International Journal of Advanced Technology in Engineering and Science
Vol. No.4, Issue No. 01, January 2016
www.ijates.com
ijates
ISSN 2348 - 7550

THE ANALYTICAL STUDY OF ANTIGENICITY AND MHC BINDER OF THE CYTOCHROME C OXIDASE SUBUNIT I

Sonu Mishra[1], Virendra S. Gomase[2], Karbhari V. Kale[3]

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

[3] Dept. of Computer Science and IT, Dr.Babasaheb AmbedkarMarathwada University,

Aurangabad, MS (India)

ABSTRACT

In this study we will summarize the potency of Cytochrome c oxidase subunit I (mitochondrion)from Dracunculus medinensis with526 amino acids. Antigenic peptide of cytochrome c oxidase subunit I (mitochondrion) protein is most suitable for subunit vaccine development because with single epitope, the immune response can be generated in large population. In this investigation, we used PSSM and SVM algorithms for the prediction of MHC class I & II binding peptide, antigenicity, solvent accessibility, polar and nonpolar residue to analyse the regions that are likely exposed on the surface of proteins which are potentially antigenic that allows potential targets to identify the active sites against infection as well as to design effective drug to treat it.

Keywords: Dracunculiasis, Antigenic Peptides; MHC-Binders;Tappred;PSSM; SVM; Nonamers; Cytochrome C Oxidase Subunit I (Mitochondrion)

I. INTRODUCTION

Cytochrome c oxidase subunit I (mitochondrion) comprised of 526 amino acid residues obtained from *Dracunculusmedinensis*for the study of MHC class I & II binding peptide, antigenicity, Solvent accessibility, polar and nonpolar residue to analyze the regions that are likely exposed on the surface of proteins.A little dragon from Medina (*D.medinesis*) is the only species of *Dracunculus*genus[1-4] which infects humans, commonly well 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 nematode undergo a very unusual life cycle of six developmental stages with incubation period last for about 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 , local skin redness , swelling and severe 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]. During emergence 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. Antigenic peptides from *D.medinensis* most suitable for the development of peptide vaccine[10] because a single protein subunit can generate sufficient immune response. In this research work we have used the phenomenon of cross-protection, whereby an individual undertaken by a mild toxin can have immunity to survive against similar strong toxic effects. MHC molecules are cell surface protein that binds to the peptides derived from host or antigenic proteins and present them to cell surface for recognition by T-cells. T cell recognition is animportant mechanism of the adaptive immune system by which the host identifies and responds to foreign antigens [10,11]. There are two types of MHC molecule and are extremely polymorphic. MHC class I molecules present peptides from proteins synthesized within the cell, whereas, MHC class II molecule present peptides derived from endocytosed extracellular proteins. MHC molecules have been well characterized due to their role in immune reactions and they take active part in host immune reactions and involvement of MHC class molecule in response to almost all antigens and it give impacts on specific sites. The involvement of MHC class-I molecule in response to almost all antigens make the study very interesting. They bind to some of the peptide fragments generated after proteolytic cleavage of antigen [12]. Identification of MHC-binding peptides and T-cell epitopes helps improve our understanding of specificity of immune responses [14-17]. Antigenic peptides are most suitable for peptide vaccine development because single epitope can generate large the immune response [17-19].

II. METHODOLOGY

2.1 Database searching

The antigenic protein sequence of cytochrome c oxidase subunit I (mitochondrion)from *Dracunculus medinensis* was retrieved from www.ncbi.nlm.nih.gov, UniProt databases are initially the most important [20-22].

2.2 Prediction of antigenicity

Prediction of antigenicity program predicts those segments from cytochrome c oxidase subunit I (mitochondrion)protein that are likely to be antigenic by eliciting an antibody response. In this research work antigenic epitopes of *Dracunculus medinensis*- cytochrome c oxidase subunit I (mitochondrion) are determined by using the Hopp and Woods, Welling, Parker, Bepipred ,Kolaskar and Tongaonkar antigenicity methods[23-27].

3.3 Prediction of Mhc Binding Peptide

The major histocompatibility complex (MHC) peptide binding of cytochrome c oxidase subunit I predicted using neural networks trained on C terminals of known epitopes. Rankpep predicts peptide binders to MHC-I ligand whose C-terminal end is likely to be the result of proteosomal cleavage using Position Specific Scoring Matrices (PSSMs). Support Vector Machine (SVM) based method for prediction of promiscuous MHC class II binding peptides from protein sequence; SVM has been trained on the binary input of single amino acid sequence [28-30].

3.4 Prediction of Antigenic Peptides by Cascade SVM based TAPPred method

In the present study, we predict cascade SVM based several TAP binders which was based on the sequence and the features of amino acids [31]. We found the MHCI binding regions (Table- 3), the binding affinity of *Dracunculus medinensis*.

3.5 Solvent Accessible Regions

We also analyzed the solvent accessible regions of proteins having highest probability that a given protein region lies on the surface of a protein Surface Accessibility, backbone or chain flexibility by Emini et al., [32] and Karplus and Schulz [33]. By using different scale we predict the hydrophobic and hydrophilic characteristics of amino acids that are rich in charged and polar residues i.e. Sweet et al. (1983), Kyte& Doolittle (1982), Abraham & Leo(19987), Bull and Breese (1974), Guy (1985), Miyazawa, et al (1985), Roseman (1988), Wolfenden et al. (1981), Wilson et al. (1981), Cowan (1990),Chothia (1976) [34-43].

III. RESULTS AND INTERPRETATIONS

The *Dracunculusmedinensis* Antigency to chrome c oxidase subunit I (mitochondrion), contain a long residue of526 amino acids with 518nonamers.

MKVLLFSYNNWYSVWFESTNHKDIGSMYLIFGFWSGMVGAGLSILIRAELCKPGFFFGSGQLYN
AVITSHAIMMIFFMVMPSLIGGFGNWMVPLMLGAPDMSFPRLNNVSYWLMPVSLMLILSACLVD
SSCGTSWTIYPPLSTSGHPGNSVDLAIFSLHCSGVSSILGGINFMTTVKNMRSASISLEHLSLFVWT
VFVTVFLLILTLPVLAGAITMLLMDRSFNTSFFDSSSGGNPLTYQHLFWFFGHPEVYILILPAFGIV
SQSSLYLTGKKEVFGSLGMIYAILSIALIGCVVWAHHMYTVGMDLDSRAYFSAATMVIAVPTGVK
VFSWLATLYGTRMIFQPVLLWVLGFIFLFTMGGFTGVILSNSSLDVVLHDTYYVVSHFHYVLSMG
AVFGIFCGISLWWTFLTGYVYDKIFMSVVFFVVFVGANLTFFPLHFAGLHGFPRKYVDYPDIYSF
WNVISSYGSMLSLFGALMFLVVLFDSFFSGRSFIYDYSGSSGLESGYSGYVFSHSYQEEVYYSGNY
KMF

3.1 Prediction of Antigenic Peptides

In this study, we found the antigenic determinants by finding the area of greatest local hydrophilicity. The Hopp-Woods scale Hydrophilicity Prediction Result Data found high at position:21 with score:0.756(max) in a protein, assuming that the antigenic determinants would be exposed on the surface of the protein and thus would be located in hydrophilic regions (Fig. 1). Welling antigenicity plot gives value as the log of the quotient between percentage in a sample of known antigenic regions and percentage in average proteins and Prediction

Result Data found high at position:380, score: 0.687(max)(Fig. 2).We also study Hydrophobicity plot of HPLC / Parker Hydrophilicity Prediction Result Data found at position:232 (residue S) i.e. 229-DSSSGGN-235(Maximum Score-6.843) (Fig. 3),BepiPred predicts the location of linear B-cell epitopes Result found at position:146(Residue H) and the maximum score 1.953(Fig.4), Kolaskar and Tongaonkar antigenicity methods (Fig. 5) Predicted peptides result found i.e 25-GSMYLIF-31,38-VGAGLSILIRAELCKPGF-55,61-QLYNAVITS-69,75-IFFMVMPSLI-84,90-WMVPLML-96,105-RLNNVSYWLMPVSLMLILSACLVDSSCGT-133,135-WTIYPPLST-143,147-PGNSVDLAIFSLHCSGVSSILG-168,182-ASISLEHLSLFVWTVFVTVFLLILTLPVLAGAITM-216,236-PLTYQHLFWF-245,247-GHPEVYILILPAFGIVSQSSLYL-269,274-EVFGSLGMIYAILSIALIGCVVWAHHMY-301,308-DSRAYFSAATMVIAVPTGVKVFSWLATL-335,341-IFQPVLLWVLGFIFL-355,361-FTGVILSNSSLDVVLHDTYYVVSHFHYVLSMGAVFGIFCGI-401,407-FLTGYVYDKIFMSVVFFVVFVGANLTFFPLHFAGLHGFPRKYVDYPDI-454,456-SFWNVISSYGSMLSLFGALMFLVVLFDS-483,486-SGRSFIYDY-494,504-YSGYVFSHSYQEEVYYS-520andthe predicted antigenic fragments can bind to MHC molecule is the first bottlenecks in vaccine design.

3.2 Solvent Accessible Regions

We also predicted the solvent accessible regions in proteins; different measurement was performed for the prediction of antigenic activity, surface region of peptides. Emini et al., (Fig. 6) predicts the highest probability i.e. found at position: 513(residue:Y)is 511- HSYQEE-516(High score:6.404), at position:19(Residue: T) 17-ESTNHK-22(score:6.325)that a given protein region lies on the surface of a protein and are used to identify antigenic determinants on the surface of proteins. Karplus and Schulz (Fig. 7) high score is found i.e. at position: 232(Residue: S) 229- DSSSGGN-235 (score:1.152),at position :233(Residue:G)230-SSSGGNP-236(score:1.145). Predict backbone or chain flexibility on the basis of the known temperature B factors of the a-carbons. The hydrophobicity and hydrophilic characteristics of amino acids is determined by using different scales that are rich in charged and polar residues i.e. Sweet et al. hydrophobicity prediction result data found high at Position:76 Score: 1.259 (max) (Fig. 8),Kyte& Doolittle result high atPosition: 200Score: 3.289 (max) (Fig. 9), Abraham & Leo result high at position Position: 352 Score: 2.143 (max) (Fig. 10), Bull and Breese result high atPosition: 146 Score: 0.509 (max) (Fig. 11),Miyazawa result high at Position:76 Score: 8.794 (max) (Fig. 12),Guy result high atPosition: 449Score: 0.516 (max) (Fig. 13), Wolfenden result high atPosition: 257Score: 1.620 (max) (Fig. 14), Roseman result high at Position: 352Score: 1.744 (max) (Fig. 15),Wilson et al., atPosition: 352 Score: 6.111 (max) (Fig. 16), Cowan at Position: 351with high Score: 1.499(Fig. 17),Chothiaat Position: 425 with Score: 0.489 (Fig. 18).

3.3 Prediction of MHC Binding Peptide

We found the binding of peptides to a number of different alleles using Position Specific Scoring Matrix. cytochrome c oxidase subunit I (mitochondrion) of *Dracunculusmedinensis* antigen, with sequence 526 amino acid residues long, having 519nonamers. MHC molecules are cell surface proteins, which actively participate in host immune reactions and involvement of MHC-I and MHC-II in response to almost all antigens. We have predicted MHC-I peptide binders of cytochrome c oxidase subunit I (mitochondrion)from *Dracunculus medinensis*was tested with on a set of 4 different alleles i.e. H2-Db (mouse) 8mer, H2-Db (mouse) 9mer, H2-Db

(mouse) 10mer and H2-Db (mouse) 11mer (Table-1a,1b,1c & 1d) and MHC-II peptide binders for I_Ab.p, I_Ad.p,I_Ag7.p alleles highlighted in red represent predicted binders (Table-2a,2b & 2c). Here RANKPEP report PSSM-specific binding threshold and is obtained by scoring all the antigenic peptide sequences included in the alignment from which a profile is derived, and is defined as the score value that includes 85% of the peptides within the set. Peptides whose score is above the binding threshold will appear highlighted in red and peptides produced by the cleavage prediction model are highlighted in violet. We also use a cascade SVM based TAPPred method which found 165 High affinity TAP Transporter peptide regions which represents predicted TAP binders residues which occur at N and C termini from *Dracunculusmedinensis (*cytochrome c oxidase subunit I (mitochondrion))(Table-3).

Table 1a- Promiscuous MHC ligands, having C-terminal ends are proteosomal cleavage sites of Dracunculusmedinensis. The binding thresholds is 33.04 and the optimal score is 52.494 of antigenic peptide to the MHC-1 Allele i.e. 8mer_H2_Db.(All rows highlighted in red represent predicted binders & A peptide highlighted in violet has a C-teminus predicted by the cleavage model used).

MHC-I Allele	RANK	POS.	N	SEQUENCE	C	MW (Da)	SCORE	% OPT.
8mer_H2_Db	1	232	DSS	SGGNPLTY	QHL	789.84	19.702	37.53%
8mer_H2_Db	3	394	MGA	VFGIFCGI	SLW	837.05	11.872	22.62%
8mer_H2_Db	7	50	RAE	LCKPGFFF	GSG	940.18	10.043	19.13%
8mer_H2_Db	9	485	DSF	FSGRSFIY	DYS	958.1	9.757	18.59%
8mer_H2_Db	12	262	FGI	VSQSSLYL	TGK	878	7.616	14.51%
8mer_H2_Db	13	288	ILS	IALIGCVV	WAH	769.01	7.012	13.36%
8mer_H2_Db	14	221	LMD	RSFNTSFF	DSS	987.09	7.007	13.35%

Table 1b- Promiscuous MHC ligands, having C-terminal ends are proteosomal cleavage sites of NADH dehydrogenase subunit5 from *Dracunculusmedinensis.* The binding potential (score) of antigenic peptide to the MHC-1 Allele i.e. 9mer_H2_Db.Matrix: 9mer_H2_Db.p.mtx, Consensus: FCIHNCDYM, Optimal Score: 50.365, Binding Threshold: 17.96(All rows highlighted in red represent predicted binders & A peptide highlighted in violet has a C-teminus predicted by the cleavage model used.

MHC-I Allele	RANK	POS.	N	SEQUENCE	C	MW (Da)	SCORE	% OPT.
9mer_H2_Db	**1**	**6**	**VLL**	**FSYNNWYSV**	**WFE**	**1138.24**	**25.193**	**50.02%**
9mer_H2_Db	**2**	**426**	**FVV**	**FVGANLTFF**	**PLH**	**997.16**	**19.872**	**39.46%**

Table 1c- Promiscuous MHC ligands, having C-terminal ends are proteosomal cleavage sites of NADH dehydrogenase subunit5 from Dracunculusmedinensis. The Optimal Score: 58.858, BindingThreshold: 41.32 of antigenic peptide to the MHC-1 Allele i.e. 10mer_H2_Db.(All rows highlighted in red represent predicted binders & A peptide highlighted in violet has a C-teminus predicted by the cleavage model used.

MHC-I Allele	RANK	POS.	N	SEQUENCE	C	MW (Da)	SCORE	% OPT.
10mer_H2_Db	1	97	LML	GAPDMSFPRL	NNV	1072.26	10.794	18.34%
10mer_H2_Db	3	459	SFW	NVISSYGSML	SLF	1052.21	10.104	17.17%
10mer_H2_Db	4	167	SSI	LGGINFMTTV	KNM	1034.22	9.981	16.96%
10mer_H2_Db	7	20	EST	NHKDIGSMYL	IFG	1159.32	8.175	13.89%
10mer_H2_Db	8	237	GNP	LTYQHLFWFF	GHP	1360.62	7.712	13.10%

Table 1d- Promiscuous MHC ligands, having C-terminal ends are proteosomal cleavage sites of NADH dehydrogenase subunit5 from Dracunculusmedinensis. TheMatrixOptimal Score: 79.495, Binding Threshold: 56.96of antigenic peptide to the MHC-1 Allele i.e. 11mer_H2_Db.(All rows highlighted in violet has a C-teminus predicted by the cleavage model used.

MHC-I Allele	RANK	POS.	N	SEQUENCE	C	MW (Da)	SCORE	% OPT.
11mer_H2_Db	1	167	SSI	LGGINFMTTVK	NMR	1162.39	20.946	26.35%
11mer_H2_Db	2	497	YSG	SSGLESGYSGY	VFS	1088.11	17.937	22.56%
11mer_H2_Db	3	458	YSF	WNVISSYGSML	SLF	1215.42	14.488	18.23%
11mer_H2_Db	4	391	VLS	MGAVFGIFCGI	SLW	1096.37	14.248	17.92%
11mer_H2_Db	6	160	SLH	CSGVSSILGGI	NFM	974.14	12.41	15.61%
11mer_H2_Db	7	291	IAL	IGCVVWAHHMY	TVG	1274.55	11.597	14.59%
11mer_H2_Db	8	5	KVL	LFSYNNWYSVW	FES	1414.61	11.436	14.39%

Table. 2a- Prediction of MHCII ligands all rows highlighted in red represent predicted binders to the MHC-II Allele i.e. MHC-II I_Ab.TheConsensus: YYAPWCNNA,MatrixOptimal Score: 35.632, Binding Threshold: 9.52 (All rows highlighted in red represent predicted binders.

MHC-II Allele	RANK	POS.	N	SEQUENCE	C	MW (Da)	SCORE	% OPT.
MHC-II I_Ab	1	313	RAY	FSAATMVIA	VPT	892.08	14.036	39.39%
MHC-II I_Ab	2	292	ALI	GCVVWAHHM	YTV	998.21	12.739	35.75%
MHC-II I_Ab	3	310	LDS	RAYFSAATM	VIA	999.16	10.743	30.15%
MHC-II I_Ab	4	398	FGI	FCGISLWWT	FLT	1048.29	10.181	28.57%
MHC-II I_Ab	5	63	GQL	YNAVITSHA	IMM	957.05	10.099	28.34%
MHC-II I_Ab	6	101	APD	MSFPRLNNV	SYW	1059.25	10.012	28.10%
MHC-II I_Ab	7	228	TSF	FDSSSGGNP	LTY	848.83	9.725	27.29%
MHC-II I_Ab	8	208	LTL	PVLAGAITM	LLM	854.07	9.53	26.75%

Table. 2b- Prediction of MHCII ligands all rows highlighted in red represent predicted binders to the MHC-II Allele i.e. MHC-II I_Ad.TheConsensus: QMVHAAHAE with Optimal Score: 53.145 and Binding Threshold: 7.10.

MHC-II Allele	RANK	POS.	N	SEQUENCE	C	MW (Da)	SCORE	% OPT.
MHC-II I_Ad	1	64	QLY	NAVITSHAI	MMI	907.03	18.11	34.08%
MHC-II I_Ad	2	380	DTY	YVVSHFHYV	LSM	1132.29	13.816	26.00%
MHC-II I_Ad	3	317	SAA	TMVIAVPTG	VKV	870.06	10.174	19.14%
MHC-II I_Ad	4	383	YVV	SHFHYVLSM	GAV	1102.28	9.055	17.04%
MHC-II I_Ad	5	187	ISL	EHLSLFVWT	VFV	1090.28	8.935	16.81%
MHC-II I_Ad	6	293	LIG	CVVWAHHMY	TVG	1104.34	8.541	16.07%
MHC-II I_Ad	7	505	SGY	SGYVFSHSY	QEE	1028.1	8.291	15.60%

Table. 2c- Prediction of MHCII ligands all rows highlighted in red represent predicted binders to the MHC-II Allele i.e. MHC-II I_Ag7.

MHC-II Allele	RANK	POS.	N	SEQUENCE	C	MW (Da)	SCORE	% OPT.
MHC-II I_Ag7	1	439	LHF	AGLHGFPRK	YVD	964.14	14.957	36.59%
MHC-II I_Ag7	2	373	SLD	VVLHDTYYV	VSH	1090.24	14.285	34.95%

MHC-II I_Ag7	3	380	DTY	YVVSHFHYV	LSM	1132.29	13.145	32.16%
MHC-II I_Ag7	4	518	EEV	YYSGNYKMF		1154.31	10.96	26.81%
MHC-II I_Ag7	5	238	NPL	TYQHLFWFF	GHP	1247.46	10.723	26.23%
MHC-II I_Ag7	6	254	VYI	LILPAFGIV	SQS	924.2	9.815	24.01%

Table 3- Cascade SVM based High affinity TAP Binders of *Dracunculusmedinensis*

Peptide Rank	Start Position	Sequence	Score	Predicted Affinity
1	3	VLLFSYNNW	8.654	High
2	208	PVLAGAITM	8.644	High
3	38	VGAGLSILI	8.644	High
4	319	VIAVPTGVK	8.643	High
5	30	IFGFWSGMV	8.642	High
6	318	MVIAVPTGV	8.641	High
7	373	VVLHDTYYV	8.638	High
8	329	FSWLATLYG	8.638	High
9	368	NSSLDVVLH	8.635	High
10	60	GQLYNAVIT	8.635	High

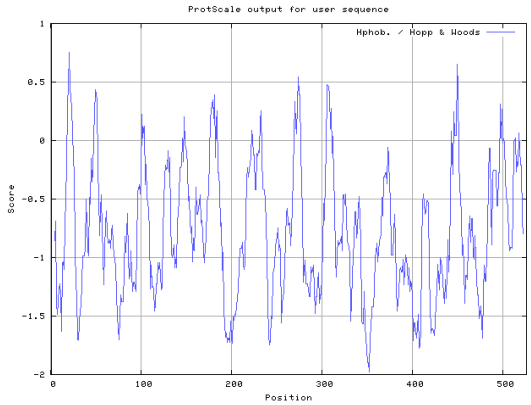


Fig. 1-Hydrophobicity plot of Hopp and Woods (1981)

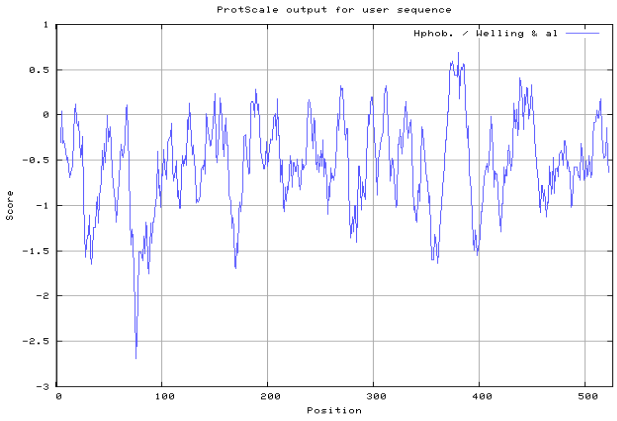


Fig. 2- Hydrophobicity plot of Welling et al. (1985)

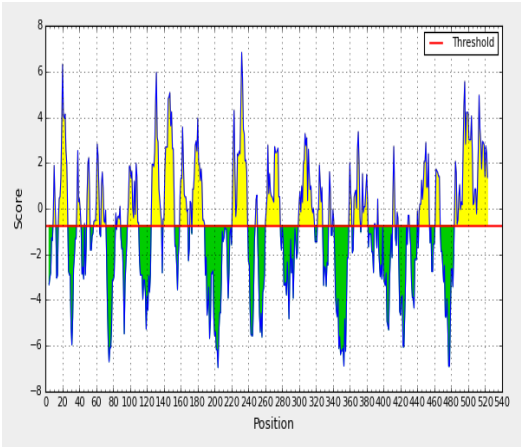


Fig. 3- Hydrophobicity plot of HPLC / Parker et al. (1986)

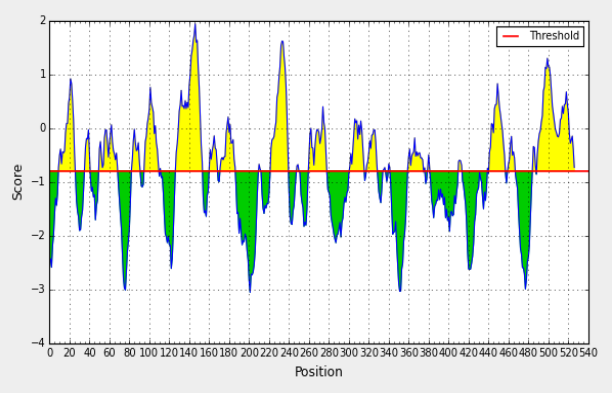
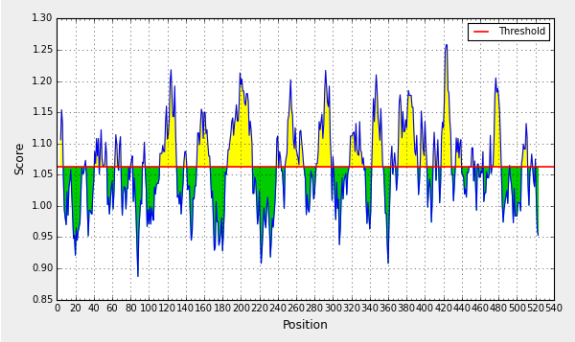


Fig. 4- Bepipred Linear Epitope Prediction plot

International Journal of Advanced Technology in Engineering and Science
Vol. No.4, Issue No. 01, January 2016
www.ijates.com
ijates
ISSN 2348 - 7550



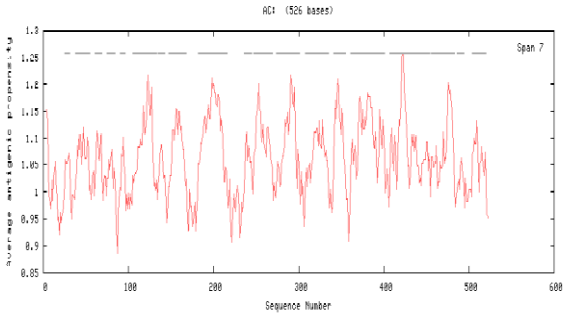


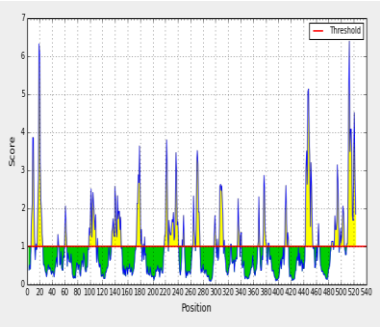
Fig. 5- Kolaskar and Tongaonkarantigenicity plot

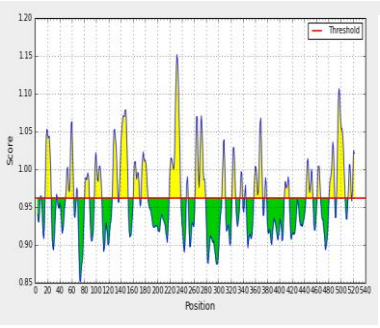
Fig 5a- Kolaskar and Tongaonkarantigenicityplot(propensity-1.0938

There are 19 antigenic determinants in cytochrome c oxidase subunit I (mitochondrion) protein sequence:

n	Start Position	Sequence	End Position
1	25	GSMYLIF	31
2	38	VGAGLSILIRAELCKPGF	55
3	61	QLYNAVITS	69
4	75	IFFMVMPSLI	84
5	90	WMVPLML	96
6	105	RLNNVSYWLMPVSLMLILSACLVDSSCGT	133
7	135	WTIYPPLST	143
8	147	PGNSVDLAIFSLHCSGVSSILG	168
9	182	ASISLEHLSLFVWTVFVTVFLLILTLPVLAGAITM	216
10	236	PLTYQHLFWF	245
11	247	GHPEVYILILPAFGIVSQSSLYL	269
12	274	EVFGSLGMIYAILSIALIGCVVWAHHMY	301
13	308	DSRAYFSAATMVIAVPTGVKVFSWLATL	335
14	341	IFQPVLLWVLGFIFL	355
15	361	FTGVILSNSSLDVVLHDTYYVVSHFHYVLSMGAVFGIFCGI	401
16	407	FLTGYVYDKIFMSVVFFVVFVGANLTFFPLHFAGLHGFPRKYVDYPDI	454
17	456	SFWNVISSYGSMLSLFGALMFLVVLFDS	483
18	486	SGRSFIYDY	494
19	504	YSGYVFSHSYQEEVYYS	520

Fig 5b- The 19 antigenic determinants





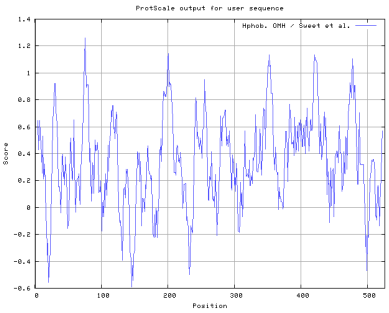


Fig. 6- Emini Surface Accessibility Prediction plot

Fig. 7- Karplus& Schulz Flexibility Prediction

Fig. 8- Hydrophobicity plot of Sweet et al. (1983)

International Journal of Advanced Technology in Engineering and Science
Vol. No.4, Issue No. 01, January 2016
www.ijates.com
ijates
ISSN 2348 - 7550

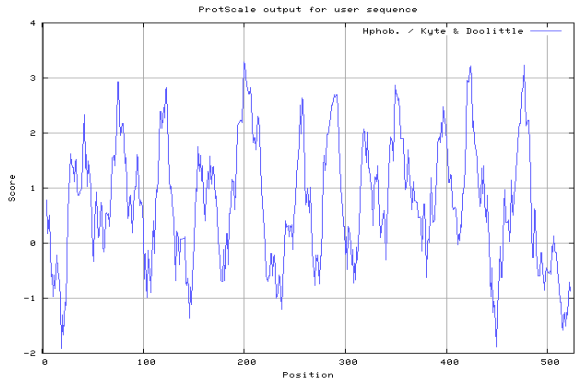


Fig. 9- Kyte& Doolittle hydrophobicity plot

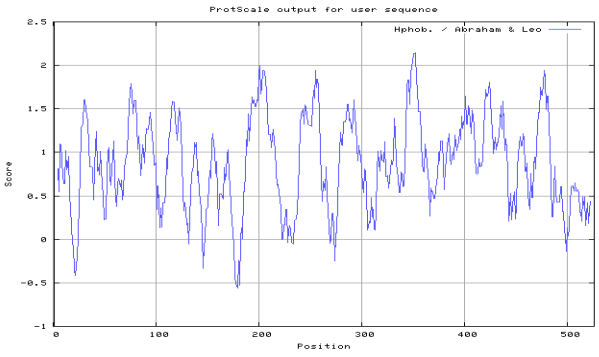


Fig. 10- Abraham & Leo hydrophobicity plot

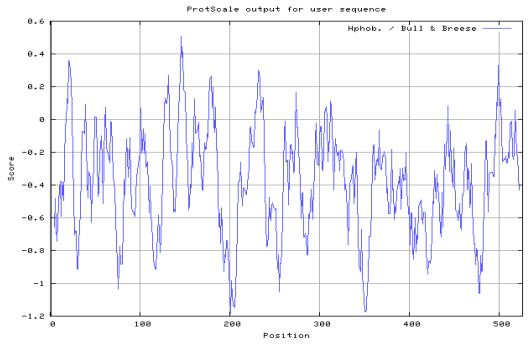


Fig. 11- Bull & Breese use surface tension to measure hydrophobicity values to describe

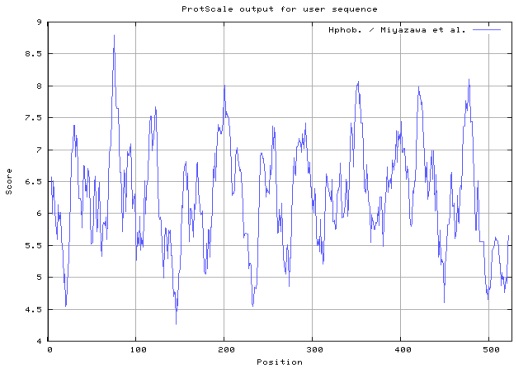


Fig.12- Hydrophobicity plot of and also uses negative the hydrophobicity Miyazawa et al. (1985)

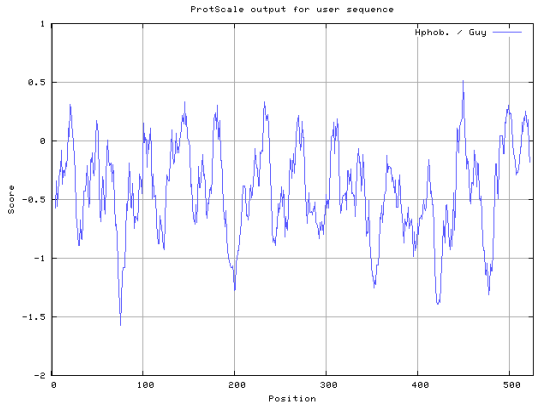


Fig. 13- Hydrophobicity plot of Guy (1988)

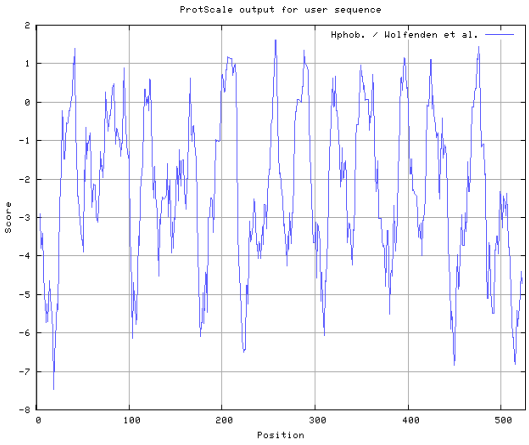
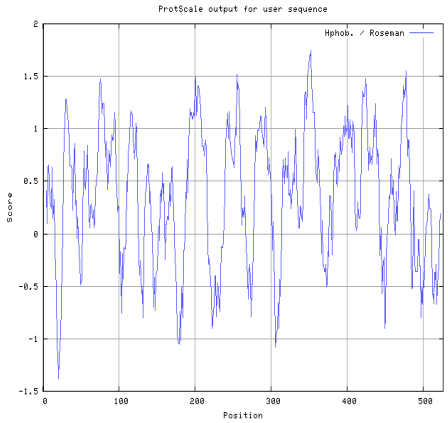


Fig.14- Hydrophobicity plot of Wolfenden et al.(1981)



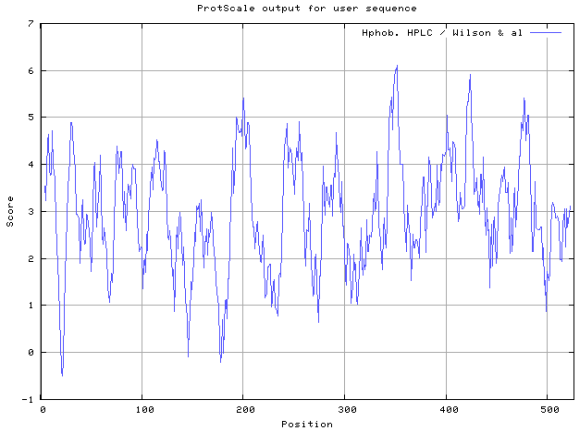
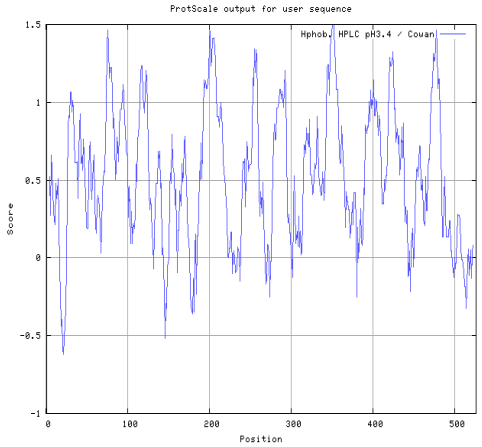


Fig. 15- Hydrophobicity plot of RosemanM.A.. (1988)

Fig. 16- Hydrophobicity/HPLC plot of Wilson & al (1981)



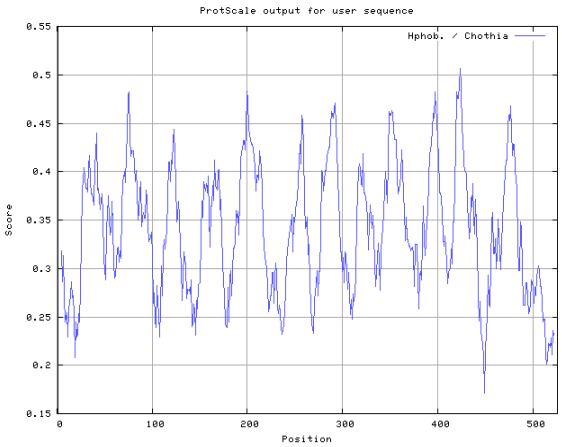


Fig. 17- Hydrophobicity/HPLC pH 3.4/ plot of Cowan (1990)

Fig. 18- Hydrophobicity plot of Chothia (1976)

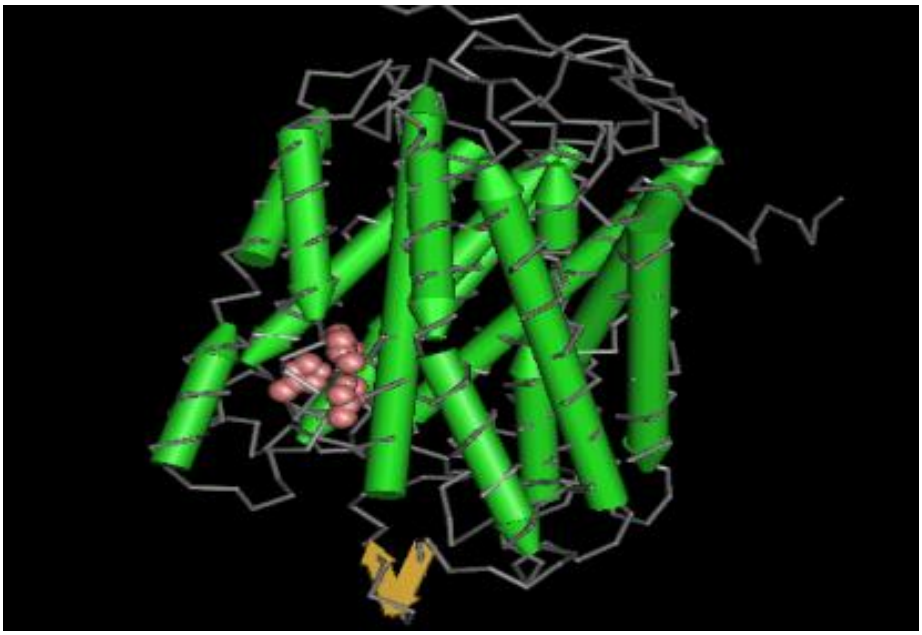


Fig.19- X-Ray Diffraction with Resolution 1.49 Å 3D Structure of the antigencytochrome c oxidase subunit I.

IV. DISCUSSION

In this study, we found the antigenic determinants by finding the area of greatest local hydrophilicity. Hopp and Woods hydrophobicity scale is used to identify of potentially antigenic sites in proteinsby analyzing amino acid sequences in order to find the point of greatest hydrophilic. Hydrophilicity Prediction result data found high in sequence position at position:21 with score:0.756(max) in a protein this scale is basically a hydrophilic index where apolar residues have been assigned negative values. The Window size of 5-7 is good for finding hydrophilic regions, greater than 0 values are consider as hydrophilic which is consider as antigenic. Welling used information on the relative occurrence of amino acids in antigenic regions to make a scale which is useful for prediction of antigenic regions and the predicted result data found high in sequence at position:380, score: 0.687(max). Welling antigenicity plot gives value as the log of the quotient between percentage in asample of known antigenic regions and percentage in average proteins. We also study Hydrophobicity plot of HPLC / Parker Hydrophilicity Prediction Result Data found in position:232 (residue S) i.e. 229-DSSSGGN-

235(Maximum Score-6.843).BepiPred predicts the location of linear B-cell epitopes Result found at position:146(Residue H) and the maximum score 1.953. There are 19 antigenic determinant sequences is found by Kolaskar and Tongaonkar antigenicity scales(Fig. 5a & 5b) the results show highest pick at position 25-GSMYLIF-31,38-VGAGLSILIRAELCKPGF-55,61-QLYNAVITS-69,75-IFFMVMPSLI-84,90-WMVPLML-96,105-RLNNVSYWLMPVSLMLILSACLVDSSCGT-133,135-WTIYPPLST-143,147-PGNSVDLAIFSLHCSGVSSILG-168,182-ASISLEHLSLFVWTVFVTVFLLILTLPVLAGAITM-216,236-PLTYQHLFWF-245,247-GHPEVYILILPAFGIVSQSSLYL-269,274-EVFGSLGMIYAILSIALIGCVVWAHHMY-301,308-DSRAYFSAATMVIAVPTGVKVFSWLATL-335,341-IFQPVLLWVLGFIFL-355,361-FTGVILSNSSLDVVLHDTYYVVSHFHYVLSMGAVFGIFCGI-401,407-FLTGYVYDKIFMSVVFFVVFVGANLTFFPLHFAGLHGFPRKYVDYPDI-454,456-SFWNVISSYGSMLSLFGALMFLVVLFDS-483,486-SGRSFIYDY-494,504-YSGYVFSHSYQEEVYYS-520. Result of determined antigenic sites on proteins has revealed that the hydrophobic residues if they occur on the surface of a protein are more likely to be a part of antigenic sites. This method can predict antigenic determinants with about 75% accuracy and also gives the information of surface accessibility and flexibility. Further this region form beta sheet which show high antigenic response than helical region of this peptide and shows highly antigenicity. X-Ray Diffraction with Resolution 1.49 Å 3D Structure of the *Dracunculusmedinensis a*ntigen- cytochrome c oxidase subunit I (mitochondrion)is predicted by PDB vive(Fig.19). We generate a purified protein for analysis of the chosen target and then structure determined the target experimentally to evaluate their similarity to known protein structures and to determine possible relationships that are identifiable from protein sequence alone. The target structure will also serve as a detailed model for determining the structure of peptide within that protein structure. We predict Solvent accessibility by using Emini et al., the result found the highest probability i.e. found at position: 513(Residue:Y) is 511-HSYQEE-516(High score:6.404), at position:19(Residue: T) 17-ESTNHK-22(score:6.325), that a given protein region lies on the surface of a protein and are used to identify antigenic determinants on the surface of proteins. This algorithm also used to identify the antigenic determinants on the surface of proteins and Karplus and Schulz predict backbone or chain flexibility on the basis of the known temperature B factors of the a-carbons here we found the result with High score at position: 232(Residue: S) 229- DSSSGGN-235(score:1.152),at position :233(Residue:G)230- SSSGGNP-236(score:1.145). We predict Solvent accessibility of cytochrome c oxidase subunit I of *Dracunculus medinensis a*ntigen cytochrome c oxidase subunit I (mitochondrion)for delineating hydrophobic and hydrophilic characteristics of amino acids. Solvent accessibility used to identify active site of functionally important residues in membrane proteins. Solvent-accessible surface areas and backbone angles are continuously varying because proteins can move freely in a three-dimensional space. The mobility of protein segments which are located on the surface of a protein due to an entropic energy potential and which seem to correlate well with known antigenic determinants. We also found the i.e. Sweet et al. hydrophobicity prediction result data found high high in Position:76 Score: 1.259 (max), Kyte& Doolittle result high atPosition: 200 Score: 3.289 (max), Abraham & Leo result high at Position: 352 Score: 2.143 (max),Bull and Breese result high atPosition: 146 Score: 0.509 (max), Guy result high atPosition: 449 Score: 0.516 (max), Miyazawa result high at Position:76 Score: 8.794 (max), Roseman result high atPosition: 352 Score: 1.744 (max),Wolfenden result high atPosition: 257 Score: 1.620 (max),Wilson et al., at Position: 352 Score: 6.111 (max), Cowan at Position: 351 with high Score: 1.499,Chothiaat Position: 425 with Score:

0.489. These scales are a hydrophilic with a polar residues assigned negative value. Because the N- and C-terminal regions of proteins are usually solvent accessible and unstructured, antibodies against those regions recognize the antigenic protein. In this study, we found predicted MHC-I peptide binders of protein for 8mer_H2_Db alleles with the consensus sequence QNWNCCTI that yields the maximum score i.e. 52.494, 9mer_H2_Db with, the consensus sequence FCIHNCDYM that yields the maximum score i.e. 50.365, 10mer_H2_Db with, the consensus sequence SGYYNFFWCL that yields the maximum score i.e. 58.858, 11mer_H2_Db with, the consensus sequence CGVYNFYYCCY that yields the maximum score i.e. 79.495 and I_Abwith the consensus sequence YYAPWCNNA that yields the maximum score i.e. 35.632,I_Ad with the consensus sequence QMVHAAHAE that yields the maximum score i.e. 53.145, MHC-II I_Ag7 with the consensus sequence WYAHAFKYV that yields the maximum score i.e. 40.873 for MHC II allele was tested. We also use a cascade SVM based TAPPred method which found 160 High affinity TAP Transporter peptide regions which represents predicted TAP binders residues which occur at N and C termini from *Dracunculus medinensis a*ntigen cytochrome c oxidase subunit I. TAP is an important transporter that transports antigenic peptides from cytosol to ER. TAP binds and translocate selective antigenic peptides for binding to specific MHC molecules. The efficiency of TAP-mediated translocation of antigenic peptides is directly proportional to its TAP binding affinity. Thus, by understanding the nature of peptides, that bind to TAP with high affinity, is important steps in endogenous antigen processing. The correlation coefficient of 0.88 was obtained by using jackknife validation test. In this test, we found the MHCI and MHCII binding regions. T cell immune responses are derived by antigenic epitopes hence their identification is important for design synthetic peptide vaccine. T cell epitopes are recognized by MHCI molecules producing a strong defensive immune response against *Dracunculus medinensis a*ntigen cytochrome c oxidase subunit I. Therefore, the prediction of peptide binding to MHCI molecules by appropriate processing of antigen peptides occurs by their binding to the relevant MHC molecules. Because, the C-terminus of MHCI-restricted epitopes results from cleavage by the proteasome and thus, proteasome specifity is important for determing T-cell epitopes. Consequently, RANKPEP also focus on the prediction of conserved epitopes. C-terminus of MHCI-restricted peptides is generated by the proteasome, and thus RANKPEP also determines whether the C-terminus of the predicted MHCI-peptide binders is the result of proteasomal cleavage. Moreover, these sequences are highlighted in purple in the output results. Proteasomal cleavage predictions are carried out using three optional models obtained applying statistical language models to a set of known epitopes restricted by human MHCI molecules as indicated here.

V. CONCLUSION

From the above result and discussion it is concluded that the ability of RANKPEP to predict MHC binding peptides, and thereby potential T-cell epitopes, Antigenic peptide that binds to MHC molecule are antigenic that means hydrophilic in nature. This means the increase in affinity of MHC binding peptides may result in enhancement of immunogenicity of *Dracunculus medinensis a*ntigen cytochrome c oxidase subunit I (mitochondrion)and are helpful in the designing of synthetic peptide vaccine. This approach can help reduce the time and cost of experimentation for determining functional properties of *Dracunculus medinensis a*ntigen cytochrome c oxidase subunit I (mitochondrion). Overall, the results are encouraging, both the ‘sites of action’

and 'physiological functions' can be predicted with very high accuracies helping minimize the number of validation experiments.

REFERENCES

[1] Muller ,R.(1971). Dracunculus and dracuneuliasis. Adv. Parasitol., 9,73.

[2] Muller,R.(1979). Guinea worm disease: epidemiology, control and treatment .Buel.WHO,57.683.1979.

[3] Jones,h.I., and Mulder E.(2007). *Dracunculusmulbus*n.sp.(nematode:Spirurida) from water Python *Liasisfuscus*(sperpentes:Boidae) in northern .Australia .Syst. Parasitol., 66.195.

[4] Moravee,F, and Santos C.P.(2009). *Dracunculusbrasiliensis*sp.n.(Nematoda:*Dracunculidae*)from the anaconda.*Eunectesmurinus* (Ophidia: Boidae).Parasitol.Res., 104,589,2009.

[5] Bimi,L., et al.,(2005). Ginea worm infection of urinary bladder manifesting as obstructureuropathy in rural Maharastra.Trop.DOct ., 35,242.

[6] Greenaway C. (2004).Dracunculiasis (Guinea worm disease). CMAJ 170(9), 495-500.

[7] Miillner A, HelferA ,KotlyarD.Oswald J, EfferthT. (2011).Chemistry and pharmacology of neglected helminthic disease.Curr Med Chem.,18(5), 767-789.

[8] Ruiz-Tiben E, Hopkins DR.(2006).Dracunculiasis (Guinea worm disease) eradication .AdvParasitol., 61:275-309.

[9] IriemenamNC,Oyibo WA, Fagbenro-BeyiokuAF.(2008).Dracunculiasis – The saddle is virtually ended. Parasitol Res., 102(3): 343-347.

[10] Flower, D.R. (2008). "Vaccines: how they work," in Bioinformatics for Vaccinology.Wiley-Blackwell, Oxford, UK. pp. 73–112.

[11] Batalia, Michael. (1997). "Peptide Binding by Class 1 and Class II MHC Molecules." Biopoly. 43: pp. 281-302,

[12] Marrack, P., Scott-Browne JP, Dai S, Gapin L, Kappler JW (2008). Review Evolutionarily conserved amino acids that control TCR-MHC interaction. Annu Rev Immunol. 26:171-203.

[13] Chapman, H.A. (1998). Endosomal proteolysis and MHC class II function. CurrOpinImmunol. 10: 93–102.

[14] Watts, C. (2004). The exogenous pathway for antigen presentation on major histocompatibility complex class II and CD1 molecules. Nat Immunol. 5: 685–92.

[15] Neefjes, J., Jongsma, M.L., Paul, P., Bakke, O. (2011).Review towards a systems understanding of MHC class I and MHC class II antigen presentation. Nat Rev Immunol. 11(12):823-36.

[16] Kumar, M., Gromiha, M.M., Raghava, G.P. (2007) Identification of DNA-binding proteins using support vector machines and evolutionary profiles. BMC Bioinformatics 8: 463.

[17] Gomase, V.S., Chitlange, N.R. (2012). Prediction of MHC Class Antigen Peptides from EchinococcusMultilocularis: Application of Computer Intelligence. 1: 191.

[18] Gomase, V.S., Kale, K.V., Chikhale, N.J., Changbhale, S.S. (2007). Prediction of MHC Binding Peptides and Epitopes from Alfalfa mosaic virus.Curr Drug DiscovTechnol 4: 117-215.

[19] Sherkhane, A.S., Changbhale, S.S., Chitlange, N.R., Waghmare,S.,Gomase, V.S., RamraoChavan and Kale, K.V. (2012). Prediction of Major Histocompatibility Complex Binding Peptides and Epitopes from NajanajaCardiotoxin (CTX). Drug Invention Today; 4(8): 435-438.

[20] http://www.ncbi.nlm.nih.gov

[21] Sayers, E.W.et al. (2012). Database resources of the National Center for Biotechnology Information. Nucleic Acids Res. 40(Database issue): D13-25.

[22] Bairoch, A., Apweiler, R., Wu, CH., Barker, W.C., Boeckmann, B., Ferro, S., Gasteiger, E., Huang, H., Lopez, R., Magrane, M., Martin, M.J., Natale, D.A., O'Donovan, C., Redaschi, N., Yeh, L.S. (2005).The Universal Protein Resource (UniProt). Nucleic Acids Res. 33: D154-159.

[23] Hoop, T.P & Woods, K.R. (1978). Prediction of protein antigenic determinants from amino acid sequences. Proc. Natl. Acad. Sci. USA. 78: 3824.

[24] Welling, G.W., Weijer, W.J., van der Zee R, Welling,-Wester S. (1985). FEBS Lett. 188: 215-218 .

[25] Parker, K.C., Bednarek, M.A., Coligan, J.E. (1994). J. Immunology. 152:163-75.

[26] Jens,Erik., Pontoppidan, Larsen, Ole Lund and Morten, Nielsen. (2006). Improved method for predicting linear B-cell epitopes.Immunome Res. 2: 2.

[27] Kolaskar, A.S., Tongaonkar, P.C. (1990). A semi-empirical method for prediction of antigenic determinants on protein antigens.FEBS Lett. 276(1-2):172-4.

[28] Reche, P.A., Glutting, J.P and Reinherz, E.L. (2002).Prediction of MHC Class I Binding Peptides Using Profile Motifs. Human Immunology. 63: 701-709.

[29] RecheP.AandReinherz, E.L. (2003). Sequence variability analysis of human class I and class II MHC molecules: functional and structural correlates of amino acid polymorphisms.JMol Biol. 331(3): 623-41.

[29] Craiu, A., Akopian,T. et al. (1997). "Two distinct proteolytic processes in the generation of a major histocompatibility complex class I-presented peptide." ProcNatlAcadSci U S A. 94(20): 10850-5.

[30] Pieters, J. (2000). "MHC class II-restricted antigen processing and presentation." AdvImmunol. 75: 159-208.

[31] Bhasin, M and Raghava, G.P.S. (2004). Analysis and prediction of affinity of TAP binding peptides using cascade SVM.Protein Sci. 13 (3): 596-607.

[32] Emini, E.A., Hughes, J.V., Perlow, D.S., Boger, J. (1985). Induction of hepatitis a virus-neutralizing antibody by a virus-specific synthetic peptide.J Virol. 55: 836-839.

[33] Karplus, P.A and Schulz, G.E. (1985). Prediction of chain flexibility in proteins: a tool for the selection of peptide antigen. Naturwissenschaften. 72: 212-213.

[34] Sweet, R.M and Eisenberg, D. (1983). Correlation of sequence hydrophobicities measures similarity in three-dimensional protein structure. J. Mol. Biol. 171: 479–488.

[35] Kyte, J., Doolittle, R.F. (1982). A Simple Method for Displaying the Hydropathic Character of a Protein. J. Mol. Biol. 157: 105-132.

[36] Abraham, D.J., Leo, A.J. (1987). Extension of the fragment method to calculate amino acid zwitterions and side chain partition coefficients.Proteins. 2: 130-152.

[37] Bull, H.B., Breese, K. (1974). Surface tension of amino acid solutions: A hydrophobicity scale of the amino acid residues. Arch. Biochem. Biophys.161: 665-670.

[38] Miyazawa, S., Jernigen, R.L. (1985). Estimation of Effective Interresidue Contact Energies from Protein Crystal Structures: Quasi-Chemical Approximation. Macromolecules. 18: 534-552.

[39] Roseman, M.A. (1988). Hydrophilicity of polar amino acid side-chains is markedly reduced by flanking peptide bonds. J. Mol. Biol. 200: 513-522.

[40] Wolfenden, R.V., Andersson,.L, Cullis,P.M., Southgate, C.C.F .(1985). Affinities of amino-acid side-chains for solvent water.Biochemistry. 20(4): 849-855.

[41] Wilson, J., Honegger, A., Stotzel, R.P., Hughes, G.J. (1981). The behaviour of peptides on reverse-phase K.supports during high-pressure liquid chromatography.Biochem. J. 199: 31-41.

[42] Cowan. R., Whittaker, R.G. (1990). Hydrophobicity indices at ph 3.4 determined by HPLC.Peptide Research. 3: 75-80.

[43] Chothia, C. (1976). The nature of accessible and buried surfaces in proteins. J. Mol. Biol. 105: 1-14.