

## Analysis of Inhibitory Activity of Ricin Extracted from *Ricinus Communis* on Human Cancer Cell Lines and testing its activity on HIV receptor CD4 using Insilico analysis

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### Abstract:

Castor (*Ricinus communis* L.) is one of the oldest cultivated oil crops in the world. The unique oil produced in castor seeds contains ricinoleic acid, an essential product that is widely used as a raw material in numerous commercial applications. Ricin one of the storage protein of the seed is a cytotoxin used in various medicinal preparations in low concentrations, whereas the same in high concentrations is highly hazardous. The purpose of the current work is to extract the ricin protein from the castor seeds and after through purification testing its activity on Cancer Cell lines. The work was also extended to perform the insilico study of the same. The structure of the protein was retrieved and the protein-protein docking was performed using Hex software. The literature survey was done to identify the proteins that are responsible for causing Cancer. The protein structures were retrieved and were used to test their docking efficacy with the ricin protein. Based upon the docking interactions the probability of using ricin as a treatment mode for cancer can be detected.

**Key words:** *Ricinus communis*, ricinoleic acid, protein- protein docking

### Introduction:

The castor bean plant is a tropical perennial shrub that originated in Africa and India, but is now cultivated in many tropical and subtropical regions around the world. It can be self and cross-pollinated and worldwide studies reveal low genetic diversity among castor bean. Approximately 90% of the oil from castor bean seeds is composed of the unusual hydroxylated fatty acid ricinoleic acid. Because of the nearly uniform ricinoleic acid content of castor oil, and the unique chemical properties that this fatty acid confers to the oil, castor bean is a highly valued oilseed crop for lubricant, cosmetic, medical and specialty chemical applications. Castor bean has also been proposed as a potential source of biodiesel; the

high oil content of its seeds and the ease with which it can be cultivated in unfavorable environments contribute to its appeal as a crop in tropical developing countries. It is believed that castor oil was first used as an ointment 4,000 years ago in Egypt, from where it spread to other parts of the world, including Greece and Rome, where it was used as a laxative 2,500 years ago. An important obstacle to widespread cultivation of castor bean is the high content of ricin, an extremely toxic protein in its seeds. Ricin is considered one of the deadliest natural poisons when administered intravenously or inhaled as fine particles in high concentration. It is very surprising to know that inspite of its hazardous nature ricin when used in mild concentrations has the property to fight against dreadful diseases like Cancer and HIV. Ricin was first isolated more than a century ago. It has been reportedly used as a weapon and attempts to use ricin as a specific immunotoxin for therapeutic purposes in different cancers have been reported. Its biochemical activity has been characterized as a type 2 ribosome-inactivating protein (RIP), composed of two subunits linked by a disulfide bond: a 32 kDa ricin toxin A (RTA) chain that harbors the ribosome-inactivating activity, and a 34 kDa ricin toxin B (RTB) chain, with a galactose-binding lectin domain. RTA is an N-glycosidase that depurinates adenine in a specific residue of the 28S ribosomal RNA. The RTB chain allows ricin to enter eukaryotic cells by binding to cell surface galactosides and subsequent endocytosis. Other RIPs are common in plants, although they are not toxic because they are usually monomeric and lack a lectin domain. These proteins constitute the type 1 RIPs. The objective of the paper was to demonstrate the anticancer activity of the ricin protein extracted from *ricinus communis*. To establish its activity cancer cell lines were used. The anticancer activity of ricin was

further analyzed using Insilco tools and software by applying the docking principle.

**Materials and Methods:**

***Collection of the Castor Seeds sample for the Extraction of Ricin Protein:***

Castor seeds were collected and crushed into fine paste to which 20 ml of 5% HgCl<sub>2</sub> is added. The solution is then filtered and incubated at 55<sup>o</sup>C for 15 min. Collect the filtrate and precipitate it with 3% Na<sub>2</sub>SO<sub>3</sub>. Set the pH using Na<sub>2</sub>SO<sub>3</sub>. Incubate the solution at 20<sup>o</sup>C and re-filter. Collect the precipitate and wash it with 16.7% Na<sub>2</sub>SO<sub>3</sub>, incubate this at 55<sup>o</sup>C for 15min and vortex and filter. Again collect the filtrate and add 5% of 5ml HgCl<sub>2</sub> vortex and filter again. Collect the filtrate and add 250µl of 3% Na<sub>2</sub>SO<sub>3</sub> and 12% Na<sub>2</sub>CO<sub>3</sub> in cool pack and incubate at -200C for 30 min and filter. The collected filtrate should be subjected for Dialysis for ensuring the purification.

***SDS PAGE for Purification of the sample:***

In order to separate the different proteins in the crude extract of the proteins 8% SDS Page was run. Using the ladder of known molecular weight the required protein can be identified as a band on the gel.

***Quantitative Estimation of ricin from Crude Samples of castor seed by Folin-Lowry's Method:***

6 various aliquots of the protein sample were taken in six test tubes in one of the tube add 0.6ml of the purified extract. Make up the level to 1ml using distilled water. The blank was prepared by taking 1ml of complete distilled water. To all the test tubes add 3 ml of Alkaline Copper sulphate reagent was added to each tube and mixed well. These solutions were incubated at room temperature for 10 minutes. 0.5 ml of Folin-Ciocalteu reagent was added to each tube and incubated at room temperature for 30 min. The OD values of the samples were read at 540nm. The absorbance was plotted against protein concentration to get a standard calibration curve. The absorbance of unknown samples was checked and the concentration of the unknown sample was determined using the standard curve plotted below.

***Testing the Efficacy of the compound Ricin on cancer cell lines:***

Cancer cell lines obtained from MTCC were used as targets to test the anticancer property of the RICIN

protein. To the cell lines the ricin protein extract is added and is incubated for a prescribed period of time. Once the incubation period is over the Optical density of the samples is read using spectrophotometer. Based upon the reading the activity of the protein can be analyzed.

***Testing for the anti HIV activity of ricin protein by insilico analysis:***

To confirm the anti HIV activity of ricin wrt to docking ability Insilico analysis was undertaken. To test the same the prerequisites are the structure of the receptor protein that is involved in HIV and the structure of the ricin.

***Receptor Protein sequence and structure retrieval:***

NCBI data base has been used to retrieve the protein sequence of CD4, having major role in cell proliferation and recognition. Once the sequence is obtained the structure is downloaded from the PDB data base. The PDB ID selected for the CD4 structure is 3o2d.

***Structure retrieval for the RICIN protein:***

To analyze the docking efficacy between the ricin and CD4 the structure of the ricin protein is required. The structure is also retrieved from the PDB. The id of the selected structure was 2vlc.

***Identification of Target Site in CD4:***

To increase the accuracy in the docking analysis, targeted docking was preferred. In order to identify the exact amino acid/ site in the CD4 protein to which the ricin protein has to bind several analysis has been performed.

***Antigenic Site prediction for CD4 Protein:***

Emboss antigenic was selected to identify the antigenic sites within the CD4 cells that are responsible for the major effects on the protein. Based on the analysis of the Emboss results the target region can be identified.

***PVS Server to identify the most antigenic site:***

Antigenic plot was designed and the antigenic propensities were calculated from the antigenic sites present within the CD4 protein. PVS server was used to plot the propensity curve for the protein.

***Disorder prediction using RONN:***

In order to finalize a site within the pathogenic region based upon the degree of disorderness it has RONN has been used. The RONN results would indicate the

probability of the disorderness possible for a given sequence.

**Docking:**

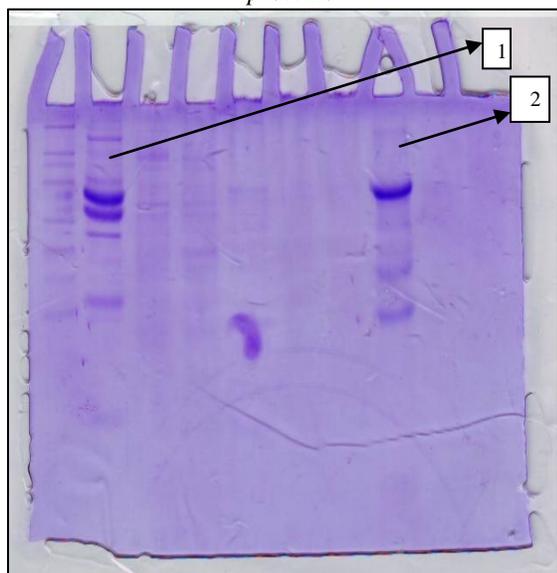
Argus lab has been used to dock the selected site on the CD4 receptor protein with the ricin protein. This is one type of the targeted protein-protein docking.

Based upon the docking energy the results can be analyzed.

**Results and Discussion:**

The results of SDS PAGE indicate the presence of the ricin protein in the crude mixture based on the band formation pattern compared with the standard ricin protein of known molecular weight.

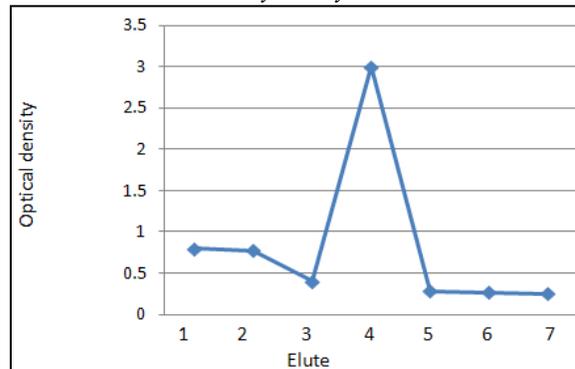
**Fig 1:** Showing the band pattern obtained for the crude protein extract along with the standard ricin proteins



In the Fig 1: 1 Band represents the sample protein and the band 2 represents the standard ricin.

**Estimation of Protein Concentration by Lowry's Method:**

**Fig 2:** Showing the graph plotted against the cell line sample and the Optical density obtained fro protein estimation by Lowry's Method

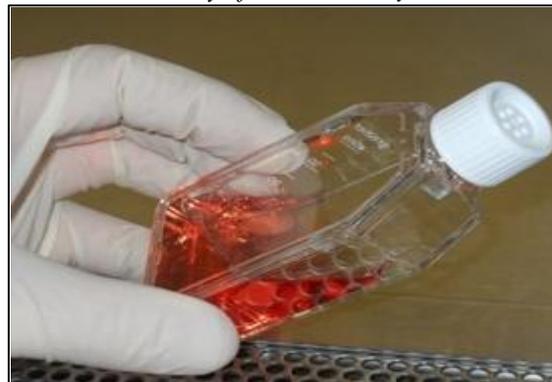


From the above graph the concentration of the extracted protein was calculated to be 400 µl.

**Testing for the anticancer activity of the Ricin protein on Cancer Cell Lines:**

To test the efficacy of ricin on the selected cancer cell lines inoculation and incubation was preferred followed by reading the Optical density values. The OD values were than plotted in the graph whose analysis would infer about the activity of the protein on the cell lines.

**Fig 3:** Showing the cancer cell lines used for the assay of ricin's activity



**Fig 4:** Testing for the anticancer activity with various sample aliquots:



**Table 1:** The OD value of cell line at 540 nm

Normal cell lines –	0.2601nm
Elute 1	-0.1597nm
Elute 2	-0.0762nm
Elute 3	-0.1001nm
Elute 4	-0.0521nm
Elute 5	0.0354nm
Elute 6	-0.0639nm
Elute 7	0.3448nm
Elute 8	0.2323nm

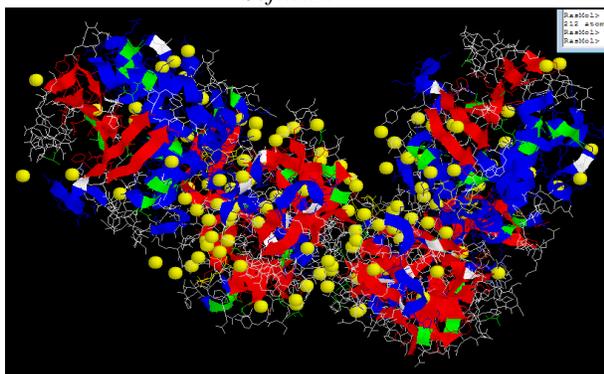
From the above table it can be inferred that, in the cell lines negative OD value indicates complete lysis of the cell. A positive value indicates controlled cell line and direct binding to target cell.

**Insilico Analysis:**

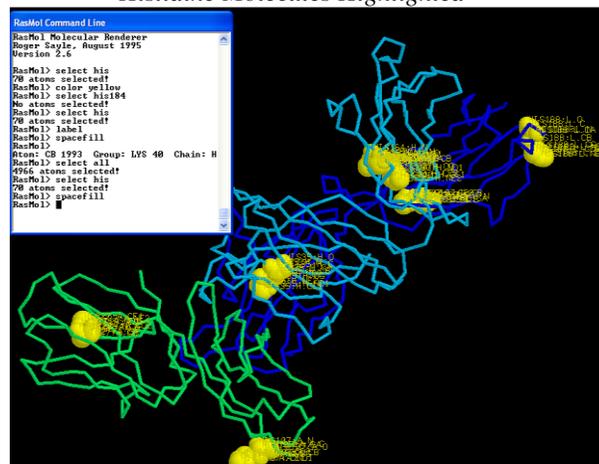
**Structure prediction of the Ricin and CD4 receptor proteins using PDB:**

To target the CD4 receptor protein responsible for HIV infection, with the ricin protein the structures of both were collected from Protein data Bank. The structures are shown below

**Fig 5:** 3D Structure of Ricin protein shown in Rasmol Software



**Fig 6:** 3D Structure of DC4 shown along with its Histidine Molecules Highlighted



**Fig7:** Results of Antigenic Sites prediction along with their antigenic propensity shown for CD4 protein

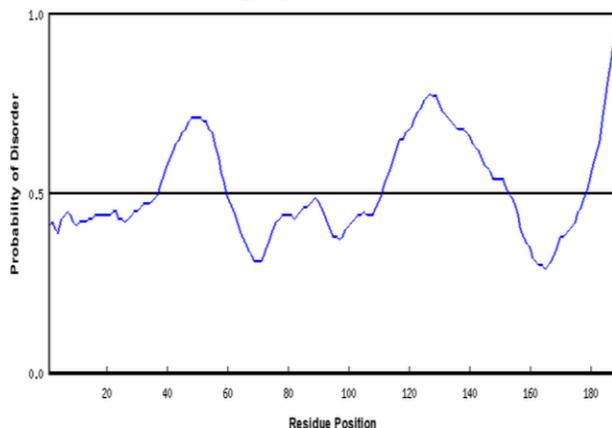
n	Start Position	Sequence	End Position
1	17	CEKKVVVLG	24
2	28	DTVELTCTA	36
3	84	NFPLIKN	91
4	98	DTYICEV	104
5	109	EEVQLLVFG	117
6	123	DTHLLQGQSLTLTLES	138
7	140	PGSSPSVQCRS	150
8	160	KTLSVSQLEL	169
9	175	WTCTVLQN	182
10	185	KVEFKIDIVVLAFAQKAAH	202

In the results shown above the best antigenic peptide is highlighted

**Detection of the final Target site based on the disorder probability as shown by RONN:**

Based on the results on RONN the best site that is falling in the disorder region as well the epitopic site can be selected. From the results of RONN the best id selected was Histidine 18 4 falling in the region of “KAHH” is selected as the best target with its disorder probability of 0.71

**Fig 8:** RONN disorder Probability Prediction for CD4 Protein



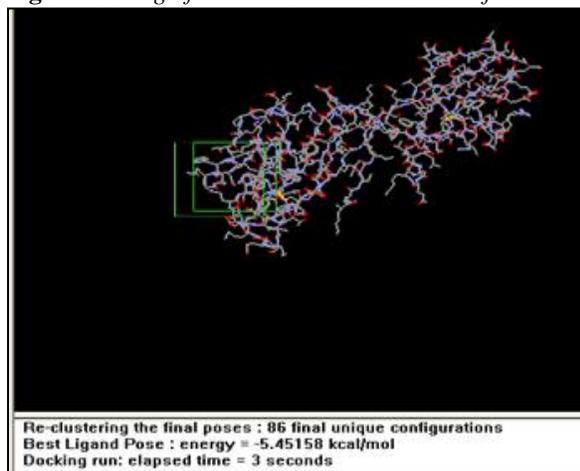
**Selection of the target site:**

As shown by the RONN results the best target site falling in the epitopic region with maximum disorder probability is **Histidine 184**. This was later used to target and bind with the selected ligand **Ricin** using **Argus Lab**

**Docking Analysis using Argus Lab:**

The CD4 His 184 is the target site where the Ricin protein has to dock. To perform the Targeted Docking Argus Lab is used.

**Fig 9:** Docking of Ricin Protein to HIS 184 of CD4



Based upon the docking output it can be inferred that the protein-protein docking of CD4 His 184 to ricin releases docking energy of -5.451 kcal/mol. This indicates a good docking pair.

**Conclusion:**

From the above study of ricin and its inhibitory activity against cancer and HIV it can be stated that ricin had good anticancer activity upon the tested cancer cell lines as indicated by their Optical density values measured by spectrophotometer. Similarly the anti HIV action has been tested in insilico conditions using Protein interaction studies or docking analysis. The HIV receptor selected here is the CD4 receptor protein to which the ricin is docked using Argus lab. The target site upon the CD4 was selected based on the antigenicity and disorderness as analysed from PVS and RONN. The docking results indicate that the interaction between the two proteins was acceptable as the docking energy released was -5.451 kcal/mol. Further clinical studies can be undertaken to conform the activity of ricin in the treatment of HIV infections and control of cell proliferation in cancer conditions.

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