phosphatidylcholinemore effectively than phosphatidylethanolamine. Scorpion venom contains phospholipase A2 which is toxic in nature. These are cardiotoxin, which affect the cardiovascular and pulmonary system.

Rhopilema nomadic is a Nemotocyst, belongs to the family of Cnidaria. The function of the cnidarians phospholipase A2 (PLA2) may include roles in the capture and digestion of prey and defense of the animal [4]. In this study, the sequences of PLA2 from different species were obtained and using Clustal W, multiple sequence alignment was done. The structures for these PLA2 sequences were predicted using the Modellar software and verified by Ramachandran plot. The evolutionary analysis of PLA2 for these species was done by using the Phylogenetic Tree Prediction by Gee Bee service.

MATERIALS AND METHODS:

Retrieval of sequences

The Phospholipase A2 sequences from different species were obtained from Uniprot, a protein database (http://www.uniprot.org/). The accession id's are as follows: Q91133, *Najaatra*; Q6T178, *Mesobuthus tumulus*; B7UUK1, *Apismellifera*; E2E4K9, *Varanusscalaris*; Q9U8P9, *Asterinapectinifera*; P43318, *Rophilemanomadic*. The protein sequences from these species were retrieved in the FASTA format. To minimize the confusion all the sequences obtained in this study are referred to by their GenBank accession numbers (http://blast.ncbi.nlm.nih.gov/Blast.cgi)

Sequence Analysis

The retrieved sequences were aligned using the multiple sequence alignmenttool, ClustalW.It performed the pairwise alignments of all the sequences and by using this alignment score it produced a Phylogenetic tree.Clustal exploits the fact that the similar sequences are likely to be evolutionary related. InclustalW, similar sequences are aligned first and gaps are inserted into those sequences that are more distant.

Phylogenetic Analysis

The Phylogenetic analyses were done for PLA2 of these organisms. These analyses were done by the online Phylogenetic analysis tool, Phylogenetic Tree Prediction by Gee Bee service. This online tool will do the multiple sequence alignment for the given PLA2 sequence.

3D Structure Prediction

Protein structure prediction was done for the selected organism as mentioned above. Comparative modeling was done by Modeller version 9.10. The template sequences were taken by searching through the Blast database. The best match was identified using the score value. The obtained 3D structures from the Modeller version 9.10 were verified in Structure Analysis and Verification Server (SAVS). The quality of the protein structure was checked by analyzing residue-by-residue geometry and overall structure geometry.

Secondary Structure Analysis

The alpha helices and the beta strands of the 3D structures of the PLA2 were predicted by using the online secondary structure prediction method called SOPMA (http:// npsa – pbil . ibcp .fr / cgi - bin/npsa_automat.pl?page=npsa_sopma.html).

RESULTS:

The toxic phospholipase A2 from different organism were studied and the structures for these PLA2 protein were predicted using the *in silico* methods. Information describing the venom-toxin sequences was obtained by using the primary sequence database, Uniprot. Uniprot is a database which contains various protein sequences from

different organisms[5]. The toxic phospholipaseA2 sequences from different organisms were obtained using uniprot. The protein sequences were converted into the FASTA format, as these format are widely used by most of the tools. The obtained protein sequences were aligned using Clustal W. In clustal W pairwise alignment was done and using the alignment score it will produce a Phylogenetic tree. The multiple sequence alignment result obtained by Clustal W is seen in **Fig. 1**.

```
NAJA            MTPAHLLILAAVCVSPLGASSSRPMPLNLYQFKNMIQCTVPS--RSWWDFADYGCYCGRG 58
Asterina        ---------------------------MSVYQFGKFISCYGGAGFFDGLDYNGYGCYCGYG 34
Mesobuthus      --------------MHTPKHAIRRMSKGEMEFFEGRCQRMG----EAKRTMWGTKWCGSG 42
Rhopilema       ------------------------------------G----LIKP---GTLWCGMG 13
Apis            --------------MQVVLGSLFLLLLSTSHGWQIR-DRIGDNELEERIIYPGTLWCGHG 45
Varanus         ------------------------------------G------CPLAGRQAWEPKE 14
                                                                   :

NAJA            GSGTP------VDDLDRCCQVHDHCYNEAEK--------------ISGC---WPYSKTYS 95
Asterina        GQGTP------LDDTDRCCLVHDNCYGKATA--------------EADCGSWDPYIIVYD 74
Mesobuthus      NEAINYTDLGYFSNLDSCCRTHDHC-DSIPAGETKYGLTNEGKYTMMNCKCESAFEKCLR 101
Rhopilema       NNAETYDQLGPFADVDSCK---------------------------------------- 32
Apis            NKSSGPNELGRFKHTDACCRTHDMCPDVMSAGESKHGLTNTASHTRLSCDCDDKFYDCLK 105
Varanus         IAVVKNLDMEEQHEREKAPRAAPLPEPGTET---------------------------- 45
                          . : .

NAJA            YECSQGT-----LTCK--------GGNNACAAAVCDCDRLAAICFAG-APYNNNN-YNID 140
Asterina        YEQTTDASGNCVIKCKKAADYSWYSTNPECREFMCECDRAGAQCFAEKRPTYNQA-YESY 133
Mesobuthus      DVRGILEGKAAAAVRKTYFDLYGNGCFNVKCPSGARSARSE-ECTNGMATYTGETGYGAW 160
Rhopilema       ------------------------------------------------------------
Apis            NSADTIS---SYFVGKMYFNLIDTKCYKLEHPVTGCGERTEGRCLHYTVDKSKPKVYQWF 162
Varanus         ------------------------NKDRKPRKRKLQGPEQPGVPWQGGRPGSRRKLLL-- 79


NAJA            LKARCQ- 146
Asterina        DKDSC-- 138
Mesobuthus      AINKLNG 167
Rhopilema       -------
Apis            DLRKY-- 167
Varanus         -------
```

FIG: 1. SEQUENCE ALIGNMENT OF *NAJAATRA* (Q91133); *MESOBUTHUS TUMULUS* (Q6T178); *APIS MELLIFERA* (B7UUK1); *VARANUSSCALARIS* (E2E4K9); *ASTERINAPECTINIFERA* (Q9U8P9); *ROPHILEMA NOMADIC* (P43318).

In the Clustal W results, the non-polar amino acids (hydrophobic) are marked in Red, the electrically charged amino acids (negative & hydrophilic) are marked in Blue, and Polar amino acids are marked in Green. The result shows that there are some conserved residues of tryptophan, aspartic acid and cysteine in the sequences. This alignment shows that there are some similarities in the PLA2 sequence of these species and it shows that these sequences may likely to be evolutionary related.

The Phylogenetic analysis of the toxic phospholipase A2 from different organisms were carried out using the online tool called Phylogenetic Tree Prediction by Gee Bee service. This Phylogenetic analysis shows how the family of toxic phospholipa seA2 sequences might have been derived during the evolution. The branching of the tree reflects the degree to which different sequences are related. The results of the Phylogenetic analysis were shown below in **Fig. 2**.

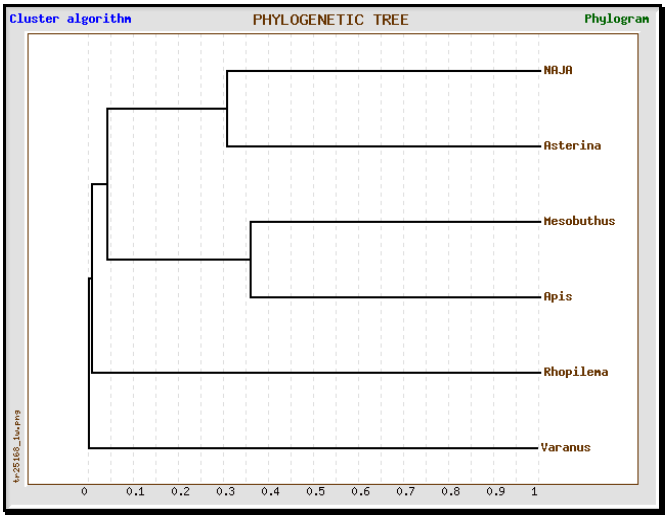

FIG. 1: PHYLOGENETIC TREE BY CLUSTER ALGORITHM.

The Evolutionary tree derived from the alignment is shown in **Fig. 2**, the cluster algorithm tree shows that the PLA2 of *Najaatra* and *Asterinapectinifera* are the most recent and the closest neighbors' are found in the cluster. The clusters correlates with

either structure or geographical location have been emphasized in **Fig.2.**The next closest neighbors'are *Mesobuthus tumulus* and *Apismellifera* whichshares a common ancestor.

This shows that the scorpion venom PLA2 is much closely related to the Bee's PLA2. The PLA2 of *Rophilemanomadic* is found on an entirely separate branch of the tree. Further-more the PLA2 of *Varanusscalaris* closely related to the PLA2 of *Rophilemanomadic*, this shows that the PLA2 of *Rophilemanomadic* and *Varanusscalaris* may be similar in function. We may predict that due to evolution the toxic content of the phospholipase A2 changes. The distance matrixes of all the organisms are show in **Table 1.**

TABLE 1: DISTANCE MATRIX

		1	2	3	4	5	6
1	*Naja*	0.000	0.698	0.957	1.000	1.000	1.000
2	*Asterina*	0.698	0.000	0.965	1.000	0.939	1.000
3	*Mesobuthus*	0.957	0.965	0.000	1.000	0.647	1.000
4	*Rhopilema*	1.000	1.000	1.000	0.000	1.000	1.000
5	*Apis*	1.000	0.939	0.647	1.000	0.000	1.000
6	*Varanus*	1.000	1.000	1.000	1.000	1.000	0.000

The analysis of 3D structures of toxic PLA2 provides a complementary approach to site directed mutagenesis for identification of functional residues in the PLA2. For the structure prediction, the unknown is compared with the known structures. The modeling of the 3D structure of protein was done by Modeller version 9.10. The template sequences were obtained from the BLAST, and the structures are obtained from the Protein Data Bank (PDB) (http:// www. rcsb. org/ pdb/home/home. do). By using the Modeller software, 3D structures of the toxic PLA2 proteins from different organisms were detected.

These structures were validated using Structure Analysis and Verification Server (SAVS). The PROCHECK method reveals that the predicted structure of *Najaatra* **(Fig.3)** has over 90.4% of the amino acids are in the most favoured regions, and 8.8% are seen in additional allowed regions and 0.8% of amino acids are seen in the generously allowed region of the Ramachandran plot **Fig. 4**.

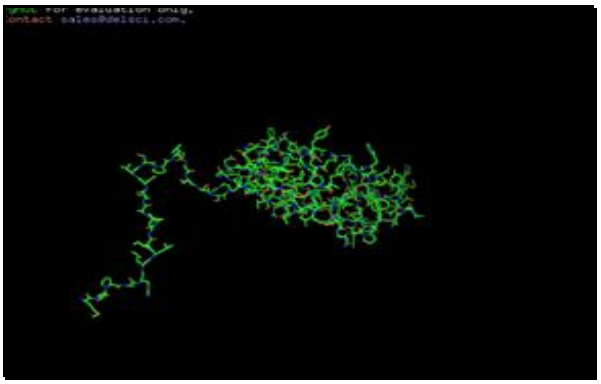

FIG. 3: PREDICTED PDB STRUCTURE OF *NAJAATRA*

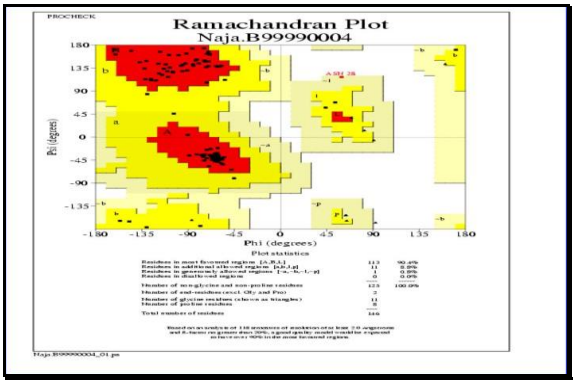

FIG. 4: RAMACHANDRAN PLOT OF *NAJAATRA*

The results of the predicted structure of *Asterinapectinifera* **Fig. 5** shows that it has over 85.5% of amino acid residues are seen in the most favoured regions, 12.8% of amino acids are seen in the additional allowed regions, and 1.7% of residues are seen in the disallowed regions **Fig. 6**.of the Ramachandran plot.

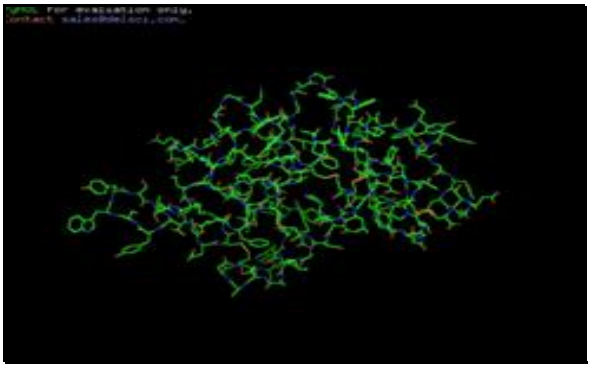

FIG. 5: PREDICTED STRUCTURE OF *ASTERINAPECTINIFERA*

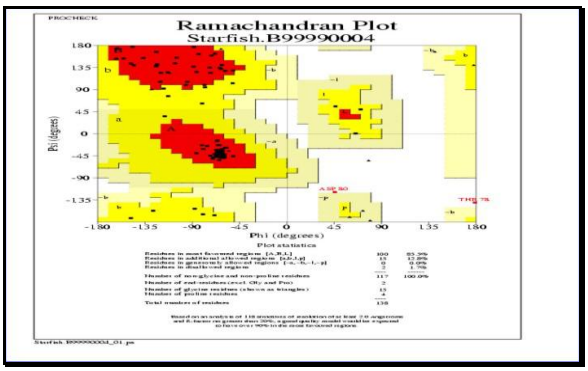

FIG. 6: RAMACHANDRAN PLOT OF *ASTERINAPECTINIFERA*

The *Mesobuthus tumulus* phospholipase A2 structure **Fig. 7** shows over 86% of amino acids residue are present in the most favoured regions, 12.6% of residues are seen in the additional allowed regions, 0.7% are seen in generously allowed region, and 0.7% of residues are seen in the disallowed regions of the Ramachandran plot **Fig. 8**.

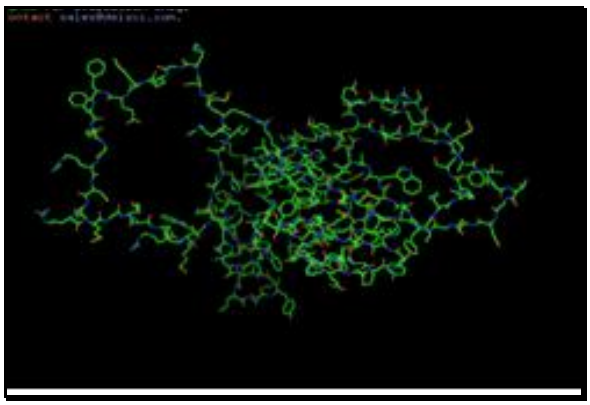

FIG. 7: PREDICTED STRUCTURE OF *MESOBUTHUS TUMULUS*

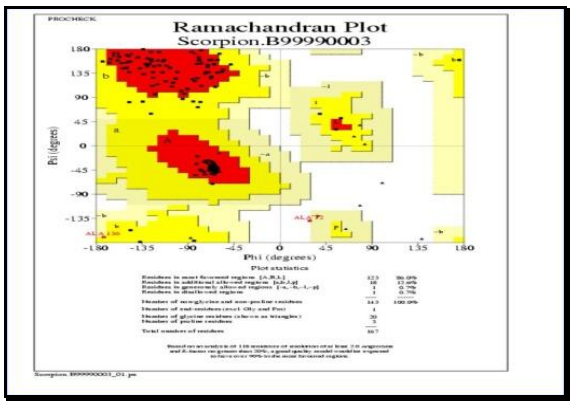

FIG. 8: RAMACHANDRAN PLOT OF *MESOBUTHUS TUMULUS*

The structure analysis **Fig. 9**.of PLA2 from *Apismellifera* shows that it has over 91.8% of amino acid residues present in the most favoured regions,8.2% of residues are seen in the additional allowed regions of the Ramachandran plot **Fig.10**.

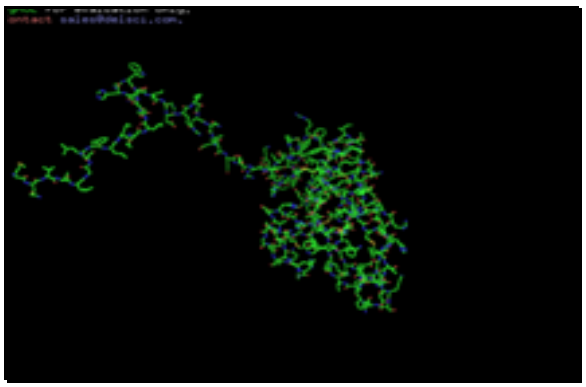

FIG. 9: PREDICTED STRUCTURE OF *APISMELLIFERA*

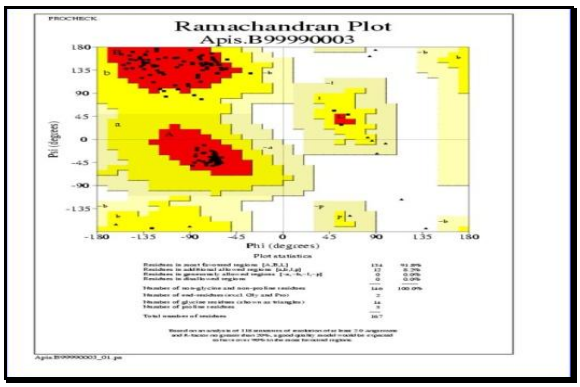

FIG. 10: RAMACHANDRAN PLOT OF *APISMELLIFERA*

The structure analysis result of PLA2 from *Rophilemanomadic* **Fig. 11**. shows that it has over 87.5% of residues in the most favoured regions, 12.5% residues in the additional allowed regions of the Ramachandran plot **Fig. 12**.

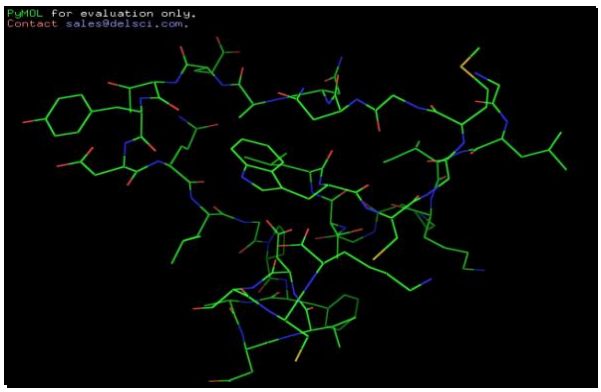

FIG. 11: PREDICTED STRUCTURE OF *ROPHILEMA NOMADIC*

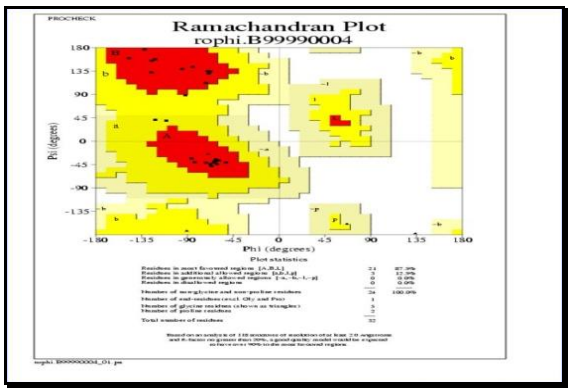

FIG. 12: RAMACHANDRAN PLOT OF *ROPHILEMA NOMADIC*

The structural analysis of the predicted phospholipaseA2 structure of *Varanusscalaris* Fig. 13shows that it has over 86.4% of residues in the most favoured region, 10.2% residues in the additional allowed regions, 1.7% residues in the generously allowed region, and 1.7% residues in the disallowed region of the Ramachandran plot **Fig. 14**.

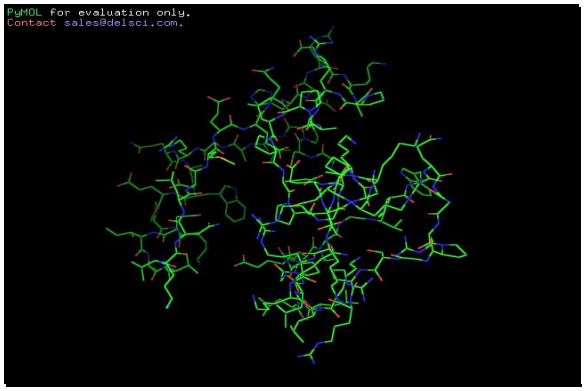

FIG. 13: PREDICTED STRUCTURE OF *VARANUSSCALARIS*

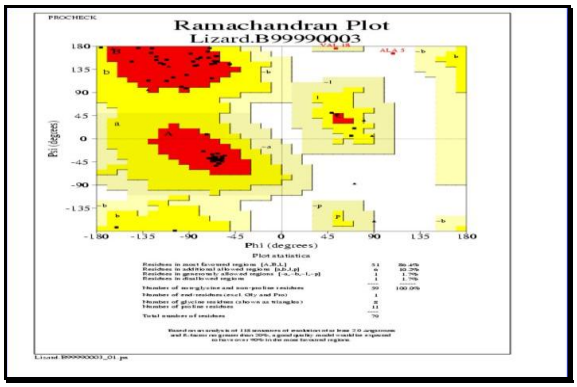

FIG. 14: RAMACHANDRAN PLOT OF *VARANUSSCALARIS*

The secondary structure prediction of phospholipase A2 from different organisms was carried out by SOPMA (online tool) [6]. Secondary protein structure prediction of *Najaatra*as follows,

```
10         20         30         40         50         60         70
|          |          |          |          |          |          |
MTPAHLLILAAVCVSPLGASSSRPMPLNLYQFKNMIQCTVPSRSWWDFADYGCYCGRGGSGTPVDDLDRC
ccchhhhhhhhheehhhccccccccccchhhhhhhhhhccttccceeectteeeeecccccccccchhhhh
CQVHDHCYNEAEKISGCWPYSKTYSYECSQGTLTCKGGNNACAAAVCDCDRLAAICFAGAPYNNNNYNID
hhhhhhhhhhhhhcttccccceeeeeecccccceeecccccchheeeehtchhhheeeecccccttcccee
LKARCQ
ecccccc
```

Here, the ‘h’ represents the helices, ‘e’ represents the extended beta sheets, ‘c’ represents the coils. The PLA2 sequence length of *Najaatra* is 146 AA. The phospholipase A2 structure of *Najaatra* has 30.82% (45 residues) of alpha helix, 21.23% (31 residues) of Beta sheets, 41.78% of coils & 6.16% of turns.

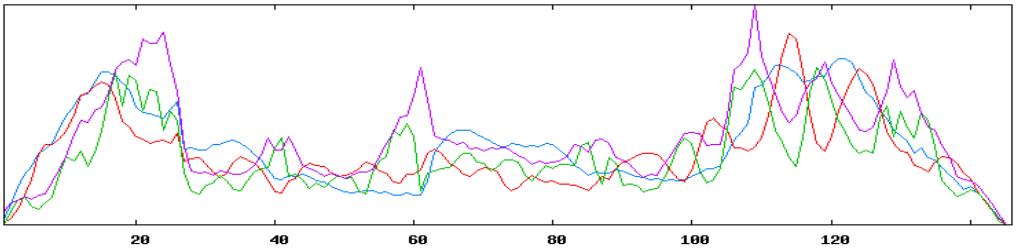

FIG. 15: SECONDARY PROTEIN STRUCTURE PREDICTION OF *NAJAATRA*

Secondary protein prediction structure of *Asterinapectinifera* is as follows,

```
10         20         30         40         50         60         70
|          |          |          |          |          |          |
MSVYQFGKFISCYGGAGFFDGLDYNGYGCYCGYGGQGTPLDDTDRCCLVHDNCYGKATAEADCGSWDPYI
hhhhhhhhheeeeccttecccceeeettcceeecccccccccccchhhhhccchhhhhhhhhccccccccee
IVYDYEQTTDASGNCVIKCKKAADYSWYSTNPECREFMCECDRAGAQCFAEKRPTYNQAYESYDKDSC
eeeeeccccccttceeeeeecccccceeeecccccchheehhtchhhheehhccccccchhhhhhcttcc
```

The PLA2 sequence length of *Asterinapectinifera*is 138 AA. The phospholipase A2 structure of *Asterinapectinifera* has 27.54% (38 residues) of alpha helix, 23.91% of (33 residues) of beta sheets, 39.13% of (54 residues) coils, and 9.42% of (13 residues) turns.

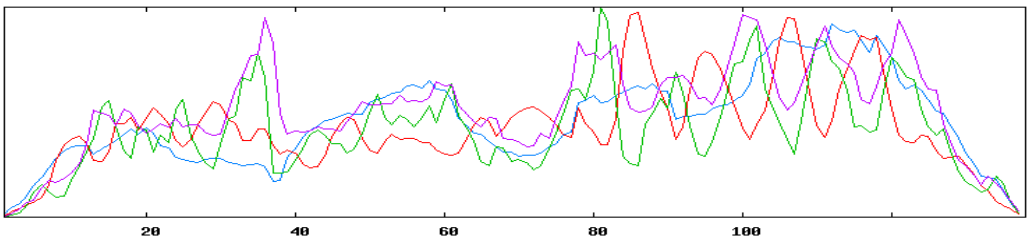

FIG. 16: SECONDARY PROTEIN STRUCTURE PREDICTION OF *ASTERINAPECTINIFERA*

Secondary protein prediction structure of *Mesobuthus tumulus* is as follows,

```
10        20        30        40        50        60        70
|         |         |         |         |         |         |
MHTPKHAIRRMSKGEMEFFEGRCQRMGEAKRTMWGTKWCGSGNEAINYTDLGYFSNLDSCCRTHDHCDSI
ccccccchhhccchhhhhhhhhhhcccccccccceeecccccccccccccccccccchhhhcccccccccc
PAGETKYGLTNEGKYTMMNCKCESAFERCLRDVRGILEGKAAAAVRRTYFDLYGNGCFNVKCPSGARSAR
chtcccceccccceeeeeccccchhhhhhhhhhhccchhhtcchhhhhhhhhhhhhtttceeeecccccccc
SEECTNGMATYTGETGYGAWAINKLNG
ccccchhheettttttcceeeeehhtt
```

The PLA2 sequence length of *Mesobuthus tumulus*is 167 AA. The phospholipase A2 structure of *Mesobuthus tumulus* has 28.74% of (48 residues) alpha helix, 13.77% of (23 residues) beta sheets, 47.90% of (80 residues) coils, and 9.58% of (16 residues) turns.

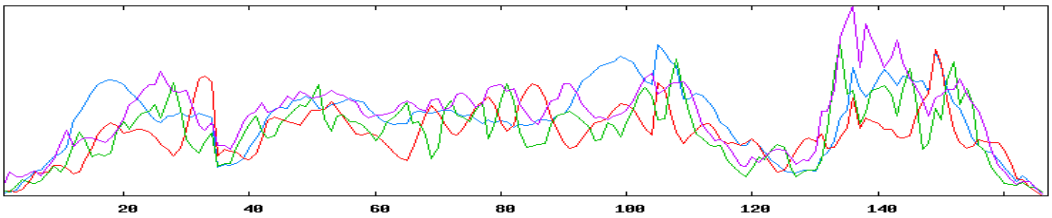

FIG. 17: SECONDARY PROTEIN STRUCTURE PREDICTION OF *MESOBUTHUS TUMULUS*

Secondary protein prediction structure of *Apismellifera* is as follows,

```
10        20        30        40        50        60        70
|         |         |         |         |         |         |
MQVVLGSLFLLLLSTSHGWQIRDRIGDNELEERIIYPGTLWCGHGNKSSGPNELGRFKHTDACCRTHDMC
hhhhhhhhheeeecccccceeechhccccccceeectteeeccccccccchhhtcccchhhhhccccccc
PDVMSAGESKHGLTNTASHTRLSCDCDDKFYDCLKNSADTISSYFVGKMYFNLIDTKCYKLEHPVTGCGE
cchhhhtcccttccccccceeecccccchhhhhhhhhhhhhhhhhhhhhhheeeehhccccccetcccccccc
RTEGRCLHYTVDKSKPKVYQWFDLRKY
Ccccchheeeecccccceeeeeccccc
```

The PLA2 sequence length of *Apismellifera* is 167 AA. The phospholipase A2 structure of *Apismellifera* has 28.41% of (48 residues) of alpha helix, 17.37% of (29 residues) beta sheets, 49.10% of (82 residues) coils, and5.39% of (9 residues) turns.

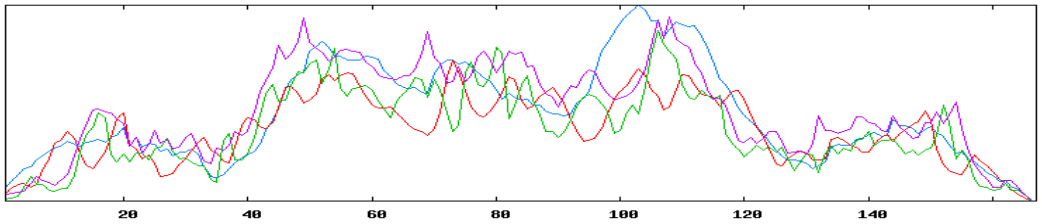

FIG. 18: SECONDARY PROTEIN STRUCTURE PREDICTION OF *APISMELLIFERA*

Secondary protein prediction structure of *Rophilemanomadic* is as follows,

```
10        20        30
|         |         |
GLIKPGTLWCGMGNNAETYDQLGPFADVDSCK
Ceecttceeccccccccchhhhccccchhhhhh
```

The PLA2 sequence length of *Rophilemanomadic* is 32 AA. The phospholipase A2 structure of *Rophilemanomadic* has 28.13% of (9 residues) alpha helix, 12.50% of (4 residues) beta sheet, 53.13% of (17 residues) coils, and 6.25% of (5 residues) turns.

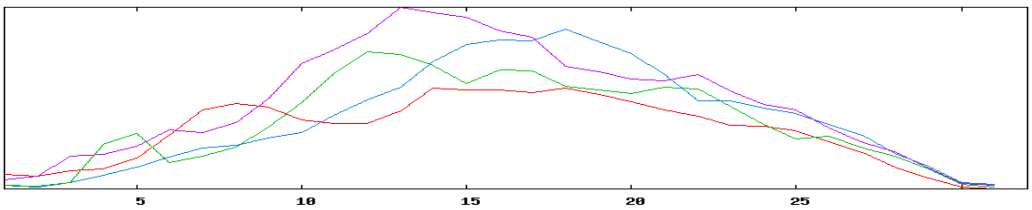

FIG. 19: SECONDARY PROTEIN STRUCTURE PREDICTION OF *ROPHILEMA NOMADIC*

Secondary protein prediction structure of *Varanusscalaris* is as follows,

```
10        20        30        40        50        60        70
|         |         |         |         |         |         |
GCPLAGRQAWEPKEIAVVKNLDMEEQHEREKAPRAAPLPEPGTETNKDRKPRKRKLQGPEQPGVPWQGGR
tccccccccctthhhhhhhhhhhhccccccccccccccccccccccccccccccccccccccccccccc
PGSRRKLLL
ccccceeee
```

The PLA2 sequence length of *Varanusscalaris* is 79 AA.The phospholipase A2 structure of *Varanusscalaris* has 13.92% of (11 residues) alpha helix, 10.13% of (8 residues) of beta sheet, 72.15% of (57 residues) coils, and 3.80% of (3residues) turns.

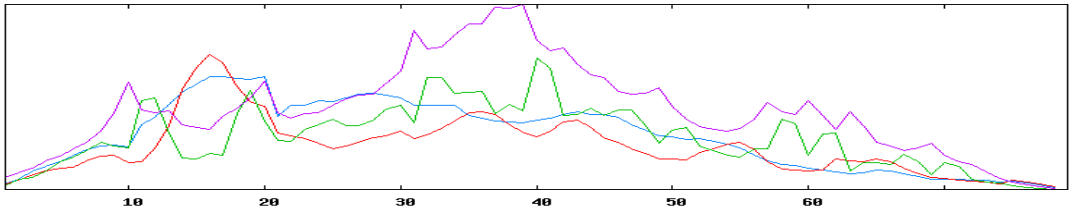

FIG. 20: SECONDARY PROTEIN STRUCTURE PREDICTION OF *VARANUSSCALARIS*

DISCUSSIONS: Phospholipase A2 is the enzyme which helps to cleave the phospholipid of the glycerol backbone [7]. These enzymes induce wide range of pharmacological activity including neurotoxic, cardiotoxic, myotoxic, anticoagulant and hemorrhagic effects [8]. PLA2 are probably the most thoroughly investigated toxins both in hemotoxic and presynaptic neurotoxic snake venom [9]. PLA2 has also been classified as a presynapticneurotoxin identified in the venom of snakes, scorpion etc.[10]. Venoms are the rich source of large number of PLA2 isoenzymes. Kini (2006) [11] which have pharmacological effects *in -vivo*[12]. Bee Venom, snake venom, scorpion venom are rich in toxic Pla2 content. Both the bee venom and the snake venom will block the pre-synaptic membrane[13].

Toxic phospholipase A2 not only seen in reptiles but also in invertebrates. Toxic phospholipase A2 are also seen in the classes of Anthozoa, Hydrozoa, Scyphozoa and Cubozoa of the phylum Cnidaria. The functions of Cnidaria include the digestion of the prey. High PLA2 have been reported in hard corals, fire corals, crown-of-thorns starfish, sea cucumber and marine sponges [14, 15].

Amino acids sequences of toxic phospholipase A2 were selected from 6 different organisms like Q91133, *Najaatra*; Q6T178, *Mesobuthus tumulus*; B7UUK1, *Apismellifera*; E2E4K9, *Varanusscalaris*; Q9U8P9, *Asterinapectinifera*; P43318, *Rophilemanomadic* from Uniprot, Protein database. To minimize the confusion their accession numbers were given next to the organism name. Multiple sequence alignment were done for these sequences by the clustal W.

The result shows that there are some conserved residues of tryptophan, aspartic acid and cysteine in the sequences. Conserved residues of Asp are seen in *Najaatra, Mesobuthus tumulus, Apismellifera, Asterinapectinifera,* and *Rophilemanomadic* sequences. The Phylogenetic analysis were done for the aligned sequences by using the online Phylogenetic analysis tool called Phylogenetic Tree Prediction by Gee Bee service. The cluster algorithm tree shows that the PLA2 of *Najaatra* and *Asterinapectinifera* are the most recent and the closest neighbors' are found in the cluster.

The next closest neighbors' are *Mesobuthus tumulus* and *Apismellifera*. This shows that the scorpion venom PLA2 is much closely related to the Bee's phospholipase A2. The PLA2 of *Rophilemanomadic* is found on an entirely separate branch of the tree.

The phospholipase A2 of *Varanusscalaris* closely related to the PLA2 of *Rophilemanomadic*, this shows that the PLA2 of *Rophilemanomadic* and *Varanusscalaris* may be similar in function. From these results we may predict that due to evolution the toxic content of the phospholipase A2 in all these organisms changes. As these phospholipase A2 sequence do not have a 3D structure, we predicted the structures of toxic phospholipase A2 in these organism with the help of Modeller version 9.10. The template sequences for these

phospholipase A2 were selected from BLAST. The PDB files of these template sequences were taken. We kept these sequence as the template and started predicted the three dimensional structure of the PLA2 sequence.

The obtained 3 D structure were analyzed in Structure Analysis and Verification Server (SAVS). The validated results shows that the predicted 3D structure of *Najaatra* **(Fig.3)** has over 90.4% of the amino acids are in the most favoured region, *Asterinapectinifera* **(Fig.5)** shows that it has over 85.5% of amino acid residues are seen in the most favoured region, *Mesobuthus tumulus* phospholipase A2 structure **(Fig.7)** shows over 86% of amino acids residue are present in the most favoured region, The structure analysis **(Fig.9)** of PLA2 from *Apismellifera* shows that it has over 91.8% of amino acid residues present in the most favoured region, the structure analysis result of PLA2 from *Rophilema nomadic* **(Fig.11)** shows that it has over 87.5% of residues in the most favoured region, and the structural analysis of the predicted phospholipaseA2 structure of *Varanusscalaris* **(Fig.13)** shows that it has over 86.4% of residues in the most favoured region. After predicting the three dimensional structures of the phospholipase A2, the secondary structure prediction of protein were done in SOPMA, an online tool.

In the secondary structure prediction the alpha helices, beta sheets, turns and coils of the predicted structures were found. This study reveals that the evolution is the major factor that plays in these organisms. An individual organism's phenotype results from both its genotype and the influence from the environment it has lived in. By this study we may predict the toxic phospholipase A2 in different organism were more or less related to one another. So, by this conclusion we may determine the organisms that are listed in this study may have originated from the same ancestor. Due to the changes in the amino acids sequences and protein differentiation, the toxicity of the phospholipase A2 changes.

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