In SILICO Molecular Docking of Kappa-Carageenan Against D7 Salivary Gland Protein of Adult Female *Anopheles Stephesnsi*

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ABSTRACT

The purpose of this study was to analyze the inhibitory action of Kappa carrageenan by computational docking simulation studies. For this, natural metabolite Kappa-carrageenan was used as ligand for molecular interaction. Computational docking analysis was performed using Arguslab and Pymol based on scoring functions. Kappa-carrageenan compound showed high docking potential with the D7 salivary gland protein of female *Anopheles stephensi*. The results clearly show that the molecular docking mechanism used to detect the novel antirepellant for mosquito has been successfully obtained from a natural sulfated polysaccharide compound.

Key words: *D7 salivary gland protein, Anopheles stephensi, Kappa-carrageenan, Molecular docking.*

INTRODUCTION

Carrageenan is a common name for the family of gel-forming and viscous polysaccharides, derived from some species of red seaweed [1]. Carrageenan is derived from a number of seaweeds of the class Rhodophyceae. This unique seaweed is common in the Atlantic Ocean near Britain, Europe and North America. When used in food products, carrageenan contains the EU additive E-number E407 or E407a. The E407a has a slightly different composition; moreover, it contains considerable cellulose. Carrageenan has no nutritional value and is used in food preparation for its gelling, hardening and emulsifying properties and experimental medicine this substance is often used for the testing of anti-inflammatory, antitumor, antimicrobial, antiviral (HSV) and antithrombin agents [2-6]. Keeping the above medicinal properties in mind we are the first pioneer to identify the *in silico* molecular docking

potential of Kappa-carrageenan compound against D7 salivary gland protein of *Anopheles stephensi*.

MATERIALS AND METHODS

Preparation of protein structure

SwissProt (URL: http://expasy.org/sprot/)

The UniProt/Swissprot Knowledgebase (UniProtKB) (Fig. 1) is the central access point for extensive curated protein information, including function, classification, and cross-reference. It consists of two sections: UniProtKB/Swiss-Prot which is manually annotated and is reviewed and UniProtKB/TrEMBL which is automatically annotated and is not reviewed. The UniProt Reference Clusters (UniRef) databases provide clustered sets of sequences from the UniProtKB and selected UniProt Archive records to obtain complete coverage of sequence space at several resolutions while hiding redundant sequences.

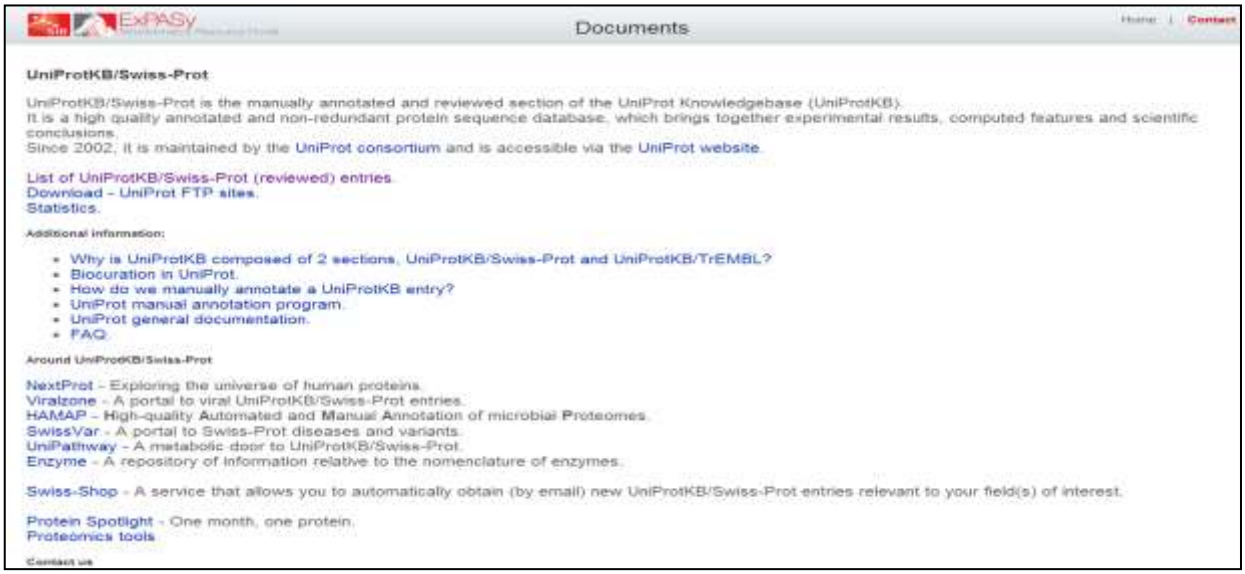

Fig. 1: SwissProt home page

Protein Data Bank (URL: http://www.rcsb.org/pdb/home/home.do)

The Protein Data Bank is a repository for the 3-D structural data of large biological molecules, such as proteins and nucleic acids. The data, typically obtained by X-ray crystallography or NMR spectroscopy and submitted by biologists and biochemists from around the world, are freely accessible on the Internet via the websites of its member organizations (PDBe, PDBj, and RCSB). The PDB is overseen by an organization called the Worldwide Protein Data Bank, wwPDB. The PDB is a key resource in areas of structural biology, such as structural genomics. Most major scientific journals, and some funding agencies, such as the NIH in the USA, now require scientists to submit their structure data to the PDB (Fig. 2).

Fig. 2: PDB homepage

PubMed (http://www.ncbi.nlm.nih.gov/pubmed/)

PubMed is a free database accessing the MEDLINE database of citations, abstracts and some full text articles on life sciences and biomedical topics. The United States National Library of Medicine (NLM) at the National Institutes of Health (NIH) maintains PubMed (Fig. 3,4 and 5) as part of the Entrez information retrieval system.

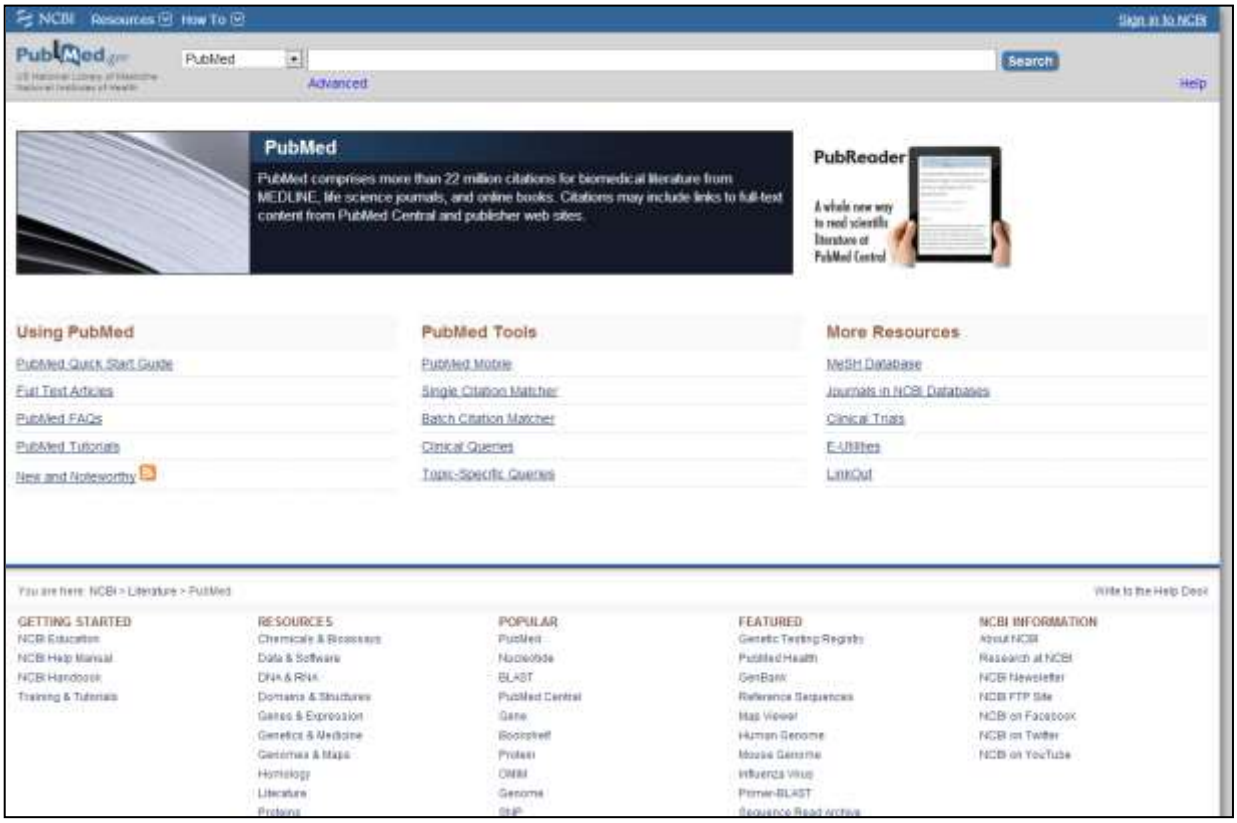

Fig. 3: PubMed home page

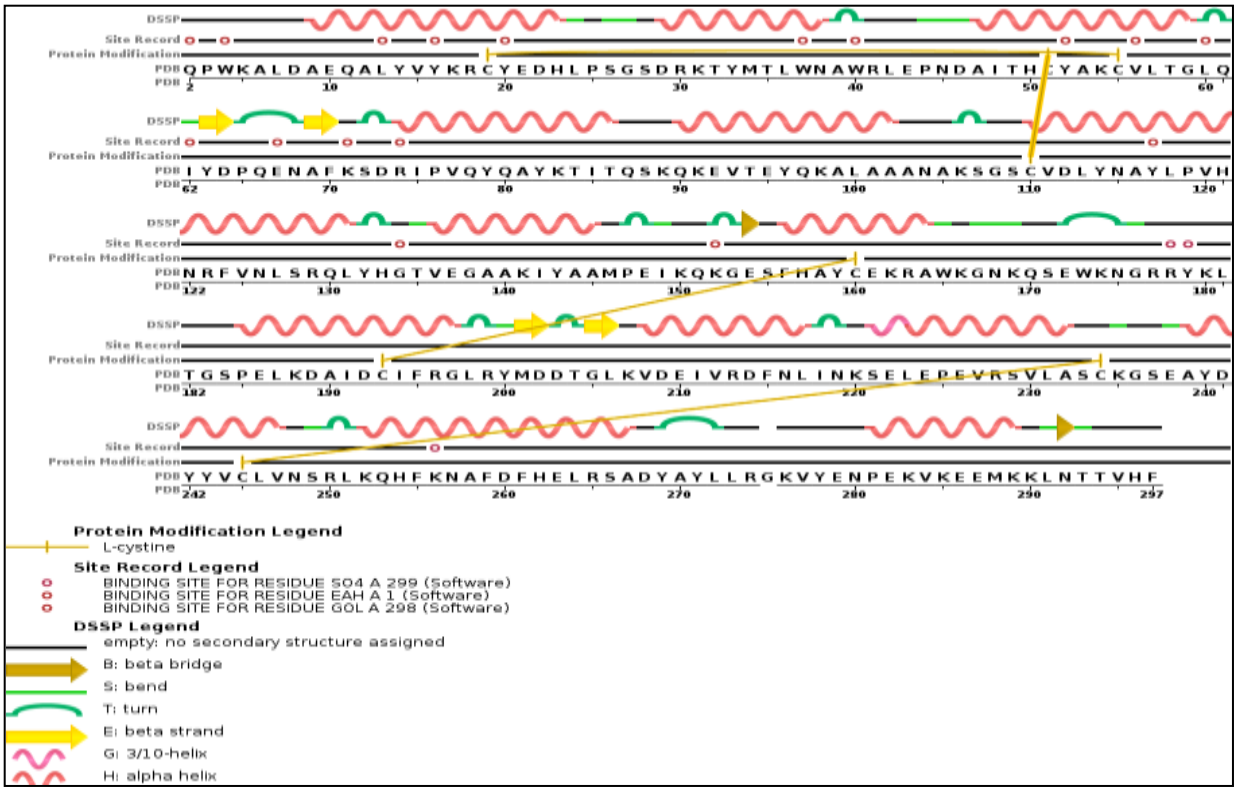

Fig. 4: Retrieve of D7 protein sequence chain view from PDB

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1      QPWKALDAEQ ALYVYKRCYE DHLPSGSDRK TYMTLWNAWR LEPNDAITHC
           HHH HHHHHHHHHH HHS SSTHHH HHHHHHHTT   SSSHHHHH

51     YAKCVLTGLQ IYDPQENAFK SDRIPVQYQA YKTITQSKQK EVTEYQKALA
       HHHHHHHHTT SEETTTTEE  TTHHHHHHHH HHHHH   HH HHHHHHHHHH

101    AANAKSGSCV DLYNAYLPVH NRFVNLSRQL YHGTVEGAAK IYAAMPEIKQ
       H   TT  HH HHHHHHHHHH HHHHHHHHHH TT SHHHHHH HHHH TTS

151    KGESFHAYCE KRAWKGNKQS EWKNGRRYKL TGSPELKDAI DCIFRGLRYM
       TTB HHHHHH HHHS SSS   TTTTS       HHHHHHH HHHHHHTTSE

201    DDTGLKVDEI VRDFNLINKS ELEPEVRSVL ASCKGSEAYD YYVCLVNSRL
       ETTEE HHHH HHHHHHTT G GGHHHHHHHH HT S  SHHH HHHHHHTSTT

251    KQHFKNAFDF HELRSADYAY LLRGKVYENP EKVKEEMKKL NTTVHF
       HHHHHHHHHH HHHHHH TTT T       H HHHHHHHHTS BS
```

Fig.5: Sequence of D7 protein and secondary structure was retrieved from PDB

PubChem Database (URL: http://pubchem.ncbi.nlm.nih.gov/)

The PubChem Substances Database contains descriptions of chemical samples, from a variety of sources, and links to PubMed citations, protein 3D structures, and biological screening results that are available in PubChem BioAssay. If the contents of a chemical sample are known, the description includes links to PubChem Compound (Fig. 6).

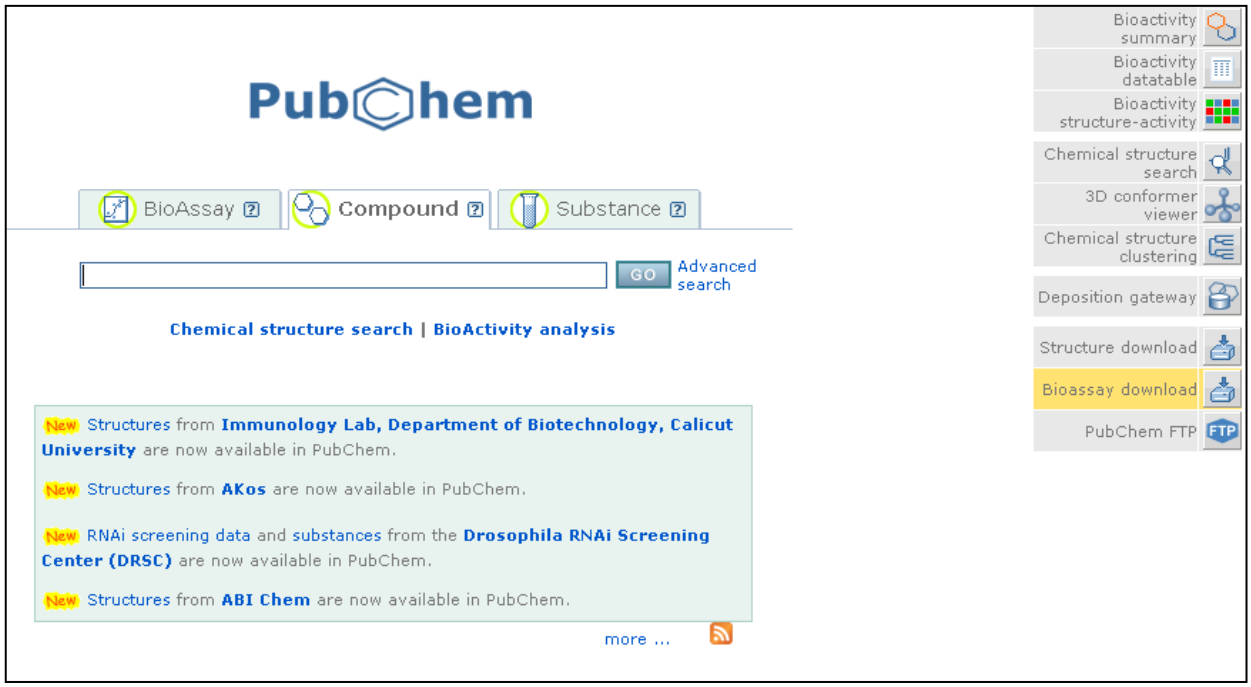

Fig. 6: PubChem home page

RasMol

RasMol (Fig. 7) is a computer program written for molecular graphics visualization intended and used primarily for the depiction and exploration of biological macromolecule structures, such as those found in the Protein Data Bank. It was originally developed by Roger Sayle in the early 90s. It was an important tool for molecular biologists since the extremely optimized program allowed the software to run on (then) modestly powerful personal computers. Before RasMol, visualization software ran on graphics workstations that, due to their expense, were less accessible to scholars. RasMol has become an important educational tool as well as continuing to be an important tool for research in structural biology.

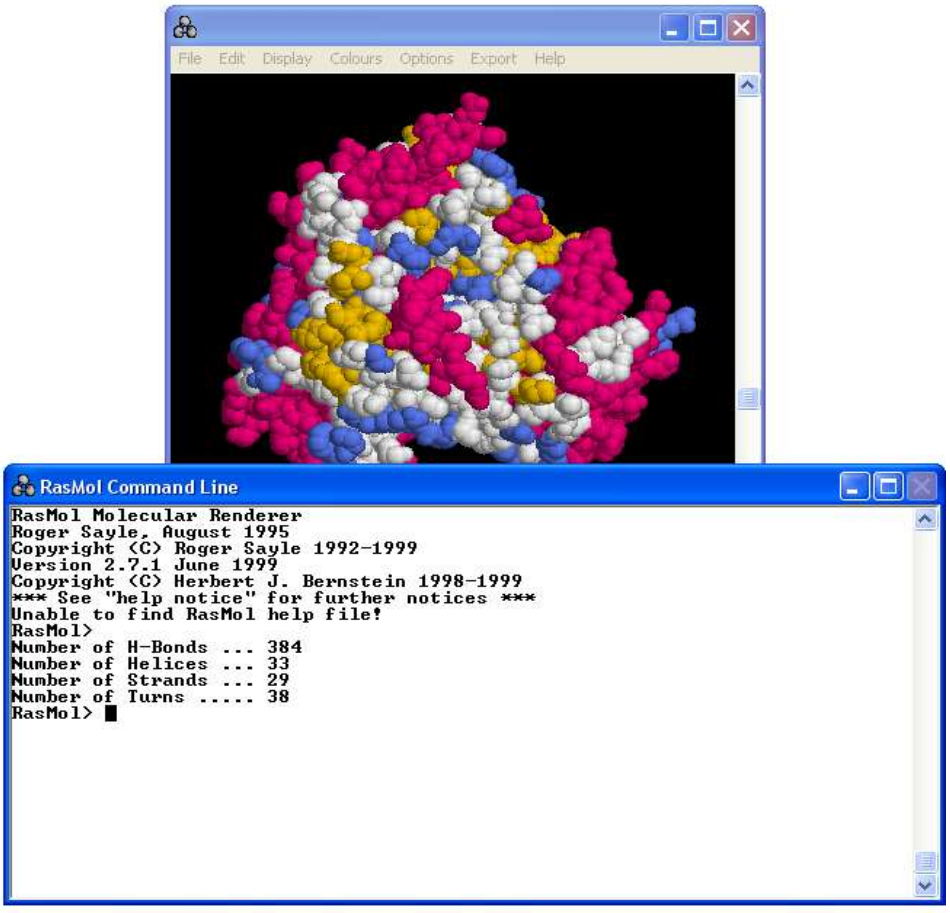

Fig. 7: RasMol tool page

Chem Sketch

ACD/ChemSketch is an advanced chemical drawing tool and is the accepted interface for the industries best NMR and molecular property predictions, nomenclature, and analytical data handling software. ACD/ChemSketch is also available as freeware, with functionalities that are highly competitive with other popular commercial software packages. The freeware contains tools for 2D structure cleaning, 3D optimization and viewing (Fig. 8).

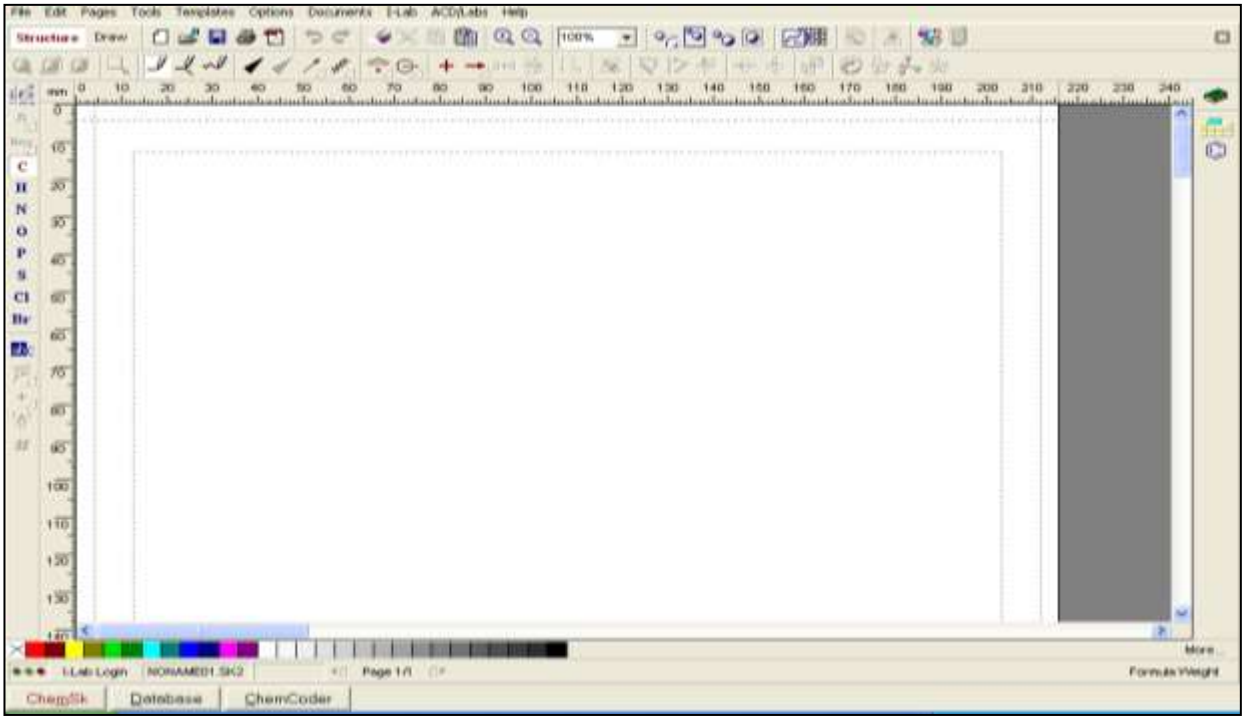

Fig. 8: Chem Sketch work space

Open Babel (http://openbabel.org/wiki/Main_Page)

Open Babel is a chemical toolbox designed to speak the many languages of chemical data. It's an open, collaborative project allowing anyone to search, convert, analyze, or store data from molecular modeling, chemistry, solid-state materials, biochemistry, or related areas as shown in the figure 9.

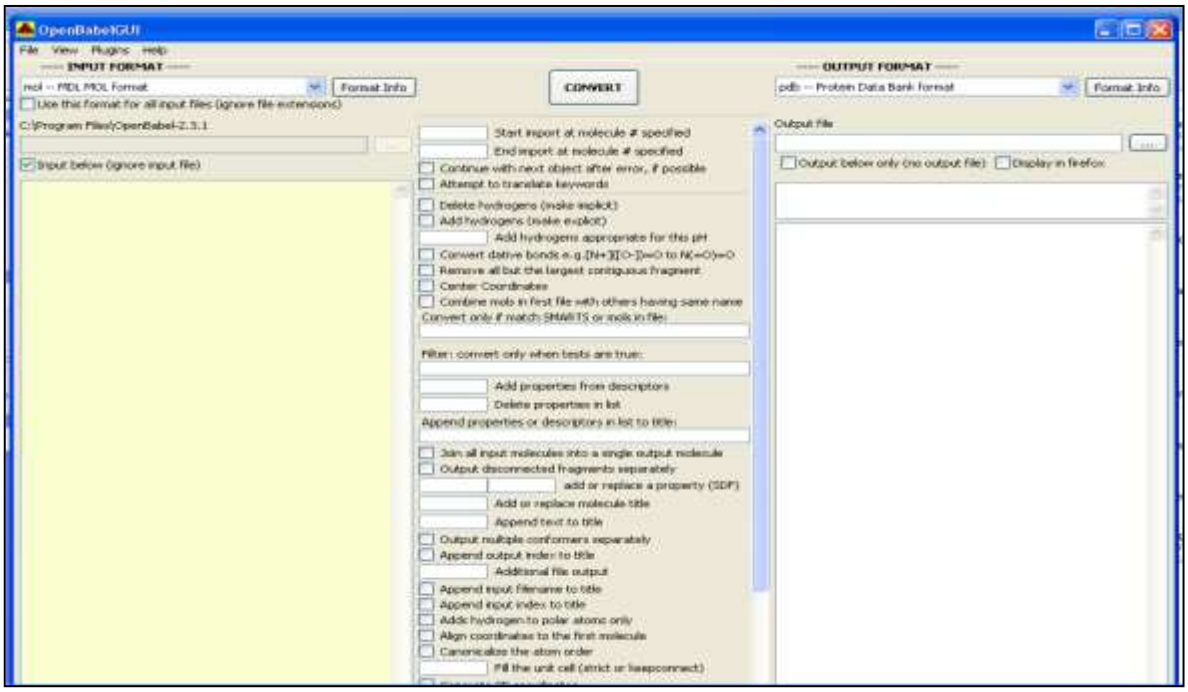

Fig. 9: Open Babel tool page

ArgusLab

ArgusLab is a program to build graphic representations of molecular models. Using this program, you will be able to show molecular models to pupils, or even design matters by combining different elements. You will be able to include in your model several atoms, residues, groups and calculations (Fig. 10).

PyMol (http://download.cnet.com/PyMOL/3000-2054_4-10914845.html)

PyMol is an open-source, user-sponsored, molecular visualization system created by Warren Lyford DeLano and commercialized by DeLano Scientific LLC, which is a private software company dedicated to creating useful tools that become universally accessible to scientific and educational communities (Fig. 11).

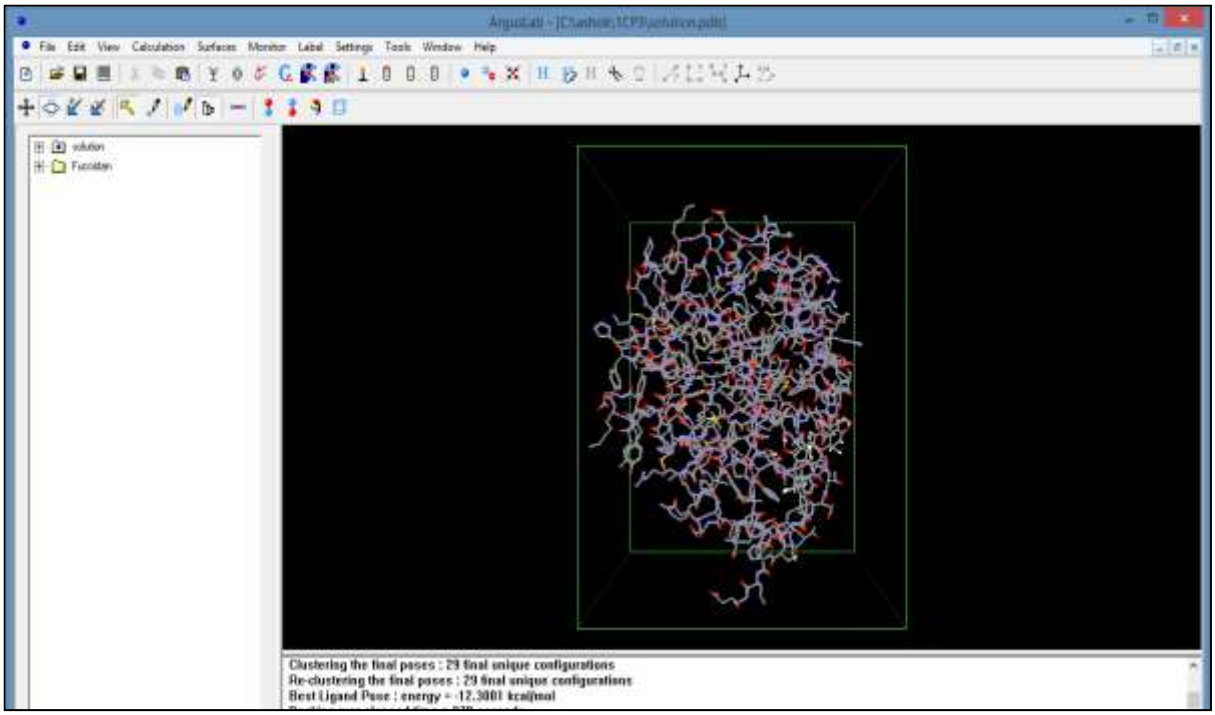

Fig. 10: ArgusLab tool page

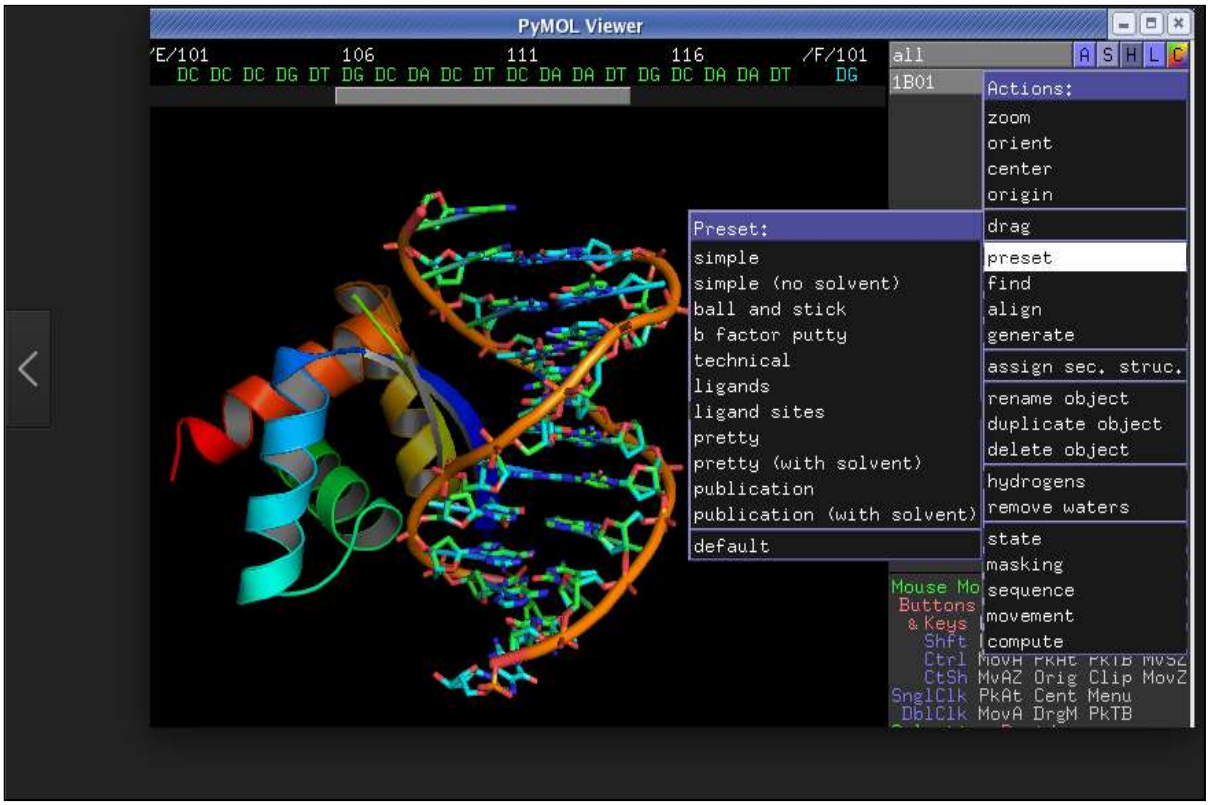

Fig. 11: PyMol tool page

RESULTS AND DISCUSSION

Protein Name: D7 protein

PDB ID: 3NGV

Alternative name(s): Long form D7clu2 salivary protein

Swissprot ID: Q95NY5

Organism: Anopheles stephensi

Fasta Sequence:

```
>tr|Q95NY5|Q95NY5_ANOST D7 protein OS=Anopheles stephensi PE=1 SV=1
MIIVAVLLSFLAHLLVQASQPWKALDAEQALYVYKRCYEDHLPSGSDRKTYMTLWNAWRL
EPNDAITHCYAKCVLTGLQIYDPQENAFKSDRIPVQYQAYKTITQSKQKEVTEYQKALAA
ANAKSGSCVDLYNAYLPVHNRFVNLSRQLYHGTVEGAAKIYAAMPEIKQKGESFHAYCEK
RAWKGNKQSEWKNGRRYKLTGSPELKDAIDCIFRGLRYMDDTGLKVDEIVRDFNLINKSE
LEPEVRSVLASCKGSEAYDYYVCLVNSRLKQHFKNAFDFHELRSADYAYLLRGKVYENPE
KVKEEMKKLNTTVHF
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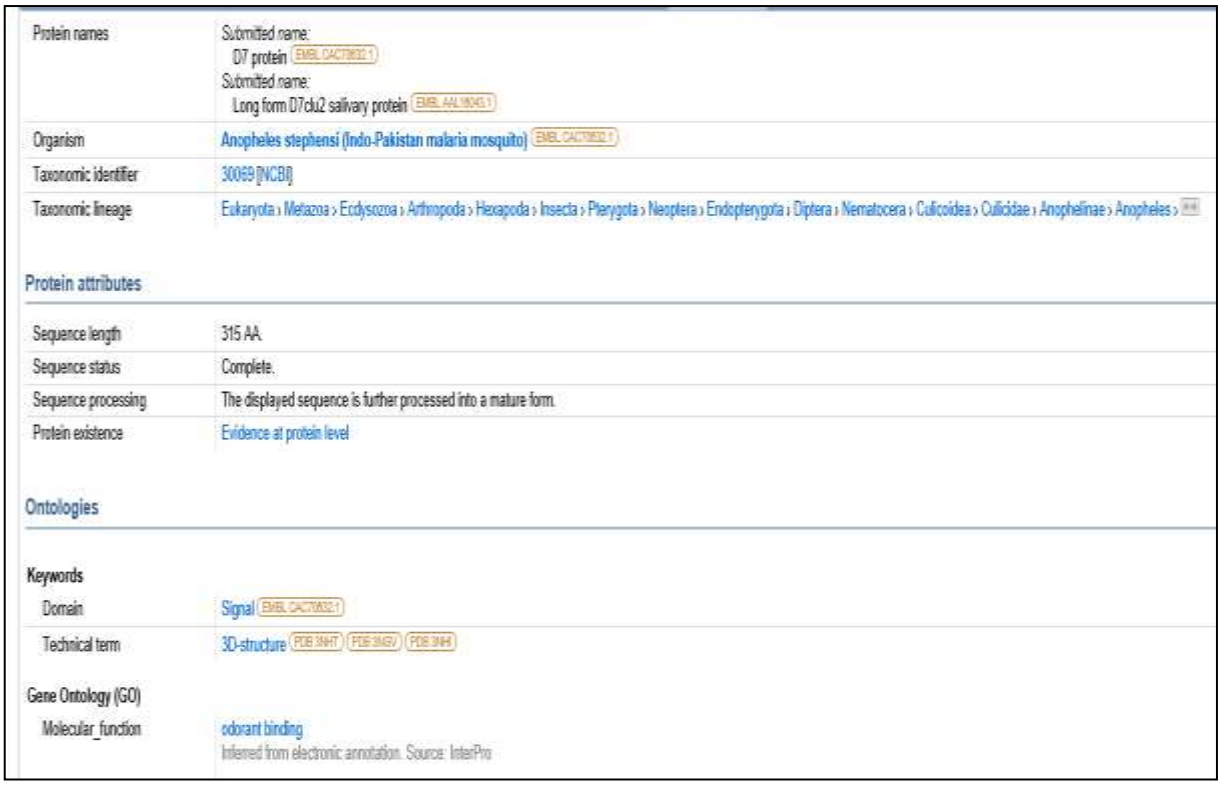

Protein names	Submitted name: D7 protein (EMBL:CAC79002.1) Submitted name: Long form D7clu2 salivary protein (EMBL:AAL19043.1)
Organism	Anopheles stephensi (Indo-Pakistan malaria mosquito) (EMBL:CAC79002.1)
Taxonomic identifier	30069 [NCBI]
Taxonomic lineage	Eukaryota › Metazoa › Ecdysozoa › Arthropoda › Hexapoda › Insecta › Pterygota › Neoptera › Endopterygota › Diptera › Nematocera › Culicoidea › Culicidae › Anophelinae › Anopheles ›

Protein attributes

Sequence length	315 AA.
Sequence status	Complete.
Sequence processing	The displayed sequence is further processed into a mature form.
Protein existence	Evidence at protein level

Ontologies

Keywords	
Domain	Signal (EMBL:CAC79002.1)
Technical term	3D-structure (PDB:3NHT) (PDB:3NGV) (PDB:3NH)
Gene Ontology (GO)	
Molecular_function	odorant binding. Inferred from electronic annotation. Source: InterPro

Fig. 12: SwissProt entry page

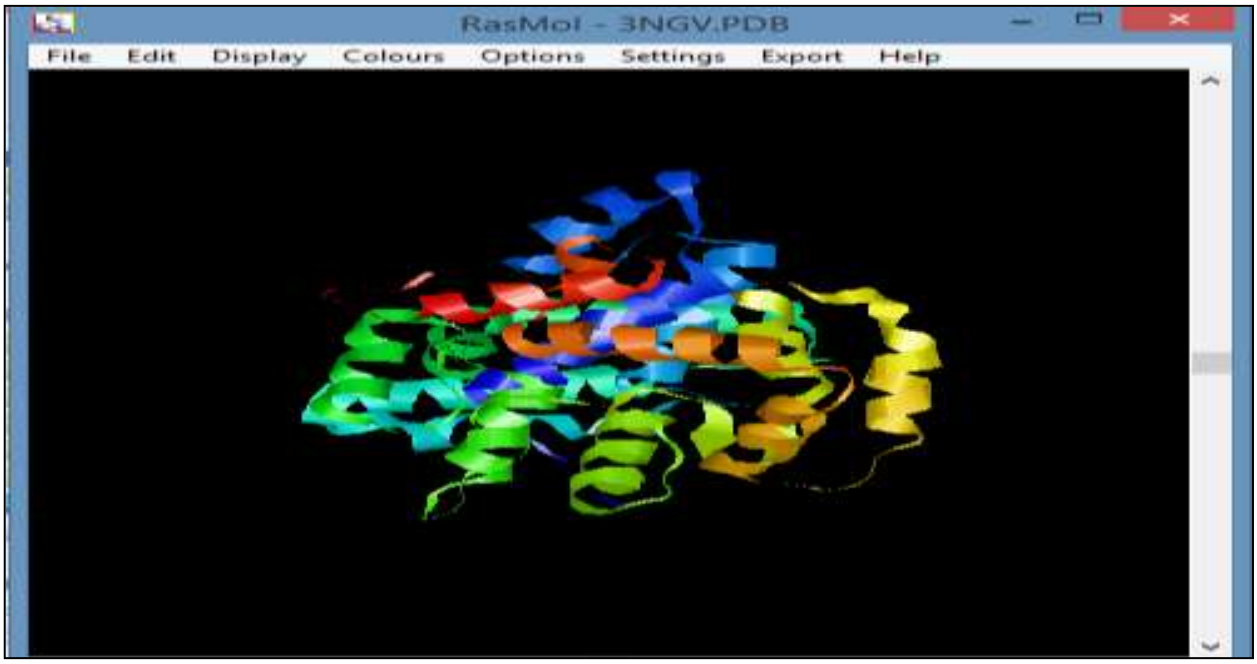

Fig. 13: Visualization of D7 protein using Rasmol tool

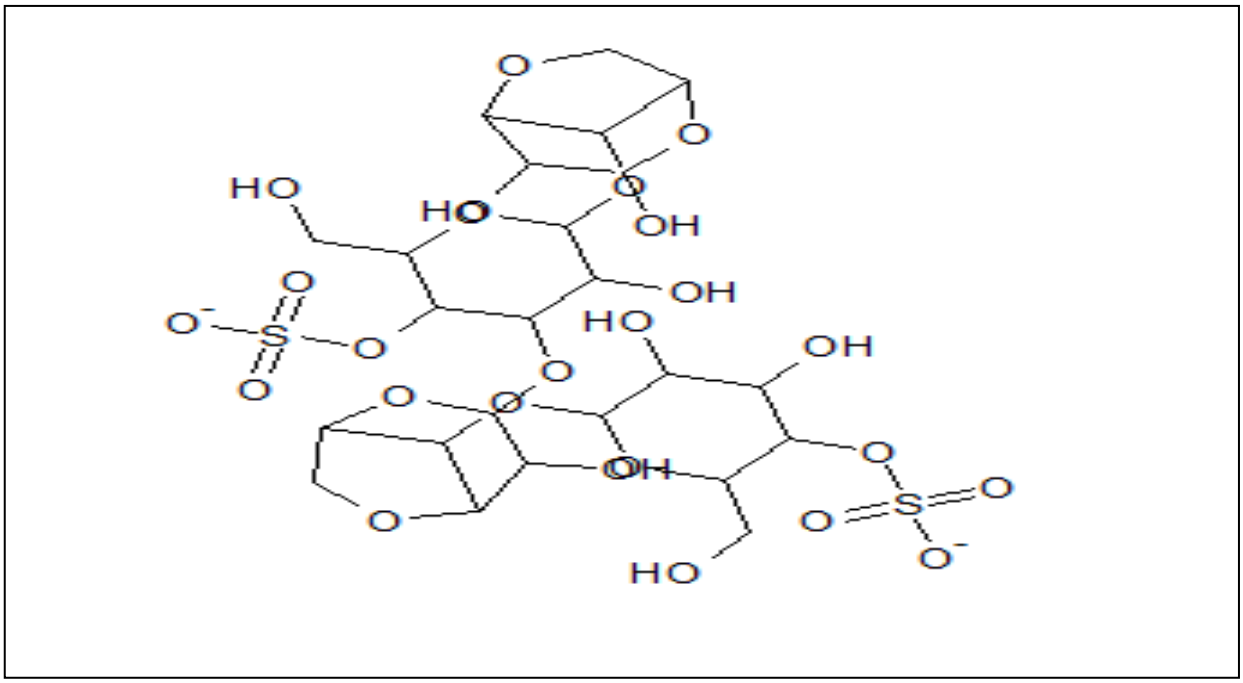

Fig. 14: 2D structure of kappa-carragennan

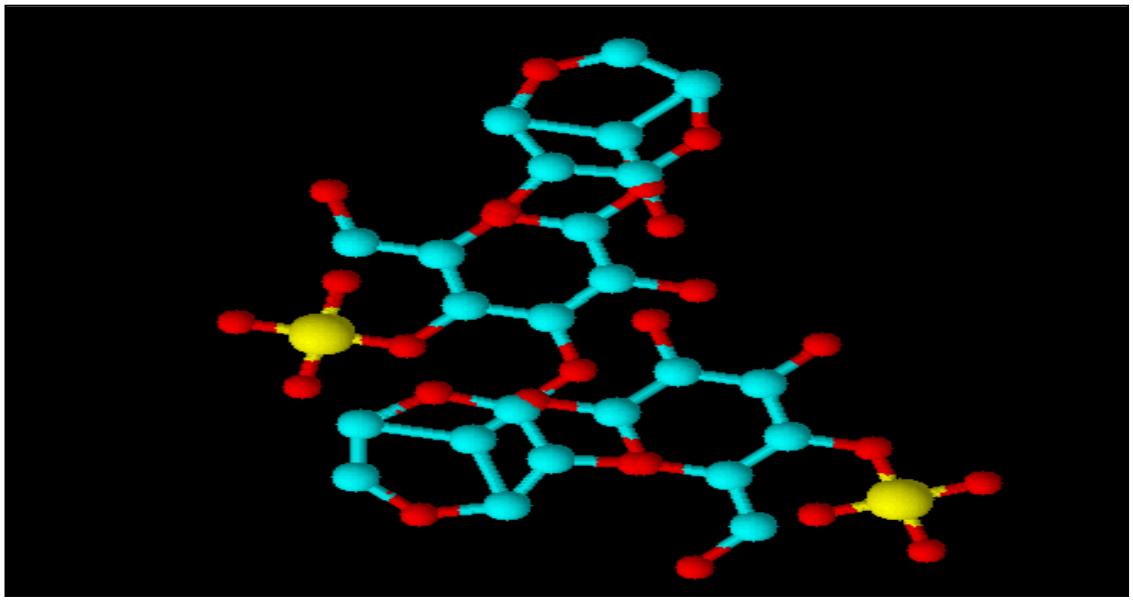

Fig. 15: 3D structure of kappa-carragennan

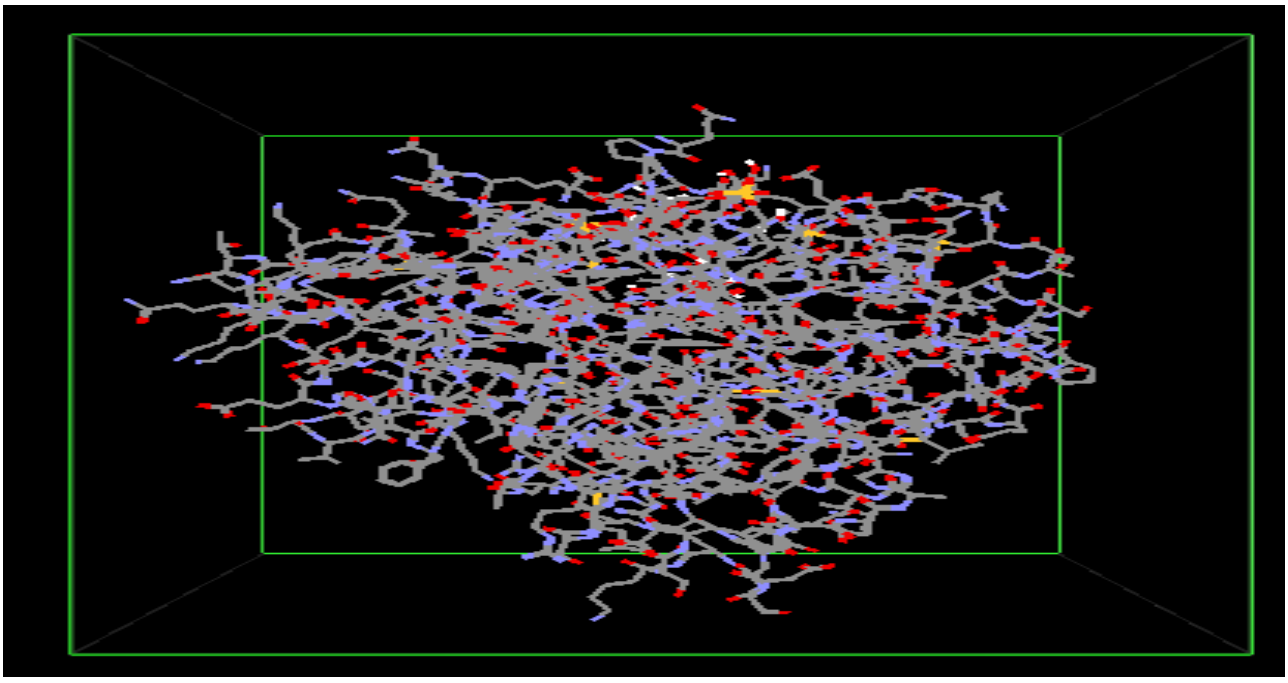

Fig. 16: Grid setting of D7 protein using ArgusLab

Fig. 17: Visualization of docked complex of kappa-carragennan with D7 protein

Table 1: Docked complex of kappa-carragennan with D7 protein

D7 protein	Kappa-carragennan		Docking score KCal/mol	Distance (Å)	H-Bond
ARG 74	NH2	O	-8.67	2.9	10
TRP 4	N	O		3.0	
GLN 2	O	H		2.3	
LYS 5	N	O		3.1	
ALA 6	O	0		3.2	
GLN 61	NE2	0		2.7	
GLN 61	NE2	O		3.0	
GLN 11	NE2	O		3.0	
GLN 11	NE2	O		2.9	
ARG 199	NE	O		2.3	

To study the binding mode of kappa-carragennan interaction with D7 protein, intermolecular flexible docking simulations were performed and. Energy values were calculated from the docked conformations of the protein-inhibitor complexes. Docking studies yielded crucial information concerning the orientation of the inhibitors in the binding pocket of the target protein. Several potential inhibitors have been identified through the docking simulation. The binding affinity of the D7 protein with the kappa-carragennan was measured by kcal/mol. The docking score was found to be -8.67 kcal/mol with the stronger interaction as showed in the table 1 and fig. 17. Analysis of ligand binding interaction with the protein can be useful for the antirepllant activity against *Anopheles stephensi* mosquito. The results obtained from this study would be useful in both understanding the inhibitory mode as well as in rapidly and accurately predicting the activities of larvicidal on the basis of docking scores.

This is the first study to demonstrate the docking simulation of sulphated polysacride (kappa-carragennan) against *Anopheles stephensi* mosquito protein Our results showed that the presence of the hydrobic interaction with 10 hydrogen bond with the interaction of ligand to form a docked complex.. This research supports the novel idelogical use of kappa-carragennan as an alternative insecticide for the control of *Anopheles stephensi* mosquito, which is a vector of severe arboviruses, such as dengue and chikungunya. Similar observations were observed by Diégina *et al.* (2019) [7] by using H. velutina extracts against Aedes aegypti and his docking results prove to valid by producing high docking score of 103.02 kcal/mol against the A. aegypti mosquito protein (1PZ4 protein).

Conclusion

In this study, the molecular docking was applied to explore the binding mechanism and to correlate its docking score with the activity of kappa-carragennan. The results of our present study can be useful for the design and development of novel compound having better inhibitory activity against the control of mosquito prevalence in the world. These potential drug candidates can further be validated in wet lab studies for its proper function.

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