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ANALYSIS OF HYDROPHOBICITY AND ANTIGENICITY OF HEAT SHOCK PROTEIN 70 FROM GWD

Sonu Mishra[1], Virendra S. Gomase[2]

[1,2]Department of Biotechnology, Mewar University, Chittorgarh, (India)

ABSTRACT

Dracunculusmedinensis (a little dragon from Medina) is responsible for Dracunculiasis, popularly known as "guinea worm disease". The Cyclops is the intermediate host that ingests the larvae of parasite (D.medinensis), that later on ingested by the human from the contaminated stagnant unfiltered water from thewater source. After ingestion these Cyclops are disintegrated by stomach digestive juices and causes the release of the larvae. These larvae travel and penetrate the digestive wall into the body cavity and get entry in abdominal cavity and retroperitoneal space. These larvae mature in adults and soon after the copulation the ovoviviparous female mature and grows in size up to 3m whereas the male dies. After an incubation period of a year these mature female worm comes towards the skin and start formation of a small round bulge on the skin by secreting an irritating chemical which causes severe pain. In order to study the antigenicity and the hydrophobicity of the protein we have taken Heat shock protein 70 (mitochondrion) proteins from D.medinensis, consist of 647aa sequence.The Identification is carried out through B- cell epitopes prediction methods .The results obtained shows that the region of maximal hydrophilicity is likely to be antigenic site having the hydrophobic characteristics and contain the segments of low complexity and high-predicted flexibility. This predicted antigenic protein from D. medinensis promises to play an important role in the further treatment of this disease and target validation in the drug development process.

Keywords*: *Dracunculusmedinensis, Dracunculiasis, Epitope, Antigen, Protein, Heat Shock Protein 70 (Mitochondrion).

I. INTRODUCTION

D.medinesis (a little dragon from Medina) is the only species from all the *Dracunculus 12* species[1-4] which infects humans, commonly known as "Guinea worm disease (GWD)". The other *Dracunculus* species generally resides in the internal tissues and body cavities of non-human mammals and reptiles (snake and turtles) [5].This little dragon undergo a very unusual life cycle of six developmental stages with incubation period last for 1 to one an half years approximately. This is one of the most neglected tropicalparasites which bears clinical importance and needs to be eradicated after small pox [6].After reaching to the maturation stage, these worms copulate and an adult female produces millions of eggs in its uterus whereas mail dies.Later on, the female worm release the larvae which induces a painful blister (1 to 6cm diameter) on the skin of lower limbs (predominantly localized in the lower extremities(80-90%) in most of the reported cases).The infected person

develops slight fever , localskinredness , swelling andsevere pruritus around the blister . Other symptoms include:diarrhea, nausea, vomiting and dizziness. The blister burst within three days and female worms one or more slowly comes out from the wounds which causes an excoriating burning sensation and pain [7].Immersing or pouring water over the blister provides pain reliever. But this the moment that adult female is exposed to the external environment [8]. Duringemergence of the limbs in open water sources it recognizes the temperature difference and releases the milky white liquid in the water which contains millions of immature larvae, when larvae released in water are ingested by copepods where they mount twice and become infective larvae within two weeks [9].The *D.medinensis* antigen peptides can be most desirable segment for the subunit vaccine development because with the single epitope, the immune response can be generated in large population. This approach is usually based on the phenomenon of cross-protection, whereby infected with the mild strain and is protected against a more severe strain of the same. The resistant transgenic host's phenotype includes of fewer centers of initial infection, following a delay development in symptom with low accumulation. In this study, Heat shock protein 70 has been used to investigate its role in antigenicityand hydrophobicity.Heat shock proteins (HSP) belong to the protein family where cell produces the response with respect to the exposure to stressful conditions such as cold [10], UV light [11] and during wound healing or tissue remodeling [12].The few groups of the HSP protein also function as the chaperone by stabilizing the new proteins in order to ensure correct folding or by assisting in refolding of those protein which undergone any damaged caused due to cell stress [13]. The virtual abundance of the HSPs can be observed in all living organisms starting from bacteria to humans.The naming of the HSP-70 protein is according to its molecular weight. Hsp 70s (70-kDa) proteins provide cooperation in wide range of folding processes that includes of protein folding, newly synthesized proteinsassembly,misfolded protein refolding and controlling of the activity of regulatory proteins [14-20]. This protein also performs the housekeeping functions in the cell in which they are built-in components of folding and signal transduction pathways, and in quality control functions. This functional activity of this protein isbased on the attribute of Hsp70 to interact with hydrophobic peptide segments of proteins in an ATP-controlled fashion. HSP 70 comprised of the two distinct functional regions: a peptide binding domain (PBD) and the amino-terminal ATPase domain (ABD). Peptide binding domain groove has the affinity for neutral, hydrophobic amino acid residues. Whereas, higher in alpha helical structure which acts as a 'lid' for the substrate binding domain found at the C-terminal /amino terminal domain–. The scenario, where HSP70 protein is ATP bound, the lid is open and peptides bind and release relatively rapidly whereas, when HSP70 proteins are ADP bound, the lid is closed, and peptides are tightly bound to the substrate binding domain. The overexpression of the HSP70 protein has been observed in malignant melanoma [21] and underexpression in renal cell cancer [22]. The presence of extracellular HSPs act as potent path of sending a "danger signal" to the immune system to generate response in order to get rid of an infection or disease.Antigen protein prediction from *D. medinensis* is necessary for paradigms of synthetic vaccine development and target validation.

II. METHODOLOGY

B-cell epitopes are the sites of molecules that are recognized by antibodies of the immune system. Knowledge of B-cell epitopes may be used in the design of vaccines and diagnostics tests. It is therefore of interest to develop improved methods for predicting B-cell epitopes[23].In this investigation we have used methods for

predicting continuous antibody epitope from Heat shock 70 protein sequences with the consideration of the parameters such as hydrophilicity, flexibility, accessibility, turns, exposed surface, polarity and antigenic propensity of polypeptides chains, hydrophobicity and have been correlated with the location of continuous epitopes. The methods used for the determination of the antigenic epitopes of the antigen protein using the Gomase in 2007,Welling,Eisenberg, Parker ,Rao& Argos, Malanvalan, Bepipred Linear Epitope Prediction, Emini Surface Accessibility Prediction, Karplus & Schulz Flexibility Prediction, Kolaskar & Tongaonkar Antigenicity, Parker Hydrophilicity Prediction[24-29].

III. RESULT AND INTERPRETATIONS

The Heat shock 70protein sequence (647 aaprotein) is analyzed through different types B- cell epitope prediction methods and the hydrophobicty, antigenicity of the protein is determined using different hydrophobic scale and antigenicity scale.In the B cell antibody epitopes prediction method such as Bepipred Linear Epitope Prediction, the highest peak with highest residue score is observed between 625-630position(Fig.1), inChou & Fasman Beta-Turn Prediction the peak was between 625-630(Fig.2), Emini Surface Accessibility Prediction-the score of the amino acid residue is between position 245-250(Fig.3), Karplus & Schulz Flexibility Prediction: between 245-250(Fig.4),whereas in Kolaskar & Tongaonkar Antigenicity thehighest score is between 140-150position (Fig.5) and in Parker Hydrophilicity Prediction between 525-535(Fig.6). All prediction calculations are based on propensity scales for each of the 20 amino acids. Each scale consists of 20 values assigned to each of the amino acid residues on the basis of their relative propensity to possess the property depicted by the scale. On the graphs[Figs. 1-6], the Y-axes depicts for each residue the correspondent score whereas X-axes depicts the residue positions in the sequence. The tables [Tables. 1-2] provide values of calculated highest scores for each residue. The larger score for the residues might be interpreted as that the residue might have a higher probability to be part of epitope (those residues are colored in yellow on the graphs[Figs. 1-6]). Considering all the above output of the result we can predict that the residue higher peak with higher score is between the positions 625-630. This in turns indicates that there might be probability of residue to be a part of the epitope. We have also found that the region of maximal Hydrophilicity is likely to be an antigenic site, having hydrophobic characteristics, because the terminal regions of antigen protein is solvent accessible and unstructured, antibodies against those regions are also likely to recognize the native protein[Fig. 7-8]. It was shown that an antigen protein is hydrophobic in nature and contains segments of low complexity and high-predicted flexibility [Figs. 9-11]. The predicted antigenic protein segments of Heat shock protein 70 can take active part in the host immune reactions. In future study the predicted antigenic protein Heat shock protein 70 fragments can be used in the investigation of MHC molecules binding and it can be the first bottlenecks in vaccine design.

IV. FIGURES AND TABLES

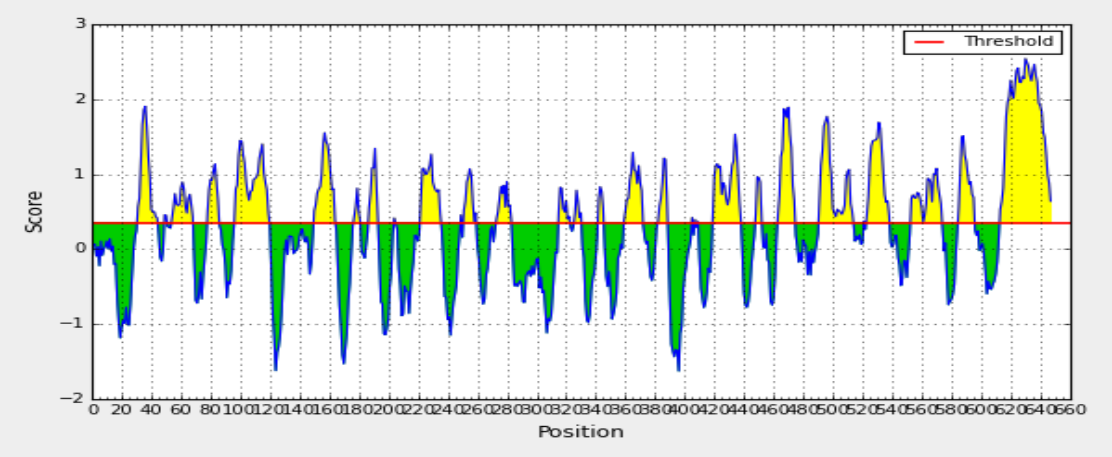

Fig. 1.Bepipred Linear Epitope Prediction Graph

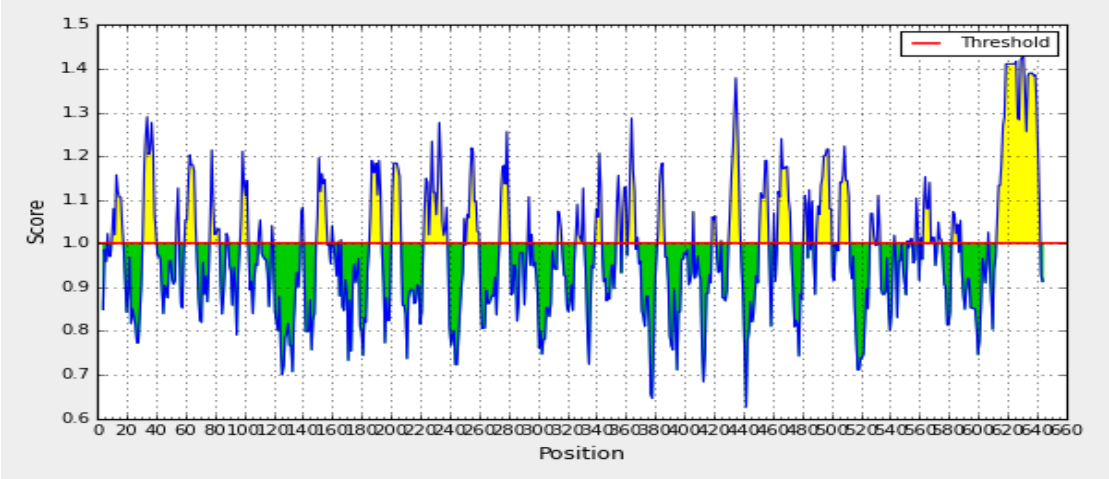

Fig.2. Chou & Fasman Beta-Turn Prediction Graph

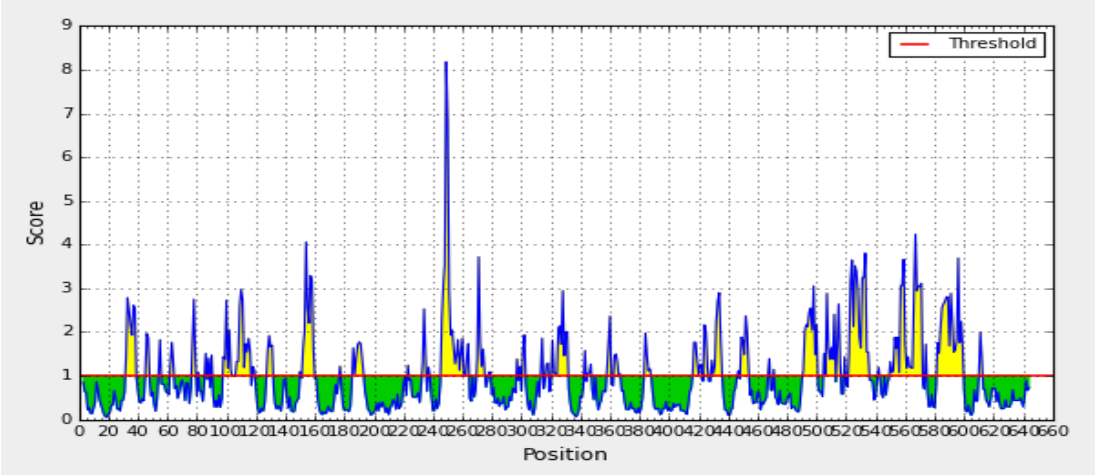

Fig.3. Emini Surface Accessibility Prediction Graph

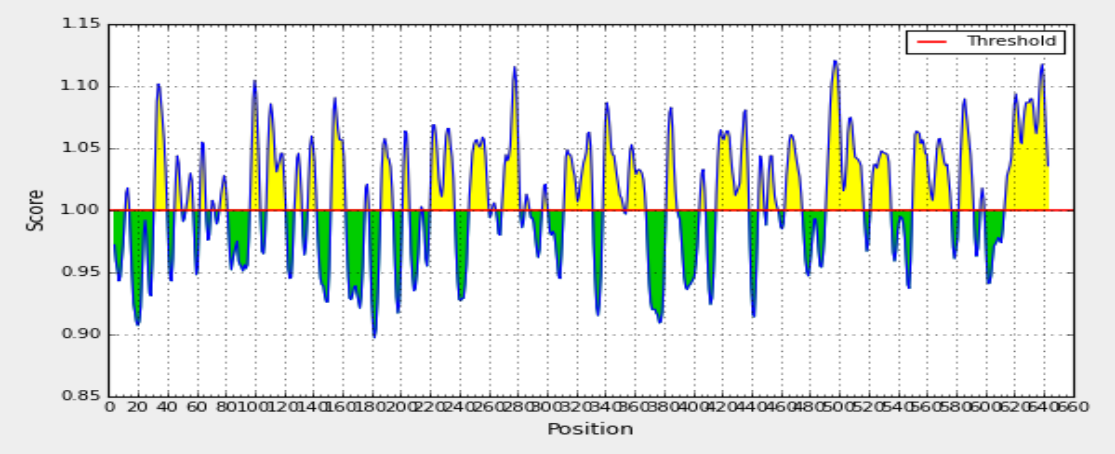

Fig.4. Karplus & Schulz Flexibility Prediction Graph

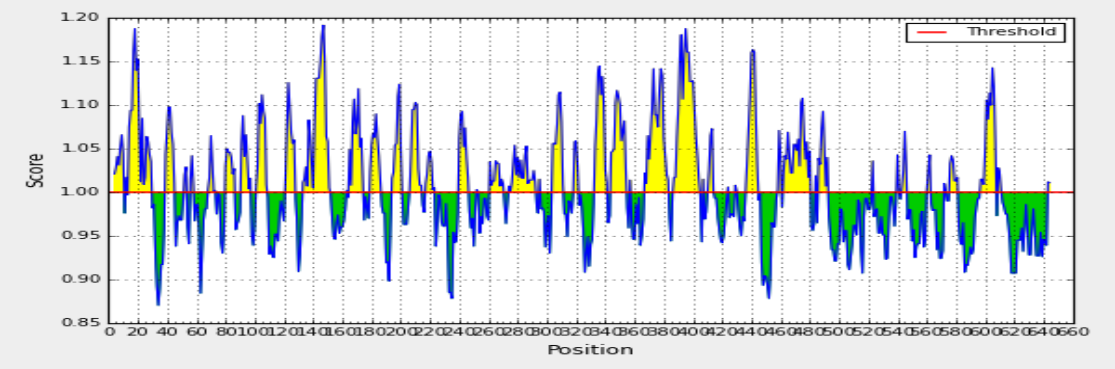

Fig.5. Kolaskar &Tongaonkar Antigenicity Graph

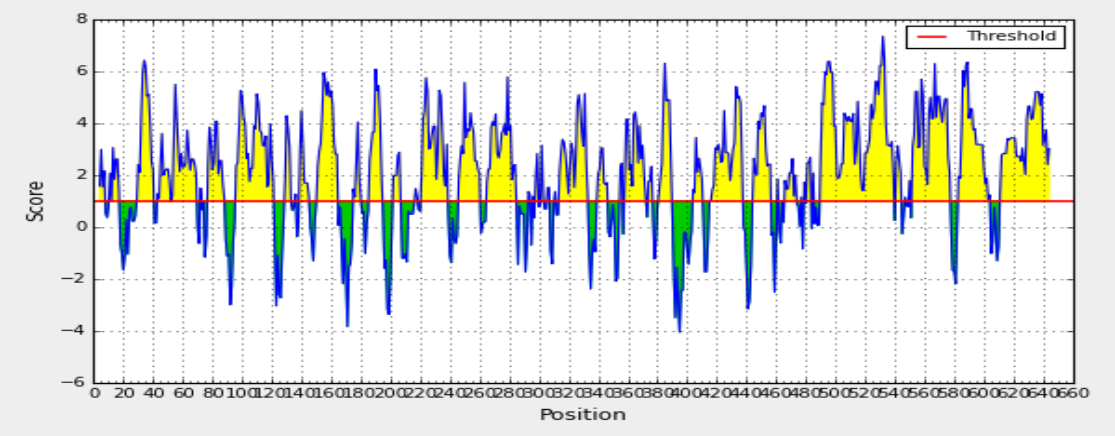

Fig.6. Parker Hydrophilicity Prediction Graph

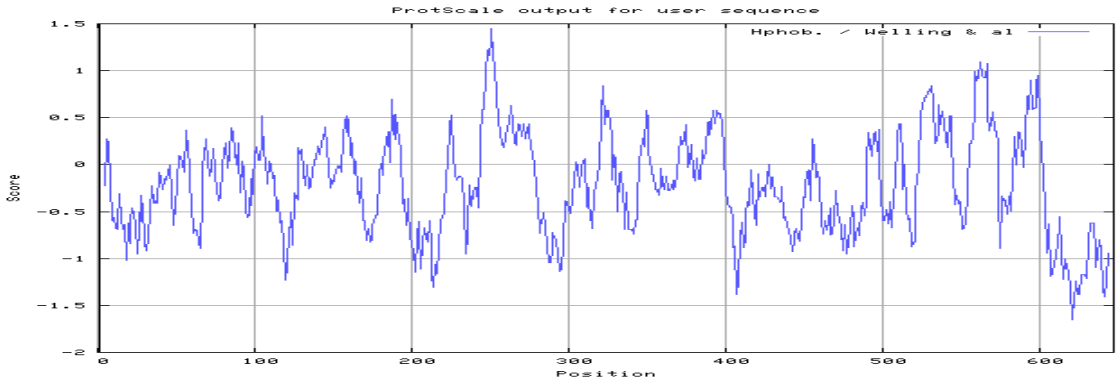

Fig.7. Hydrophobicity plot of antigen by Hphob/Welling & al., scale

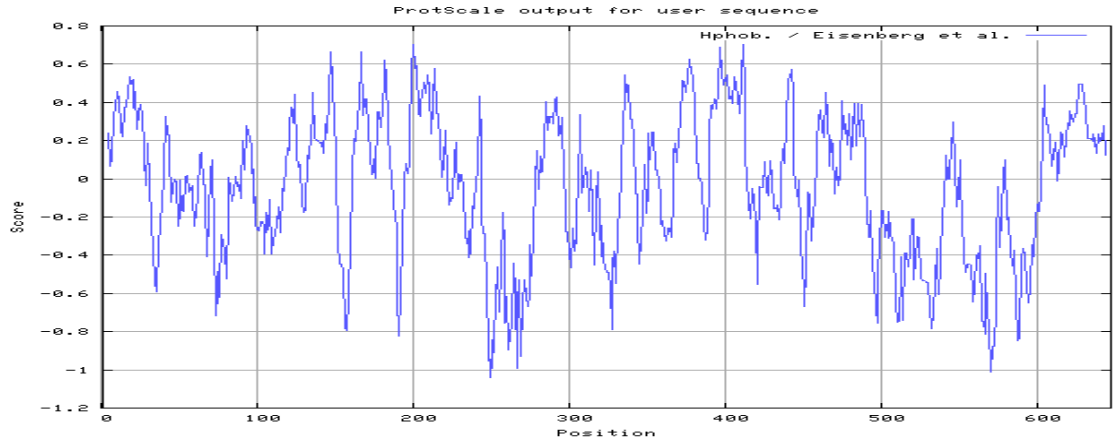

Fig.8. Hydrophobicity plot of antigen by Hphob/Eisenberg, et al., scale

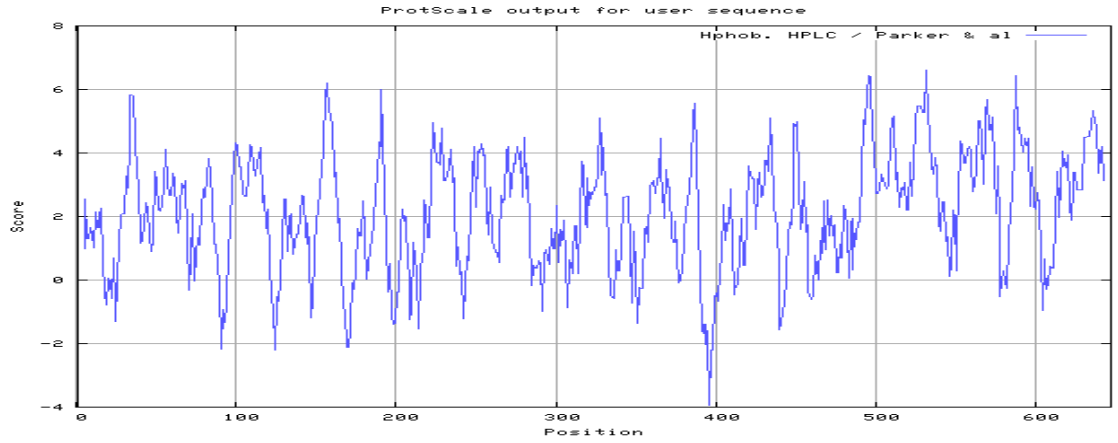

Fig.9. Hydrophobicity plot of antigen by Hphob. HPLC/Parker & et al., scale

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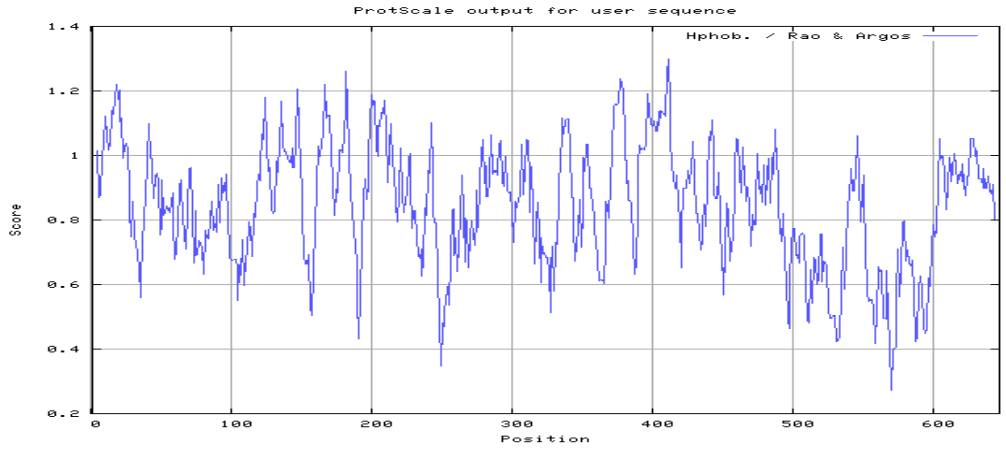

Fig.10 Antigenicity plot of antigen protein by Hphob. / Rao& Argos, scale

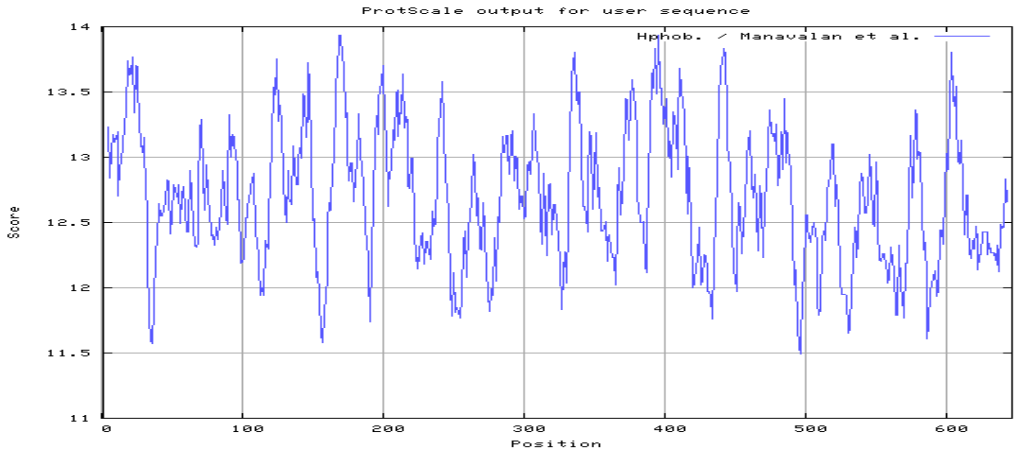

Fig.11. Antigenicity plot of antigen protein by Hphob. / Manavalan et al., scale

Table1: Bepipred Linear Epitope Prediction-Predicted Residue Scores

	Position	Residue	Score	Assignment
Bepipred Linear Epitope Prediction-Predicted residue scores	625	F	2.421	E
	626	P	2.23	E
	627	G	2.227	E
	628	G	2.308	E
	629	G	2.281	E
	630	A	2.548	E

Table2: Chou & Fasman Beta-Turn Prediction -Predicted Residue Scores

	Position	Residue	Start	End	Peptide	Score
Chou & Fasman Beta-Turn Prediction - Predicted residue scores	625	F	622	628	PGGFPGG	1.411
	626	P	623	629	GGFPGGG	1.417
	627	G	624	630	GFPGGGA	1.289
	628	G	625	631	FPGGGAP	1.283
	629	G	626	632	PGGGAPG	1.42
	630	A	627	633	GGGAPGG	1.426

V. CONCLUSION

An antigenic protein Heat shock protein 70 from *D. Medinensis*can plays an important role in vaccine development. The peptide fragments of antigen protein can be used to select nonamer for use in rational vaccine design and can develop the understanding of roles in the immune system in infectious disease.

Conflicts of Interest

The authors declare no conflict of interest.

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