Using Molecular Docking Develops Plant-Bioactive Compounds as Fungicides Against Tox-A Protein in Wheat Tan Spot Disease

Fareed Ullah[*], Salman khan[†], Ijaz Naeem[‡], Shah Fahad [§], Noreen Nawaz[**], Khalil Ur Rahman [††]

Abstract

Tan spot is a foliar disease of wheat caused by a fungus Pyrenophora tritici-repentis (Ptr) which produce Tox-A protein, results in major yield losses worldwide. Pyrenophora tritici-repentis acquire gene known as Tox-A through horizontal gene transfer from Stagonospora nodorum that code for toxins known as PTRTox-A or PTR necrosis Tox-A. Molecular docking of different bioactive compound, lycopene, zeaxanthin, lutein and vincristine with Tox-A was performed using Patchdock server. The study revealed that various bioactive compounds purified from various medicinal plants were effective against Tox-A protein. Based on Geometric shape complementarity score of first compounds (6560, 6100, 5856, 4748) of each complex, lycopene interacted best with Tox-A protein followed by zeaxanthin, lutein and vincristine. High interactability of lycopene with Tox-A protein of PTR is because of high Geometric shape complementarity (GSC) score, large Interface area (IA) and larger size of Lycopene-Tox-A complex. LEU 161, CYS 160 and PHE 147 are found in the entire three complex (Lycopene-Tox-A, Zeaxanthin-Tox-A and Lutein Tox-A complex). CYS 160 is found in Zeaxanthin-Tox-A and Lutein-Tox-A complex and is involve in Alkyl interaction while in Lycopene-Tox-A complex it involves in Vander Waal interaction. Pi-cation interaction is only limited to Vincristine-Tox-A complex and does not find in any other complex. All the bioactive compounds were screened in order of lycopene>zeaxanthin>lutein>vincristine, based on their interaction with Tox-A protein of Pyrenophora tritici-repentis.

***Keywords*:** Bioactive Compounds; Tox-A Protein; Necrosis; Molecular docking; Amino Acids.

Introduction

Wheat is an important ceral crop that fulfill the nutritional needs of billions of people worldwide, contributing about 3% to the global GDP

[*] Department of Biotechnology, University of Swabi, Swabi 23562, Pakistan, fareed23610@gmail.com

[†]Corresponding Author: Department of Biotechnology, Abdul Wali Khan University Mardan, Mardan 23200, Pakistan, salmankhanmrd48@gmail.com

[‡] Department of Biotechnology, University of Swabi, Swabi 23562, Pakistan, ijaznaeem@uoswabi.edu.pk

[§]Department of Biotechnology, Abdul Wali Khan University Mardan, Mardan 23200, Pakistan, shahfahadkhan4566@gmail.com

[**]Department of Biotechnology, Abdul Wali Khan University Mardan, Mardan 23200, Pakistan, noreennawaz21@gmail.com

[††]Department of Biotechnology, Abdul Wali Khan University Mardan, Mardan 23200, Pakistan, khalilurrahman@awkum.edu.pk

(Erenstein et al., 2022). Pakistan is the 10th largest producer in the world in term of wheat production (Shahzad et al., 2019). Globally wheat cultivation takes place on more than 220-million-hectare area. From the last seven years there is no increase in wheat production, while global population rate have increased by 160 million (Curtis et al., 2014). Yield loss of wheat due to tan spot disease are greater in older plants as compare to juvenile and can cause from 2% to 40% yield losses annually (Dinglasan et al., 2016). Tan spot disease is a foliar disease of wheat caused by fungi *Pyrenophora tritici-repentis (PTR)* (Lamari et al., 2010). The fungus causes damage to wheat leaves and lead to the formation of brown spot on the leaves, which are surrounded by chloratic spot (chloratic haloes) is due to the formation of lesion on the leaves, which results in photosynthesis slow down hence lead to yield loss (Pazdiora et al., 2016). *Pyrenophora tritici-repentis* has the property of homothallism and reproduce by mean of sexual and asexual reproduction through ascospore and conidiospore (Moreno et al., 2012). During spring season both sexual and asexual spore of *Pyrenophora tritici-repentis* grows on wheat leaf and start germination (Kayim et al., 2022). During germination the fungal spores produce a special out growth called germ tube, penetration peg and specialized cell known as appressorium that helps fungus to infect plants (Chethana et al., 2021). Cold temperature around 10°C and high humidity enhance multiplication of germ tubes from spores, successively various steps are involved in the infection process of host plant by fungi (Evans et al., 1995). When the germ tube starts formation of appressorium cells, just beneath the cell layer a penetrative peg starts to develop, which helps the fungus to enter leaf directly or via stomata to host plant (Chethana et al., 2021). This whole penetration process takes about 3hrs to complete (Abdullah et al., 2017).

Toxic compounds produced by *Pyrenophora tritici-repentis* is the causative agent of tan spot (Orolaza et al., 2019). *Pyrenophora tritici-repentis* acquire gene known as *Tox-A* through horizontal gene transfer from *Stagonospora nodorum* that code for toxin known as *PTR Tox-A* or *Tox-A* or *PTR* necrosis *Tox-A. Tox-A* is small protein of 13.2 kDa, which cause necrosis when there is light and active host metabolism (Ramos et al., 2019). While the *Tox-B* and *Tox-C* cause chlorosis in wheat (Sarova et al., 2005). Based on survey performed on cereal crops in central Asia in 1986 tan spot was considered one of the most important wheat crop disease (Maulenbay et al., 2022). Increase in incidence of disease are linked to reduced tilling practice, lower crop rotation and continuous cultivation of wheat crop (Hesston CEF 1992). In order to contain the decrease there is a need for proper tilling system, crop rotation and appropriate use of fungicides, but these methods are very laborious and costly (Simón et al .,

2020), since the only effective solution to such problem is to use genetically resistant cultivars (Kokhmetova et al., 2021). Initially *Pyrenophora tritici-repentis* were classified into four different pathotypes on the basis of symptom they develop on wheat cultivars (Andrie et al., 2007). Such as Pathotype 1 are the most common pathotypes which produce both kind of symptoms i.e. chlorosis and necrosis (chl+, nec+), while pathotype 2 induce only necrosis and does not produce chlorosis (nec+, chl-) (Friesen et al., 2001). While pathotype 3 are able to produce only chlorosis in wheat (chl+, nec-), while pathotype 4 are not able to produce any kind of symptoms (chl-, nec-) (Lamari et al., 1991).

Tox-A toxin is well studied among all the other toxic proteins, such as *Tox-B* and *Tox-C* produce by *Pyrenophora tritici-repentis* in wheat tan spot disease (Moreno et al., 2012). Response to *Tox-A* protein is assigned by single sensitive locus Tsn1 located on 5BL chromosome, *Tox-A* gene codes for pre and pro section of protein (Galagedara et al., 2018). The pre portion of protein is responsible for protein secretion while the pro region of 4.3 kDa anionic N domain and 13.2 kDa C 13 domain is responsible for protein folding (Miles-Rockenfield KB 2009). Expression of both domain of *Tox-A* protein in bacteria (Escherichia Coli) give more specific protein and will show that N domain are responsible for proper folding of mature protein, while C domain consist of two cysteine residue and responsible for the function of protein (Tuori RP 1998). Within *Tox-A* sequence of protein there is conserve region called RGD domain (Arginyl-Glycyl-Aspartic acid), this region is important for toxic effect of protein and mutation in this region (mutation from RGD to RAD or RGE) shows significant decrease in toxicity of protein (Day et al., 2015). Tox-A toxin enter into mesophyll cell of sensitive plant with the help of RGD domain and transport to chloroplast of plant cell, in chloroplast it interacts with Tox-A binding protein-1 (Tox-ABP1), which is involved in photosystem II and thylakoid formation (Ching, 2007). But current investigations suggest that *Tox-A* interact with both photosystem I, II, and lead to accumulation of reactive oxygen species (ROS) in chloroplast(Barkla, 2016).

Tox-A toxin does not require the presence of pathogen to initiate cell death and it is confirmed when mature toxin is transfer to into empty space present between cell membrane and cell wall of sensitive plants it triggers the disease own its own (Meinhardt et al., 2002). Plant bioactive molecules have great potential against plant pathogenic diseases and provides many important health benefits to plants i.e. vigor, resilience and yield (Teklić et al., 2021). Secondary metabolites contain large number of terpenoids, glycosides, alkaloids, steroids and phenolics (Ahmed et al., 2017). Several plant bioactive compounds are widely used as a green

fungicides against pathogenic fungi, as in case of wheat tans pot disease they interact with Tox-A protein and inhibit the growth of *Pyrenophora tritici-repentis* (Sánchez et al., 2016). The purpose of the study is to perform molecular docking of four Plant bioactive compounds with Tox-A protein. These bioactive compounds include lutein from Spinach, kale, collards, lycopene from tomatoes, watermelon, and pink grapefruit, vincristine from Catharanthus roseus and zeaxanthin from Spinach, kale, and collards. And to build three-dimension structure of Tox-A protein to study interaction of our target compounds with Tox-A protein using molecular docking studies. By utilizing Patchdock a geometric shape complementarity database is to identify best compound based on GSC score of each complex. To knows the interaction of Tox-A protein with various bioactive compounds. To find compounds which interact best with Tox-A protein and have important anti-fungal activity through molecular docking study.

Materials and Methods

Retrieval of Protein Sequence from Uniprot

Protein sequence of Tox-A (PDB ID: m412) was retrieve from Uniprot using the link given below. Then we downloaded our desired protein in FASTA format. FASTA sequence of protein contain the single letter amino acid sequence, containing residue 61-178.
Link: https://www.uniprot.org/uniprot/P78737

Protein Modeling using Swiss Model

A protein model was developed using Swiss Modeling. FASTA format of protein was uploaded to Swiss model. Various structure assessment option were applied during modelling and download the structure in PDB format using the following link. Value of GMQE (Global Model Quality Estimate) and QMEAN (Qualitative Model Energy Analysis) of protein model calculated as 0.42 and -0.31 respectively.
Link: https://swissmodel.expasy.org/interactive/FkU2MY/models/

Active Site Determination

Active site of Tox-A protein was determined using CASTp (http://sts.bioe.uic.edu/castp/calculation.html). Then the PDB ID of protein were uploaded to CASTp to various amino acid residues present in active site and pockets respectively. PDB file were also uploaded by selecting calculations in upper portion of CASTp. The value of radius probe was selected as 1.4Å by default and leaving the remaining parameters unchanged. CASTp usually show the entire pocket along with

amino acid, protein chain and atoms present in the protein. Active site with pocket ID is mostly involved in ligand interactions.

Ligand Structure

Ligand structure of lycopene (CID 446925), zeaxanthin (CID 5280899), vincristine (CID 5978), and lutein (CID 5281243) were retrieved from Pubchem database (https://pubchem.ncbi.nlm.nih.gov/). The compound structures were downloaded from the database in SDF format. Usually, 3D structures are obtained from Pubchem databases. Online SMILE translator (https://cactus.nci.nih.gov/translate/) were utilized to convert SDF format into PDB format. The 3D structures of ligands were then optimizing through "Biovia Discovery Studio Visualizer".

Molecular Docking through Patchdock Server

Docking of ligands with Tox-A protein was performed through Patchdock server (see the link below). Patchdock as molecular docking software which works on shape complementarity principle. According to procedure PDB ID of ligand and receptor were uploaded in corresponding section. RMSD clusters value were kept at 4.0 by default and selected protein small ligand as complex type. Docking was performed for all of the four ligands with Tox-A protein. Geometric shape complementarity (GSC) score of first solution of every complex were recorded. The complex file of first solution were obtained in PDB format and were analyzed through Discovery Studio Visualizer software.
Link: https://bioinfo3d.cs.tau.ac.il/PatchDock/php.php

Results and Discussion

GSC score of compound 1 Lycopene-Tox-A complex (6560) was higher than any other compound. High score of compounds one shows that this complex has larger size and Interface area (IA) about 844.90 (Table 1; Figure 1). Moreover, the value of transformation of compound 1 shows that the site where the ligands bind to the receptors were heterogeneous. Analysis of compound one of Lycopene-Tox-A complex through DS visualizer showed that amino acid residues LEU 161, TYR 144, ARG 140, CYS 160, ASN 162, GLY 148, ARG 169, PHE 147 and SER 166 were involved in lycopene interaction (Figure 2). The interactions found in this complex are Alkyl, Pi-alkyl, Vander Waal and some unfavorable non bond interaction. PHE 147 and TYR 144 bind through Pi alkyl interaction with lycopene and the bond distance among them was 4.50Å and 4.63Å respectively. LEU 161 bind with lycopene through Alkyl interaction with bond distance among them were 5.38Å. While CYS 160 and SER 166 are

involved in Vander Waal interaction with lycopene while other residue forms some unfavorable non bond interaction with lycopene. Hydrogen bond is found among residue CYS 64 and SER 63, while Conventional hydrogen bond interaction is found between residue CYS 160N - GLY 62O and GLY 62N-CYS 160O with bond distance between them is 2.87Å and 2.76Å (Table 1).

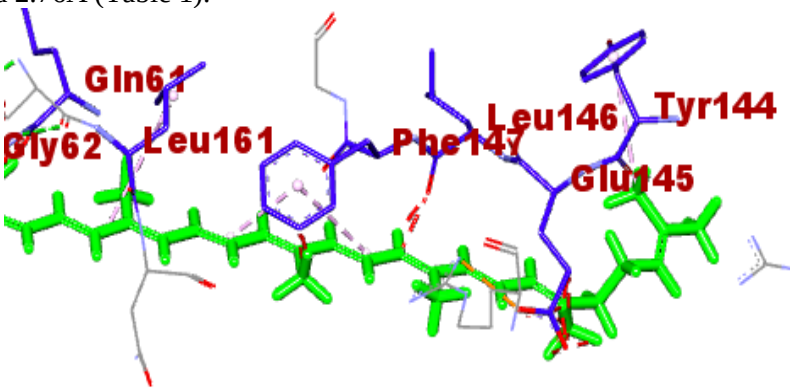

Figure 1: Structures of bioactive compound obtain from DS visualizer. White color represent hydrogen, red color represent oxygen, blue color represents nitrogen atoms and grey color represent carbon backbone.

Table 1: Value of different parameters of solution one of each complex.

S. No.	Complex Type	GSC Score	Area	ACE	Transformation
1	Lycopene-Tox-A	6560	844.90	-314.12	2.45, -0.54, -2.26, 46.37, 16.57, -78.62
2	Zeaxanthin-Tox-A	6100	724.10	-317.79	1.01, 0.44, 0.82, 47.81, 18.87, -76.78
3	Lutein-Tox-A	5856	799.50	-410.36	0.71, 0.31, 0.46, 45.33, 13.85, -82,79
4	Vincristine-Tox-A	4748	582.10	-141.39	2.29, -0.43, 1.07, 31.44, 30.51, -74.89

Zeaxanthin is the second most interactive compound with Tox-A after lycopene because GSC score of Zeaxanthin-Tox-A complex is lower than Lycopene-Tox-A complex. GSC score of compounds 2 Zeaxanthin-Tox-A complex (6100) shows that this complex has large size, however their size is lower than that of Lycopene-Tox-A complex Table 1. Lutein and zeaxanthin are important carotenoids which improve visual performance of the eyes (Kvansakul et al., 2006). Lutein and zeaxanthin cannot be synthesize by the body and must be obtain from the diet (Mares et al., 2002). The site of transformation of compound with highest scores

were 1.01, 0.44, 0.82, 47.81, 18.87 and 76.78 respectively Table 1. The site of transformation actually shows positions of different elements of ligands binding with the Tox-A proteins. Compounds with the highest score were then analyzed with DS visualizer. Compound 2 zeaxanthin and Tox-A complex were studied through DS visualizer and their analysis revealed that amino acid residues i.e., TYR 150, ARG 140, TYR 144 LEU 146, LEU 161, PHE 147, CYS 160, and THR 115 were involved in interaction with zeaxanthin. PHE 147 and TYR 144 link with zeaxanthin through Pi-alkyl interaction with bond distance 3.37Å and 5.23Å. CYS 160 and LEU 161 form Alkyl interaction with zeaxanthin with bond distance 4.71Å and 5.31Å (Figure 3). While TYR 150, ARG 140 and LEU 146 were involved in Vander Waal interaction with zeaxanthin. Hydrogen bond was found among GLY 62N and CYS 160O, while conventional hydrogen bonding was noticed between CYS 160N and GLY 62O with bonding distance of 2.87Å.

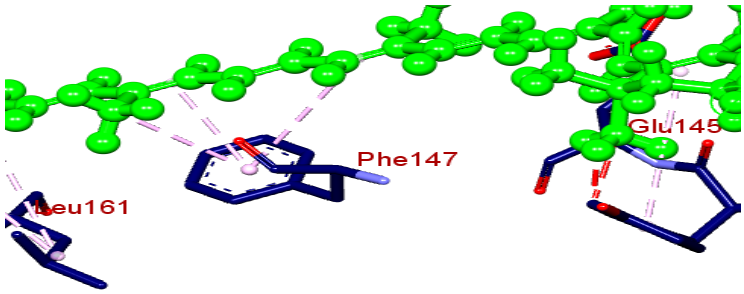

Figure 2: Amino acid residue of Tox-A protein involved in lycopene interaction.

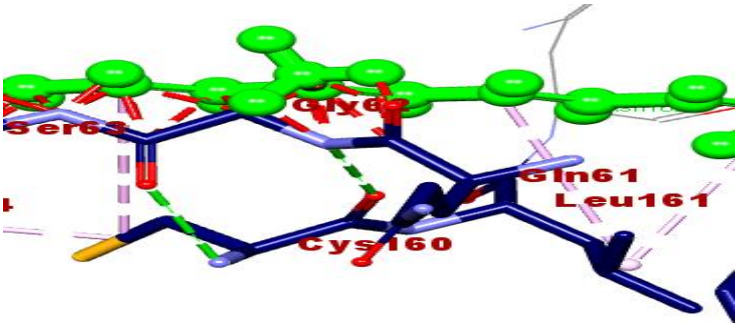

Figure 3: Amino acid residue of Tox-A protein involved in zeaxanthin interaction.

On the other hand, molecular docking of lutein with Tox-A protein, showed that this compound has lower intractability with Tox-A

protein because of lower GSC score of the complex (5856) and minimum interface area around 799.50. Analysis of compound 3 Lutein-Tox-A complex through DS visualizer showed that LEU 161, PHE 147, CYS 160, TYR 150, ILE 67, ARG 117 and GLY 148 were involved in Lutein interaction. While LEU 146, LEU 161 and CYS 160 were involve in Alkyl interaction with Lutein, and the possible bond distance between the amino acid residue and ligand were 5.37Å, 5.24Å and 5.02Å respectively. TYR 150 and PHE 147 bind through Pi-alkyl interaction with Lutein. Bond distances between these interacting residues and compounds was 5.13Å and 5.07Å respectively. Similarly, ILE 67, ARG 117 and GLY 148 linked through nonpolar Vander Waal interaction. Analysis of this complex through DS visualizer shows that there is Carbon hydrogen bond interaction present between amino acid residues in addition with hydrogen bond and conventional hydrogen bond interaction as well. Carbon hydrogen bond interaction were found between ARG 117-ILE 67 and GLY 148-TYR 150 with bond distances of 3.10 Å and 3.69 Å respectively. Hydrogen bonding were noticed between ASP 149N-ASP 149O and ILE 67N-ser 66O, while Conventional hydrogen bond interaction were found in CYS 160N-gly 62O and GLY 62N-CYS 160O with bond distance of 2.87Å and 2.76Å.

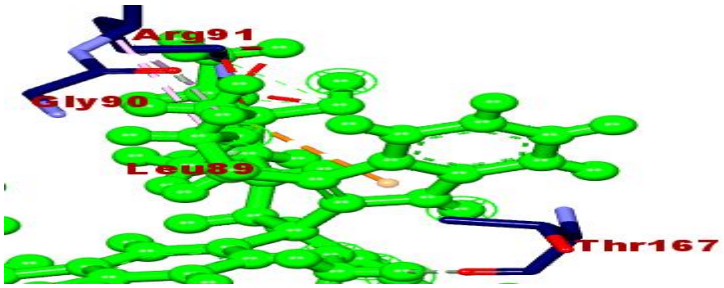

Figure 4: Amino acid residue of Tox-A protein involved in vincristine interaction.

Similarly docking of compound 4 Vincristine with Tox-A protein shows that this compound has the lowest interaction with Tox-A protein. Lowest activity of vincristine against Tox-A is because of the lower GSC value of Vincristine-Tox-A complex than any other complex. Vincristine contains specific alkaloids which are extremely toxic and used as anti-cancer agent (Dhyani et al., 2022). Anti-diabetic, anti-oxidant and anti-microbial activity of vincristine was also reported in previous studies (Dada & Nilima, 2021). Vincristine isolated from Catharanthus roseus were utilized as an anti-cancer agent for 40 years and was consider more effective than any others anti-cancer drugs available (Dhyani et al., 2022).

The size of this complex is smaller as compared to other complex and the interface area of Tox-A-Vincristine complex (582.10) is lower than the other complex such as lutein-Tox-A, zeaxanthin-Tox-A, and lycopene-Tox-A. The transformation site of vincristine on receptors are 2.29 -0.43, 1.07-31.44 and 30.51 -74.89 (Figure 4). Analysis of compounds with highest GSC scores through DS visualizer showed that Pi-cation interaction were found in Vincristine-Tox-A complex which is limited to only this complex and wasn't reported in any other complex.

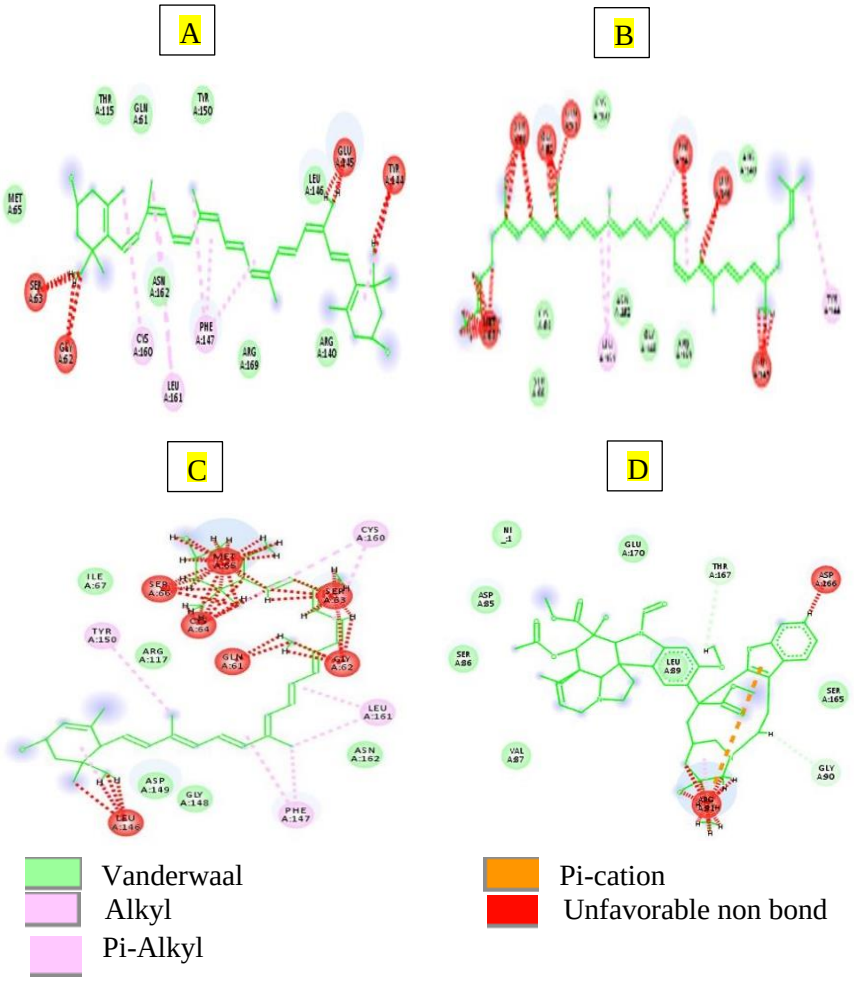

Figure 5: These figures shows various types of interaction occurs between ligands and receptor. (A) Lycopene-Tox-A (B) Zeaxanthin-Tox-A (C) Lutein-Tox-A (D) Vincristine-Tox-A

However, Alkyl and Vander Waal interaction was also found in this complex. Amino acid residues which are involved in Vincristine interaction are ARG 91, GLU 170, ASP 166, GLY 90, and THR 167. ARG 91 is involved in Alkyl interaction with bond distance of 4.98Å. Pi cation interaction is found in this complex, which is form by ARG 91 with Vincristine. Carbon hydrogen bond interaction were found in SER 165-GLY 90 and the bond distance between these residues are 3.58Å. ASP 166N-ASP 166O and VAL 87N-SER 86O form hydrogen bond, while conventional hydrogen bond interaction was found between the residue GLY 90N-VAL 87O and LEU 89N-ASP 85O and the bond distance between two residues was 3.22Å and 2.84Å (Figure 5, Table 2).

Table 2: List of amino acid residue involve in hydrophobic interaction. Hydrogen bond and conventional hydrogen bond interaction is present among the amino acid's residue.

Protein ligand complex	Amino acid residue involves in hydrophobic interaction	Hydrogen bond among the residue	Conventional Hydrogen bond interaction among the residue
Lycopene-Tox-A	TYR 144, GLU 145, LEU 146, PHE 147, LEU 161, GLN 61, GLY 62, SER 63, MET 65	CYS 64(N-O)SER 63	GLY 62 (N-O)CYS 160
Zeaxanthin-Tox-A	GLY 62, SER 63, CYS 160, LEU 161, PHE 147, GLU 145, TYR 144	ARG 169(NH2-O)GLU 145, CYS 160(N-O)GLY 62	CYS 160(N-O)GLY 62
Lutein-Tox-A	LEU 146, PHE 147, TYR 150, GLN 61, LEU 161, GLY 62, CYS 160, SER 63, CYS 64, MET 65, SER 66, GLY 148, ARG 117	ASP 149(N-O)ASP 149, ILE 67(N-O)SER 66	ARG 117(NH1-O)ILE 67
Vincristine-Tox-A	LEU 89, GLY 90, THR 167, ASP 166, ARG 91	VAL 87(N-O)SER 86, ASP 166(N-O)ASP 166	GLY 90(N-O)VAL 87, LEU 89(N-O)ASP 85

Conclusions

It was concluded from the study that lycopene is the only bioactive compound which will inhibit the activity of Tox-A protein up to greater extent, followed by Zeaxanthin, Lutein and Vincristine respectively. LEU 161, CYS 160 and PHE 147 were found in the entire

three complexes like Lycopene-Tox-A, Zeaxanthin-Tox-A and Lutein-Tox-A. PHE 147 is found to be involved in Pi-alkyl interaction in all the complexes except Vincristine-Tox-A complex. CYS 160 is found in Zeaxanthin-Tox-A and Lutein-Tox-A complex and is involved in Alkyl interaction while in Lycopene-Tox-A complex it was involved in Vander Waal interactions. Hydrogen bond and conventional hydrogen bond interaction was identified between the amino acid residues of the complex. CYS 160N-GLY 62O residue was found in all the three complexes like Lycopene-Tox-A, Zeaxanthin-Tox-A and Lutein Tox-A with bond distance of 2.87Å. Carbon hydrogen bond was found only in Lutein-Tox-A and Vincristine-Tox-A complex and was not reported in any other complex until now. Additional noncovalent interaction was found in Vincristine-Tox-A complex identified as Pi-cation interaction; such kind of interaction are produced by ARG 91 with vincristine. Lower activity of vincristine against Tox-A was because of lowest GSC score and lower Interface area of vincristine-Tox-A complex than any other complex in current study.

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