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Molecular Docking and Molecular Dynamics Simulation of Enzyme-Drug Interactions: In Silico and in Vitro Evaluation of Flavone as a Potential Inhibitor of Human Triose Phosphate Isomerase (TPI1)

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Abstract

Other choices for comprehending chemical systems are offered by computational techniques, which produce information that is challenging, if not impossible, to gain from laboratory examination. Using computational methods like docking, it is simple to verify a compound's strength of binding in the active site after it has been constructed within such computer programs. Flavonoids are a vital component of many pharmacological, cosmetic, nutraceutical, and medicinal applications due to their various health-promoting qualities. This is mainly because of their ability to regulate the activity of crucial cellular enzymes and their anti-oxidative, anti-inflammatory, anti-carcinogenic, and anti-mutagenic properties. By concentrating just on the most likely compounds, computer-aided drug design (CADD) helps scientists save time and money on chemical and therapeutic testing. Our primary goal was to employ molecular docking and molecular docking simulation programs to investigate the inhibitory effect of flavonoid drugs toward the TPI1 enzyme. This inhibitor might then be tested experimentally to stop the activity of the enzyme. Flavone were specifically selected to evaluate their binding capacities. This study also investigated the application of the flavone compound and its effect on serum samples from female breast cancer patients—breast cancer being the most common cancer among women, accounting for 22% of all malignant tumors. The enzyme triosephosphate isomerase (TPI1) has been identified as a potential oncogene and a biomarker in the serum of patients with breast cancer and other malignancies. Serum samples were collected and subjected to a multi-step partial purification process, including gel filtration, dialysis, and the use of ion-exchange resins (such as DEAE-cellulose and Sephadex G100). The purification results yielded an enzyme recovery rate of 41%, a purification factor of 1.55, and a specific activity of 0.36 U/mg. Kinetic analysis determined the optimal substrate concentration to be 5.5 mM, the K_m value to be 11.11 mM, with a maximum velocity of 12.5 units/mL (V_{max}). The enzyme showed maximal activity at 37°C with an ideal pH of 7, according to an analysis of the influence of different variables on the enzymatic reaction rate.

Keywords

Triose phosphate isomerase 1,
Molecular docking,
Flavone.



1. Introduction

Flavonoids are secondary metabolites found in a wide range of fruits, vegetables, herbs, stems, grains, nuts, flowers, and seeds. Occurring as chemical compounds in over 4,000 species, they serve to protect plants from ultraviolet radiation [1, 2]. More than 10,000 flavonoid compounds have been discovered and isolated, with particular medical significance attributed to their role as antioxidants [3,4]. Flavonoid compounds play a pivotal role in pharmaceutical applications, as they are considered among the most important antioxidants [5]. They act as antioxidants in the context of various conditions, such as cancer, heart disease, and neurological disorders, functioning as both antioxidants and anti-inflammatory agents [6, 7].

The TPI1 enzyme plays a key role in numerous biological processes and is essential to the glycolysis pathway [8]. TPI1 is located at 12p13.31 [9,10]. Other names for it include TIM, TPI, TPID, and HEL-S-49. The interconversion of di hydroxy-acetone phosphate and glyceraldehyde-3-phosphate is catalyzed by triose phosphate isomerase, which is encoded by TPI1. in the glycolysis and gluconeogenesis pathways, respectively. It consists of two identical subunits [11].

In our earlier research [12], we observed a strong link between serum TPI1 levels and newly diagnosed patients, suggesting that serum TPI1 could be a useful early diagnostic marker for diabetic individuals. A cutoff value of 16.85 ng/ml emerged as the most reliable measure to distinguish patient cases from controls.

In our current study, Flavone compounds were selected to be used as inhibitors of TPI1 enzyme in order to assess their binding capabilities and overall reactivity with the TPI1 enzyme (PDB ID: 2jk2). Later, we focus on assessing the inhibitory potential of vitexin compound against the TPI1 enzyme, which has been purified from the serum of women with breast cancer. this compound could be

then o experimentally to inhibit the enzyme from the serum of women with breast tumor.

2. Material and Methodology

2.1. Theoretical part

2.1.1. Molecular docking

Scoring calculations and molecule docking using MOE [13]. The target protein 2jk2 was taken from the pharmacological Bank and chosen for a molecular docking study as a potential pharmacological inhibitor of flavonoid compounds (Flavone). The 3D crystal structures of the enzymes were acquired from the Structural Protein Data Bank using their (PDB IDs:2jk2). The resolution of the crystal structure of the TPI1 enzyme (PDB IDs: 2jk2) was 1.70 Å. Receptors were selected as rigid and ligands as flexible for the docking simulation.

The RMSD (root mean-square distance) and -7 Kcal/mol of energy score of the criterion for the result obtained fall between 1.5 and 2.5. Receptors were selected as rigid and ligands as flexible for the docking simulation. The RMSD (root mean square distance) and -7 Kcal/mol of energy score of the criteria for the result produced from the molecular docking were measured in the range of 1.5-2.5 [14,15].

2.1.2. Molecular dynamics simulation

A 100-nanosecond molecular dynamics (MD) simulation using the Desmond module in the Schrödinger suite was used to investigate the structural stability of flavones under physiological settings [16]. An orthorhombic periodic boundary box was set up using the TIP3P water model to protect the system's integrity [17]. Sodium (Na⁺) and chloride (Cl⁻) ions were randomly distributed within the solvated system to achieve a 0.15 M salt concentration. The OPLS_2005 force field was then applied at 300 K and 1 atm to minimize and equilibrate the system [18].

Establishing an equilibrium state for the Flavone complex was the initial step, followed by final simulations recorded at 100 ps intervals.

2.2. Experimental Part

2.2.1. Methods

An initial 10 ml serum sample was collected from women with breast cancer for enzyme purification. In the preliminary step, proteins in the serum were precipitated by gradually adding ammonium sulfate until an 80% saturation level was reached specifically, 4 grams of ammonium sulfate spread over about 40 to 60 minutes. The subsequent step involved dialysis to eliminate any remaining ammonium sulfate. For this, the dissolved protein was placed into a dialysis bag, which was then immersed in 10 mM Tris-HCl buffer (pH 7.2). For 24 hours, the buffer solution was periodically replaced while maintaining a low temperature of 4°C to preserve the enzyme's activity. In the next phase, ion exchange chromatography was performed.

The resin was prepared by dissolving 2.75 g of DAEA-cellulose in 200 ml of 10 mM Tris-HCl buffer (pH 7.4), then allowing it to sit at 4°C for approximately 20 to 24 hours. Lastly, gel filtration was applied using Sephadex G-100 for purification. Specifically, 2.5 g of Sephadex G-100 was dissolved in 200 ml of 10 mM Tris-HCl buffer (pH 7.4), which was also left at 4°C for 20 to 24 hours [8]. The transformation of glyceraldehyde 3-phosphate (GAP) into dihydroxyacetone phosphate was tracked in order to assess enzymatic activity. Using a UV-Visible spectrophotometer (Shimadzu, Japan 1700), the decrease in absorbance at 340 nm was used to monitor the oxidation of NADH. The ideal

temperature was determined by analyzing temperatures between 7 °C and 57 °C. Activity was assessed in 10 mM Tris-HCl buffer with a pH range of 4.5 to 9 in order to determine the enzyme's ideal pH. Finally, to determine Km and Vmax, glyceraldehyde 3-phosphate concentrations ranged from 0.5 to 5.5 mM.

Using Esnouf's method [9,10], which links the isomerization step to an NAD-dependent enzymatic process, TPI1 activity was determined. Briefly, a 10 µl sample of TPI1 was combined with 20 µl of triethanolamine-HCl buffer (25 mM, pH 7.9) containing either 0, 100, 200, 300, 400, or 500 µg/ml of Flavone (in DMSO) and allowed to stand for 30 minutes before starting the assay. In a 1-cm quartz cuvette, 5 µl of α-glycerophosphate dehydrogenase (10 mg/ml), 25 µl of NADH (7 mM, disodium salt), and 50 µl of glyceraldehyde phosphate (10 mM) were mixed with 1.4 ml of triethanolamine-HCl buffer. After the process was initiated by adding the TPI1/inhibitor combination, the absorbance at 340 nm dropped.

3. Results and Discussion

Using MOE, Flavone compound were docked against the TPI1 enzyme (2jk2 protein) target, with the resulting docking scores. Docking scores for Flavone is (-6.2Kcal/mol) summarized in Table 1. Flavone which belongsto flavonoids compounds could be chosen for further investigation due to its strong experimental performance.

Table 1: Valid molecular docking scores with 2jk2 were demonstrated by the compound' binding affinity, RMSD, and molecular docking score.

E kcal/mol	Distance	Type of interaction bond	Involved receptor residues	Atom Of Receptor	Atom of compound	RMSD (Å)	S score (kcal/mol)	Drugs
-0.7	3.34	H-donor	GLN64	OE1	C12	2.421	-6.2	Flavone
-0.8	3.61	H-donor	GLU97	OE1	C15			
-0.8	3.44	H-donor	GLN64	O	C17			
-1.3	3.22	H-acceptor	ASN11	ND2	O28			
-0.8	3.29	H-acceptor	ARG98	CD	O29			
-1.3	4.22	Pi-cation	LYS13	NZ	6-Ring			
	3.59							

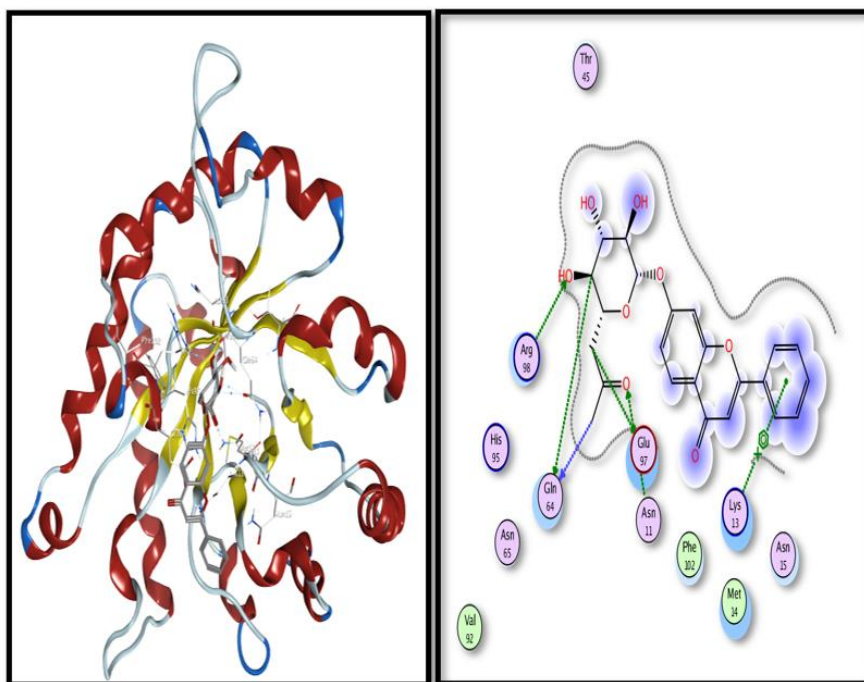


Fig.1. Flavone compounds binding with 2jk2 protein

Figure 1 displayed the significant complex poses between Flavone and 2jk2 protein and their interactions. Three hydrogen-bonded donor and Two hydrogen bonded acceptor contacts were observed by Flavone at the binding region amino acid residues of 2jk2 (GLN64, GLU97, GLN64, ASN11, ARG98). Flavone derivatives with a focus on activity and bioavailability concerning increase Insilco and experimental effectiveness. The MD simulation aids in comprehending both the stability of the Flavone and the interactions that take place between

molecules throughout the target period. Additionally, if a complex system were created in an artificial environment, it might estimate the structural evolution that would take place. A relatively equivalent RMSD value was reported in Figure 2 after a 100 ns MD simulation was performed to analyze the structural change of the target 2jk2 protein in the resulting complex of the desired chemical. Throughout the simulation, the protein and ligands in Flavone and 2jk2 stayed stable[19].

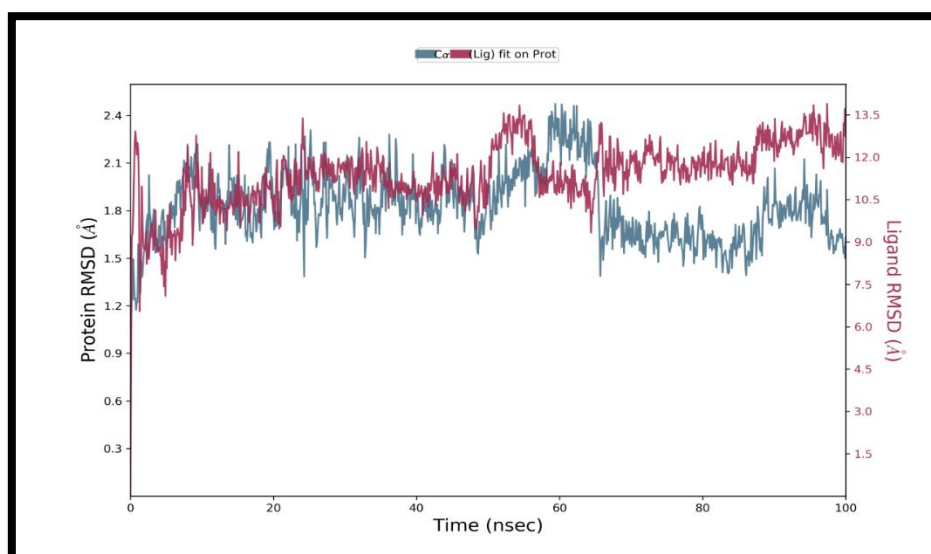


Fig.2. The complex of compound Flavone - 2jk2 with RMSD analysis

To track the change in protein structural flexibility that took place when particular molecules attached to a particular residual site, the RMSF values of the molecule Flavone complexes with the 2jk2 protein

were computed. Figure 3 shows the peak area of the 2jk2 protein at residual locations that showed the highest volatility throughout the simulation for each of the chosen chemicals.

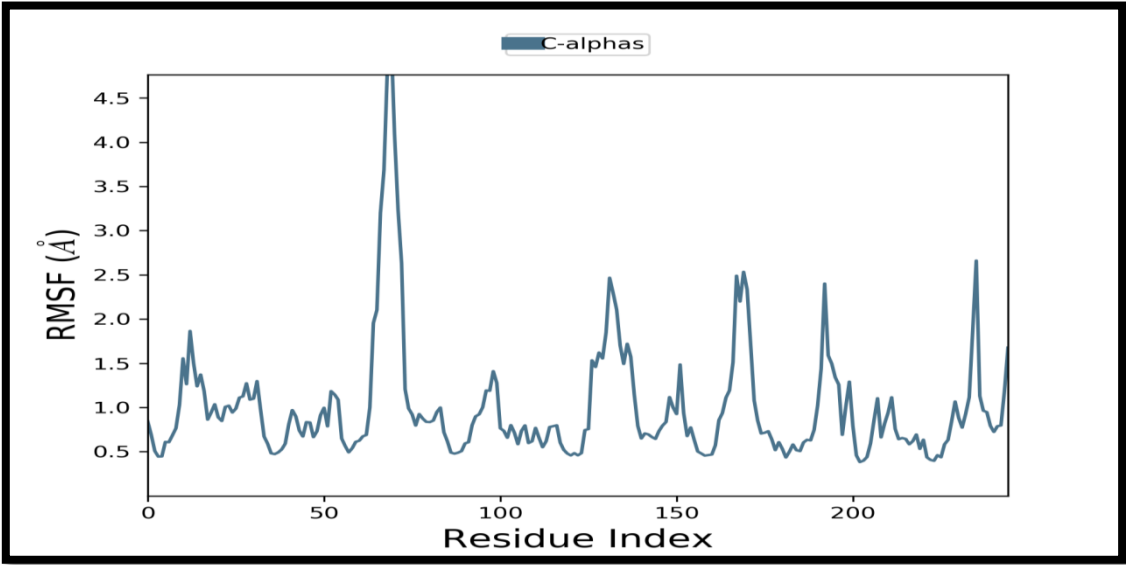


Fig. 3. The complex Flavone -2jk2 RMSF analysis

Figure 4 provides an explanation of the secondary structural elements (SSE) that were present in the 2jk2 protein during the simulation. Collectively, these constituents account for approximately 47.27 percent of the secondary structure of the 2jk2

protein in complex. More precisely, beta-strands and alpha helices make up around 15.47 and 31.08 percent of the secondary structural components, respectively for complex[20].

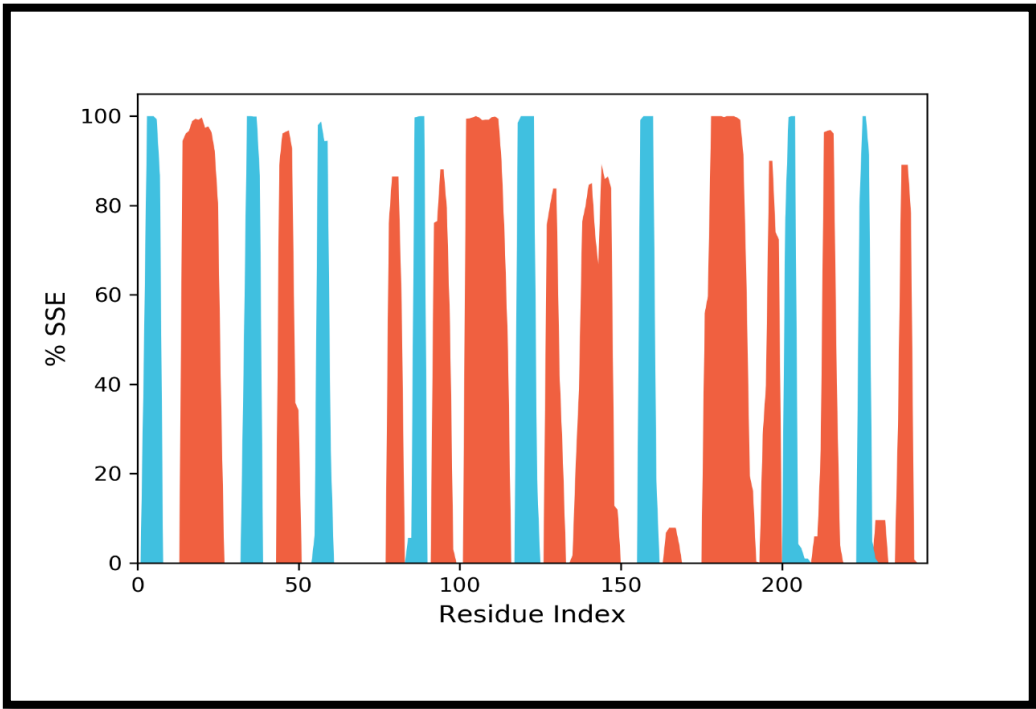


Fig.4. The SSE plots of the complex Flavone -2jk2 protein

As seen by the figure in Figure 5, the ligand repeated contacts preserve the unique interaction throughout the simulation. The chemical Flavone compound established many connections with the

residues SER96, HIS100, LEU131, ASP132, VAL167, and ILE170 that persisted for the length of the simulation.

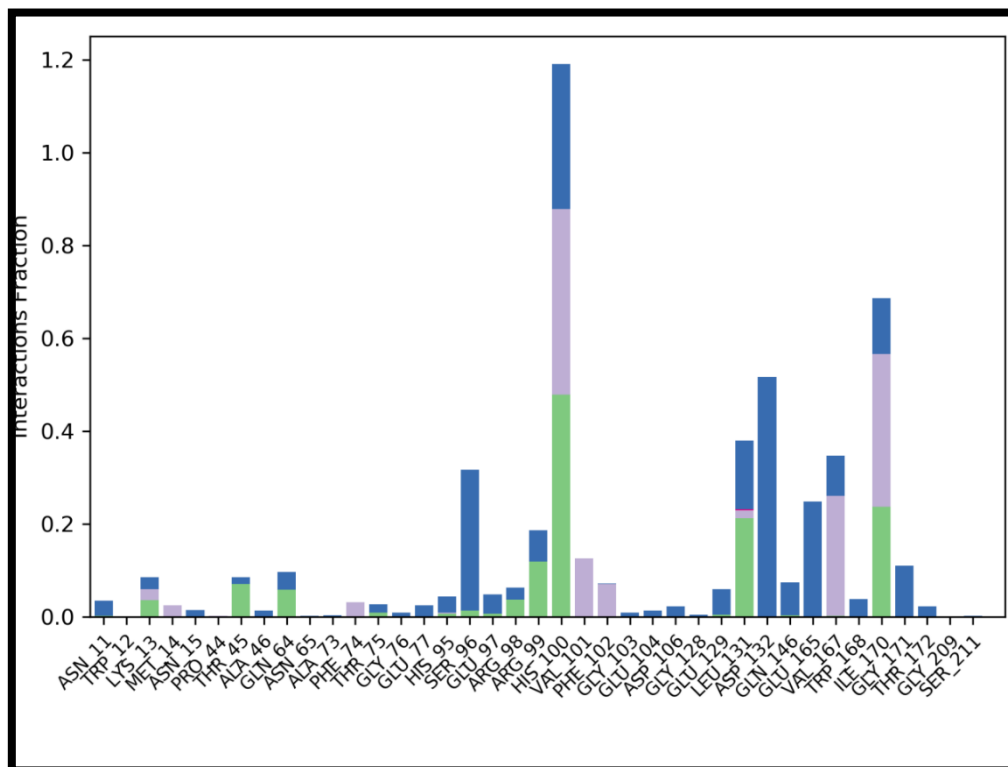


Fig.9. The interactions analysis of the complex Flavone -2jk2 protein

Table 2 shows that the enzyme purification process involved a series of steps aimed at obtaining the purest product. Initially, the enzyme was precipitated using ammonium sulfate, achieving a yield of 75.2% and a specific activity of 0.252 units/mg using a specific amount of the sulfate as a precipitating agent. This process is called salt precipitation and has been widely used in many previous studies. Following this, dialysis was performed using a dialysis bag to remove any remaining ammonium sulfate. The buffer used was Tris-HCl at a concentration of 10 mM at pH 7. This resulted in a purification of 1.294 times with a recovery rate of 53.28% and a specific activity of 0.255 units/mg. Ion exchange chromatography was performed using DEAE-Cellulose A50. Ion exchange chromatography resulted in a purification of 1.247-fold, a recovery rate of 44.02%, and a specific activity of 0.246 units/mg.

As illustrated in Figure 3, enzyme kinetics involves examining the factors that influence enzyme functionality—such as enzyme concentration, substrate concentration, pH, and temperature. By evaluating these

elements, one can gain crucial insights into the reaction mechanics and behavior of the enzyme. The findings indicate that the purified enzyme (via gel filtration) had a K_m of 11.11 mM and a V_{max} of 12.5 U/ml. Additionally, the enzyme's optimal pH was 7, and its optimal temperature optimum was 37.

The use of vitexin as an inhibitor as shown in figure 4 showed significant results for triose phosphate isomerase enzymes extracted from the serum of breast cancer patients, exhibiting inhibition at all Flavone concentrations. The highest inhibition was 74% at a concentration of 500 $\mu\text{g/ml}$, and 69% at a concentration of 400 $\mu\text{g/ml}$. The effects of substrate concentration, temperature, and pH were also investigated. Kinetic studies of the enzyme were conducted by varying the concentration of the substrate, glyceraldehyde-3-phosphate. The mechanism of inhibition was investigated using Linn over -Berck plots, comparing enzyme activity in the presence and absence of the inhibitor and under specific substrate conditions.

Table 2: purification of TPI1 enzyme from breast cancer patients

Steps of purification	Volume (ml)	Enzyme activity (U/mL)	Total activity (U)	Protein concentration (mg/ml)	Specific activity (U/mg)	Purification (folds)	Yield (%)	Total Protein (mg)
Crude enzyme	10	0.67	6.7	34	0.197	1	100	340
Ammonium sulphate	8	0.63	5.04	20	0.252	1.278806	75.2238	160
Dialysis	7	0.51	3.57	14	0.255	1.294029	53.2835	98
(Ion exchange) DEAE-Cellulose A50	5	0.59	2.95	12	0.246	1.247516	44.0298	60
(Gel filtration) Sephadex G - 100	5	0.55	2.75	9	0.306	1.550586	41.0447	45

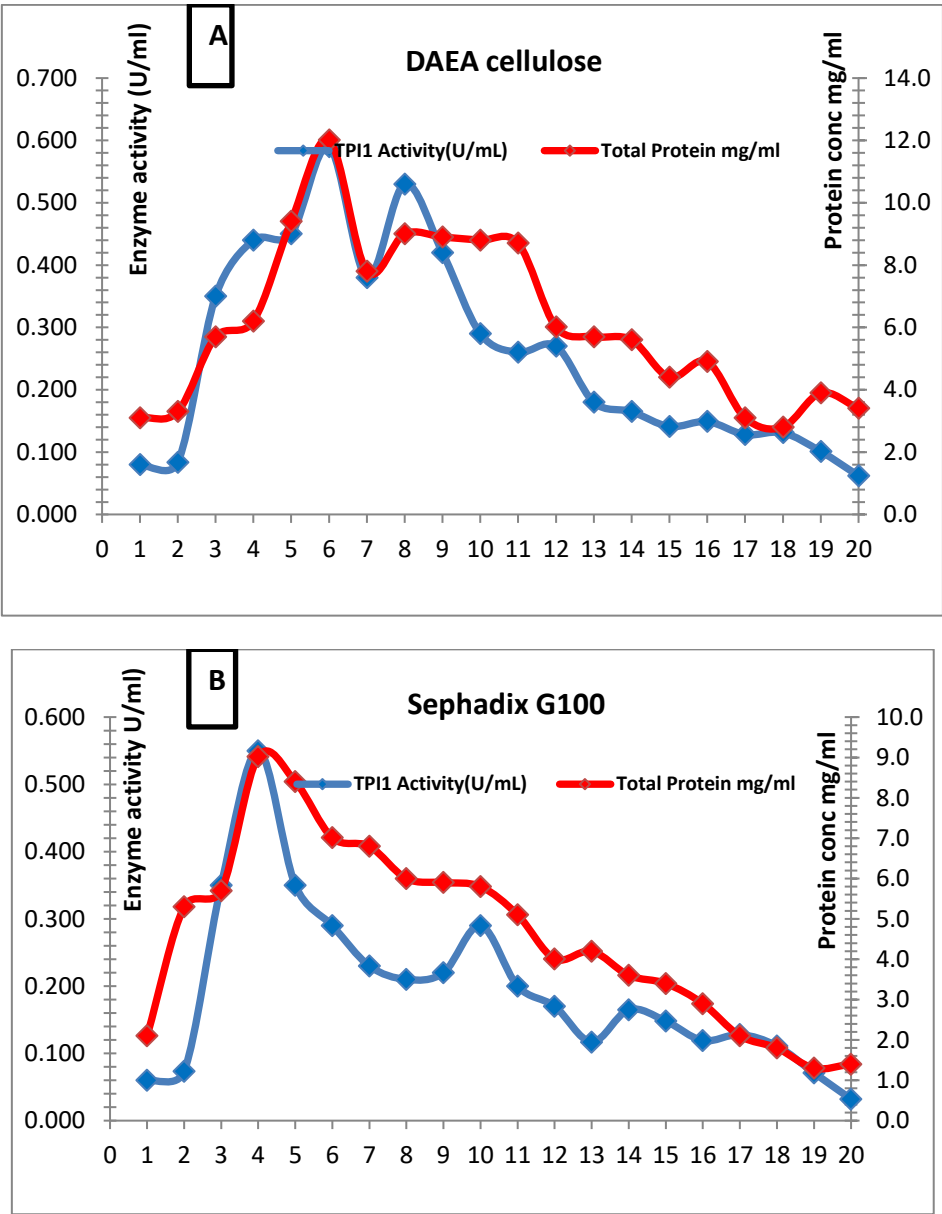


Fig.10. purification of TPI1 enzyme from breast cancer patients by: (A)DAEA Cellulose. (B). Sephadex G100.

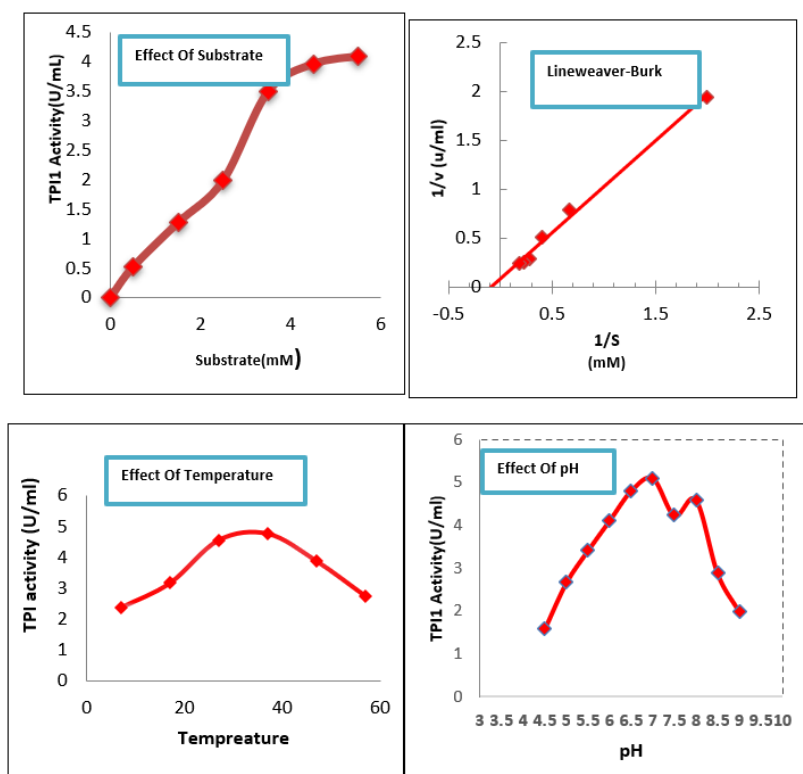


Fig.11. Enzyme kinetic studies on the activity of the TPI1 enzyme purified from the serum of women with breast cancer

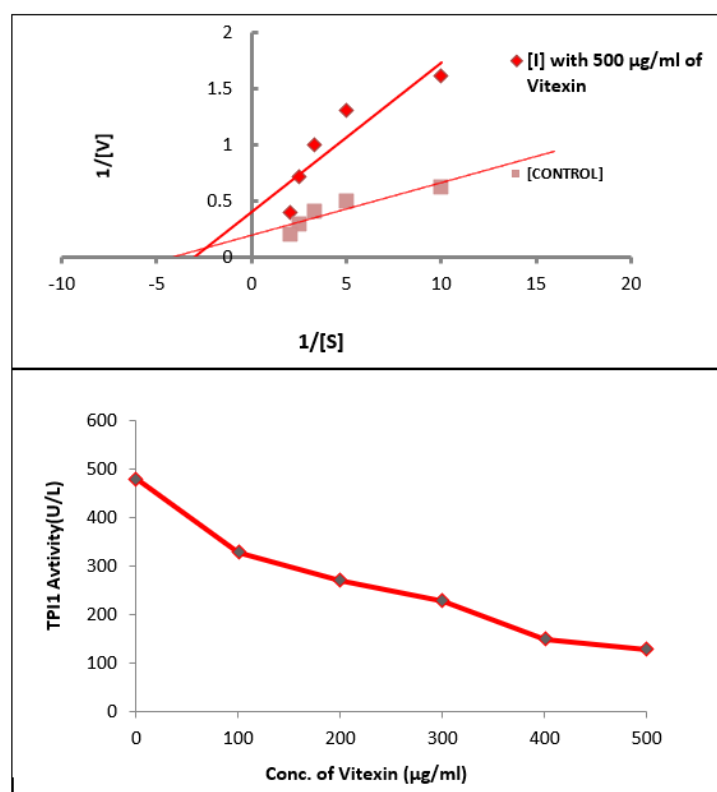


Fig.12. Enzyme kinetic studies on the Inhibition of Flavone on TPI1 enzyme purified from the serum of women with breast cancer

4. Conclusions

Flavone compound were chosen to assess their binding capabilities and overall reactivity with the TPI1 enzyme (PDB ID: 2jk2). According to the docking data, the compounds had the best root-mean-square distance and the lowest energy score. Good binding results were demonstrated by these substances. The interaction with the TPI1 enzyme's active site is facilitated by these functional groups (2jk2). This study shows us that the compound flavone has a significant effect on the TPI1 enzyme. Further research into Flavone's effects revealed non-competitive inhibition of the purified enzyme. From kinetic experiments on the enzyme extracted from the serum of women with breast cancer, the optimal conditions were pH 7, temperature 37 °C, and a substrate concentration of 5.5 mM. Using the Lineweaver-Burke method, a Km value of 11.11 mM and a Vmax of 12.5 U/ml were obtained.

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