Alternative Medicine against Herpes Simplex Virus Type-1(HSV-1) via molecular docking.

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1. ABSTRACT

Thymidine kinase enzyme of HSV-1 was selected to identify putative functional sites and alternative drug molecules through molecular docking .The functional sites were predicted by PINTS, Q-Site finder and PROFUNC servers. A total of 62 antiviral plant metabolites and 13 drug molecules were docked against both the chains of Thymidine kinase enzyme using Patchdock tool. The plant metabolite Geraniin has produced higher score (5680 (chain A), 6562(chain B)) than the commercially known anti-herpes compound Acyclovir (3504 (chain A), 3264 (chain B)). In addition, Gemdockv2.0 also produced the lower best fitness values for Geraniin (-130.123226 (chain A), -132.309075 (chain B)) as compared to Acyclovir (-102.182402 (chain A), -84.599474 (chain B)). Furthermore, docking through Autodock4 by considering the predicted site as a grid centre also produced lowest docking energy of -13.40 Kcal/mol (chain A), -15.17Kcal/mol (chain B) for Geraniin compound in comparison to Acyclovir -7.48Kcal/mol (chain A), -7.96Kcal/mol (chain B). Geraniin firmly binds at the cavity of the enzyme 3F0T (A and B chains) at these surrounding residues: H58 G59 M60 G61 K62 T63 T64 T66 Q67 L69 V70 A71 G73 E83 W88 R89 M85 E95 I97 Y101 K219 D162 R220 R222 P223 E225. Moreover, this compound makes appropriate hydrogen bonds with RA89, QA67, KA219 residues from chain A and EB83, KB62, RB163, EB225, KB219 residues of chain B. We conclude that the natural plant metabolite Geraniin can serve as an effective antiviral drug against thymidine kinase enzyme of HSV-1.

General Terms

Identifying potential functional site residues, molecular docking, Computer added drug designing.

Keywords

Herpes simplex virus type-1, Functional site, Antiviral compound, Acyclovir, Geraniin, Molecular docking

2. INTRODUCTION

Herpes simplex virus type-1 (HSV-1) is a harmful pathogen, which causes severe cold sores or fever blisters on the face, epidermal lesions in the oral cavity, eyes and mucous membrane in humans [1]. They mainly invade the neuronal part of the human body and cause recurrent infections upon reactivation in nervous system after the latent period [1]. HSV-1 mostly affects
adults (Kimberlin and Whitley, 1998) causing genital herpes [2]. Primary phenotypic symptoms mostly include fever, headache, malaise, genital itching and lesions [3]. Subjects with weak or compromised immune system are highly susceptible to HSV-1 infection, resulting in critical health conditions [4-6] .

In general, HSV-1 causes rapid mutations in their genes during therapeutic treatment in order to resist the drug action [4]. These mutations usually occur in the genes that code for thymidine kinase or DNA polymerase enzymes [7]. However, thymidine kinase enzyme is significantly involved in catalyzing the phosphotransfer reaction during the DNA synthesis of the virus.

$$Thd + ATP \rightarrow TMP + ADP$$
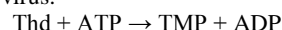
where Thd is deoxythymidine, ATP is (energy-rich) adenosine 5'-triphosphate, TMP is deoxythymidine 5'-phosphate and ADP is adenosine 5'-diphosphate.

A number of anti-viral drugs are commercially available in the market and most of them have been formulated using chemicals. For instance, Acyclovir, Ganciclovir, Valaciclovir (lvaline ester of Acyclovir), Penciclovir, Famciclovir, vidarbines are few well-known anti-herpes drugs. Among these, Acyclovir is the most commonly used one for anti-herpes treatment. However, due to its cost and drug resistance developed by the virus, there is an urgent need to develop new alternatives against the HSV-1 [8].

Our computational work proposes alternative potent anti-herpes drug compounds through structure based functional site prediction and molecular docking approach. We specifically target thymidine kinase enzyme, a key enzyme of HSV-1 virus. This enzyme is important for DNA synthesis and cell division in the HSV-1 virus, and it plays a major role in cell survival as well as regulation of DNA replication. Moreover, this enzyme has a well-defined structure available in the RCSB Protein Databank useful for functional genomics study. However, the functional site information of this enzyme is not available. Hence,, we have selected this enzyme as a potential target for our structure based drug discovery study against HSV-1 virus.

3. MATERIALS AND METHODOLOGY

3.1 Input files

Our overall approach has been illustrated schematically in Figure. 1. We have used two input files for drug discovery study against HSV-1 virus: (i) PDB structure of HSV-1 thymidine kinase (3F0T) and (ii) Anti-herpes drugs. The overall method comprises of two broad steps: (i) Prediction of functional site of HSV-1 thymidine kinase and (ii) Identification of potent drug against HSV-1 virus enzyme using molecular docking.

3.2 Functional site prediction

The functional site residues for HSV-1 Thymidine kinase enzyme were predicted using public domain servers such as PINTS [9], CSA [10], PROFUNC [11] and Q-Site finder [12]. These servers provide a list of potential functional site residues.

3.3 Antiviral compound dataset

A compound dataset of 75 antiviral drugs was generated by literature survey and DRUG bank database [13]. In this generated dataset, a total of 62 anti-viral plant metabolite compounds were obtained from the literature. The rest 13 antiviral drugs were obtained from DRUG bank database. The SMILES strings for the collected compounds were downloaded from Pubchem database (http://pubchem.ncbi.nlm.nih.gov/). The obtained SMILES strings for all the compounds were generated in to 3D structures using Corina server (http://www.molecular-networks.com/online_demos/corina_demo).

3.4 Molecular docking

The compound dataset was docked against the 3D structure of Thymidine kinase (3F0T). The docking experiment was categorized into two steps (1) blind and (2) refined docking. The blind docking was performed using Patch dock [14]and Gem dock v2.0 [15], while the refined docking was performed using Autodock (version 4.0) [16]. Docking was performed separately for chain A and B of thymidine kinase structure. During blind docking, the functional site was not specified and the complete protein was used as a docking target for the antiviral compounds. Patch dock produced an affinity score for individual compounds based on the geometry matching of compound on both the chains of 3F0T enzyme. The Gemdockv2.0 software has performed automated docking for the compounds against the complete structure of 3F0T and ranked the compounds based on the interaction energy (fitness value) with the enzyme structure.

On performing refined substrate docking using Autodock4, we selected the functional sites as a docking target and generated the grid map around them with spacing of 0.375 Å . The Autodock tool (version 1.5.2) was used to prepare input files for docking. All the polar and non polar hydrogens were added before executing the docking process. Additionally, the partial charges were assigned to the enzyme. The number of flexible bonds in the compounds was set to a maximum limit.

AUTOGRID (version 4.0) tool was used for generating the grid maps around the selected functional sites. Automated molecular docking was performed using Genetic Algorithm- Local Search (GA-LS). Autodock4.0 generated an output log file containing the information about interaction energy between compounds and the enzyme. It also provided the docking energy with the sum of intermolecular energy and internal energy. The compounds with highest affinity have shown the lowest docking energy values. Finally, the predicted functional site residues that were validated by docking were accounted for putative functional site residues. The compound that has shown lowest docking energy in refined docking was proposed as an alternate anti-herpes drug.

3.5 Binding site analysis

The antiviral compounds were analyzed for binding site prediction. We obtained putative ligand binding site for 3F0T enzyme based on lowest interaction energy using blind docking approach by including the residues with in 6Å radius from the antiviral compound (as center).

3.6 Statistical Analysis

Histograms were generated for analyzing the number of antiviral compounds that showing high range of fitness values for 3F0T enzyme. The patchdock scores (fitness values) for the respective antiviral compounds were plotted on X axis and the corresponding frequency of distribution were plotted on Y-axis. In the histogram, the height of the bar indicates the number of compounds that produce specific range of fitness values. We also calculated the Z score to evaluate the uniqueness of drug compounds that showing distinctive affinity for 2UUQ enzyme. In this case, either maximum positive Z value (Patchdock Score) or minimum negative Z value (Gemdock score) represents the best compound showing higher affinity from the random population of drug compounds. Further, the affinity of selected compound was tested by normal distribution analysis. The probability (P) values were calculated for the obtained Z values of all respective compounds. The P value was set to 0.05 at 95% confidence interval and the compounds that exhibiting lesser P value than < 0.05 were considered as best from the rest population of drug compounds.
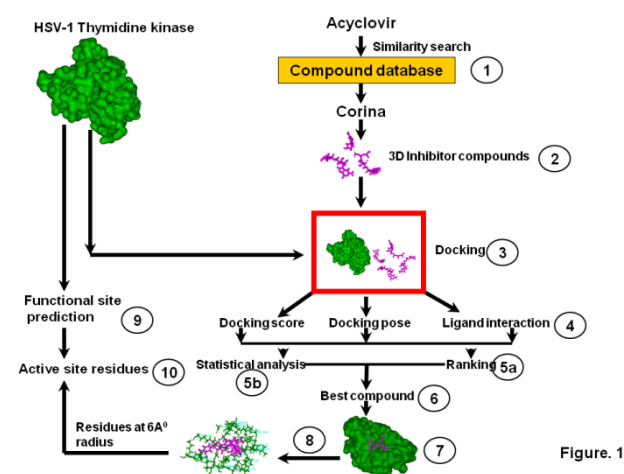

Fig. 1: Overall schematic representation of the proposed method. It can be divided into the steps: (1) Enumeration of potential anti-herpes compounds similar to Acyclovir, a well known anti-herpes drug, from ligand databases. The SMILE files were also obtained in this step. (2) The 3D structures of the ligands were obtained using Corina server. (3) The ligands were screened against Thymidine kinase of HSV-1 virus. Note that the whole protein structure was taken as a docking target. (4) Analysis of docking results based on docking score, docking pose and ligand interaction. (5a) Ranking of ligands based on scores obtained in the previous score. (5b) Statistical analysis of docking results via Z score and probability density function calculations. (6) Selection of the best ligand. (7) Analysis of the cavity of the protein where the best scoring ligand binds. (8) Extraction of residues within 6Å radius of the bound ligand. (9) Prediction of functional sites of the viral Thymidine kinase via various prediction servers. (10) The residues common in step 8 and 9 were selected as putative functional site residues of the protein.

4. RESULTS

The functional site residues such as: H58, G59, G61, K62, T63, T64, E83, W88, M85, I97, I100, Y101, Q125, M128, G129, Y132, R163, A167, A168, Y172, R176, R222, P223, G224, E225 and M231were predicted for HSV-1 thymidine kinase using various online servers such as PINTS, Q-Site Finder and PROFUNC. The predicted functional sites were further

Table 1. Docking analysis of top 10 antiviral compounds with chains A& B of 3F0T via Patchdock and Gemdock software.

S. No.	Compound	Chain A of 3F0T		Chain B of 3F0T	
		Patchdock Score	**Gemdock Fitness value**	**Patchdock Score**	**Gemdock Fitness value**
1	Geraniin	5680	-130.123	6562	-132.309
2	Hesperidin	5158	-115.002	5414	-118.454
3	Narirutin	5136	-100.428	5692	-116.345
4	Penciclovir	3852	-87.101	3432	-89.594
5	Famciclovir	4746	-102.524	4300	-103.613
6	Valacyclovir	4338	-98.942709	4316	-100.132
7	Acyclovir	3504	-102.182	3264	-84.600
8	Carbovir	3326	-88.250	3186	-86.850
9	Vidarabine	3506	-100.197	3372	-110.198
10	Ganciclovir	3634	-92.973	3375	-87.120

herpes chemical compound Acyclovir obtained least score of 3504 (Table 1, Figure. 3a). Chain B also produced high affinity for Geraniin (score 6562) in comparison to Acyclovir (score 3264) (Table 1, Figure. 3b). Interestingly, Geraniin appropriatelybound at the cavities of both the chains of HSV-1 thymidine kinase. It also scored highest rank in Gemdock tool with the lowest docking energy of -130.123226 for chain A and -132.309075 for chain B (Table 1, Figure. 4 (a,b)). On the other hand, Acyclovir has produced an increased docking energy of -102.182402 for chain A and -84.599474 for chain B (Table 1, Figure. 4 (a,b)). Note that Geraniin is known as a tannin
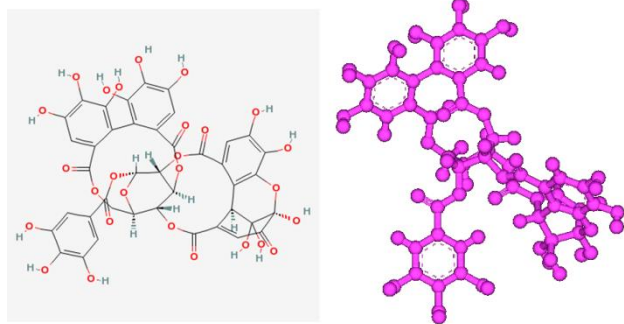

Figure. 2

Fig. 2: Structure of Geraniin

validated by docking analysis through Patchdock and Gemdock computational tools.

As mentioned earlier, each chain of the enzyme was subjected to docking analysis separately. For chain A of the enzyme, the plant metabolite Geraniin (Table 1, Figure. 2) has showed the highest patchdock score of 5680, while the well known anti-
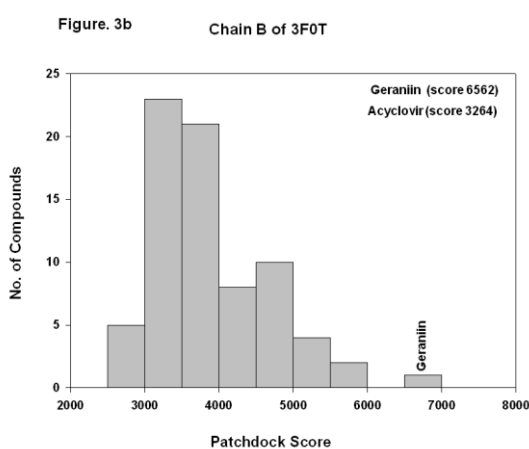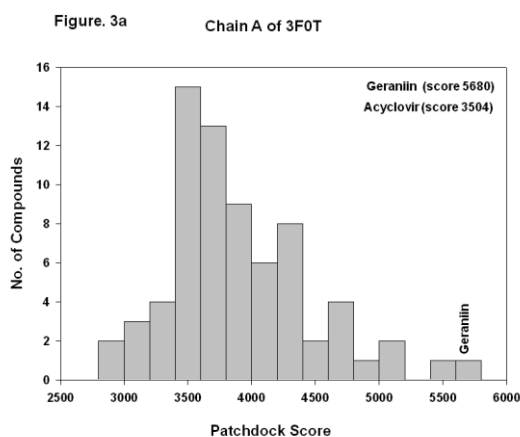
Fig. 3. Distribution of anti-viral compounds based on their affinity with both the chains of thymidine kinase enzyme of HSV-1 via Patchdock software. (a) The Geraniin produced the highest patchdock score of 5680 with chain A as compare to Acyclovir 3504 and (b) for chain B the Geraniin produced patchdock score of 6562 and the drug Acyclovir of 3264.
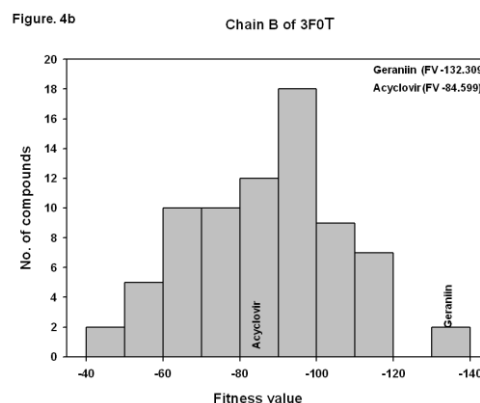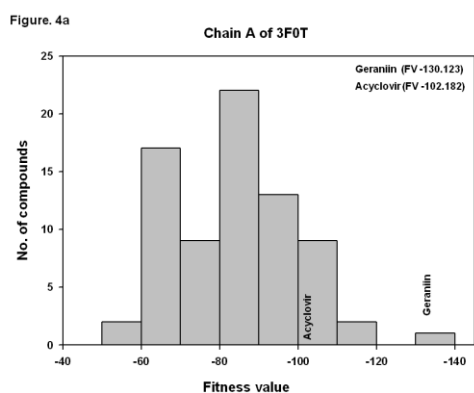

Fig. 4. Distribution of anti-viral compounds based on their affinity with both the chains of thymidine kinase enzyme of HSV-1 via Gemdockv2.0 software. (a) The Geraniin produced the lowest fitness value of -130.123 with chain A as compare to Acyclovir -102.182 and (b) for chain B the Geraniin produced lower fitness value of -132.309 and the drug Acyclovir of -84.599 .
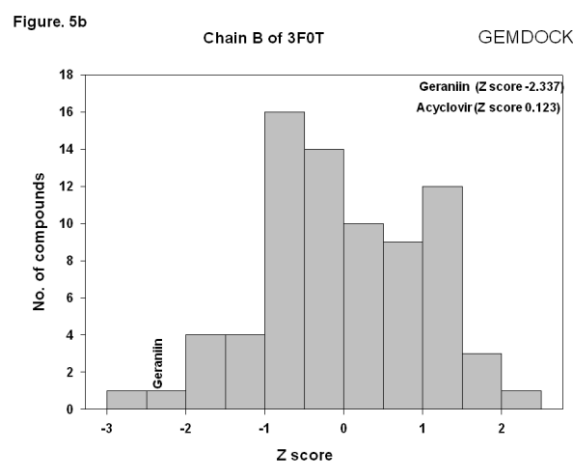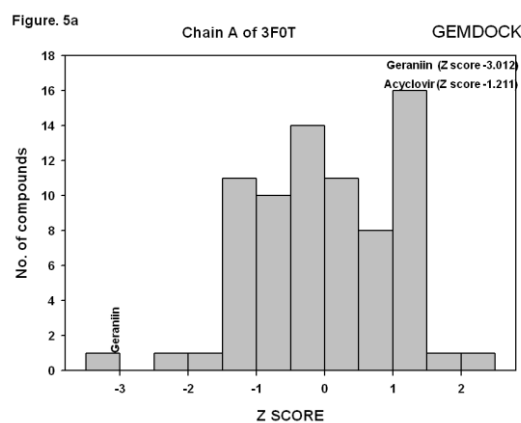

Fig. 5: The distribution of Z score analysis of the compounds for finding of best compound with unique results (for Gemdockv2.0 docking results). Here lower the Z score (more negative), more unique is the result. (a) For chain A, Geraniin Z score is -3.012 and Acyclovir Z score is -1.211 and (b) for chain B, the Geraniin Z score is -2.337 and Acyclovir Z score is 0.123. The graphs was generated by Sigma plot 10 software.

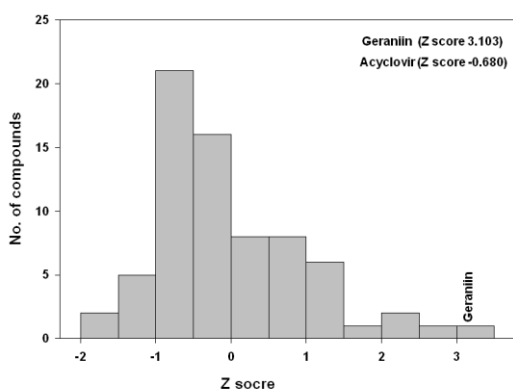Figure. 6a — Chain A of 3F0T — PATCHDOCK

Geraniin (Z score 3.103)
Acyclovir (Z score -0.680)

Figure. 6b — Chain B of 3F0T — PATCHDOCK

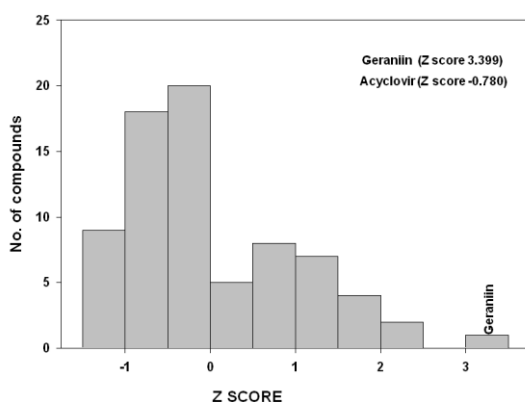Geraniin (Z score 3.399)
Acyclovir (Z score -0.780)

Fig. 6: The distribution of Z score analysis of the compounds for finding of best compound with unique results (for Patchdock docking results). Here higher the Z score (more positive), more unique is the result. (a) For chain A, Geraniin Z score is 3.103 and Acyclovir Z score is -0.680 and (b) for chain B, the Geraniin Z score is 3.399 and Acyclovir Z score is -0.780. These graphs were generated by Sigma plot 10 software

compound and is found in medicinal plant *Phyllanthus urinaria (*Euphorbiaceae) [17-20].

Statistical analysis was performed for the docking results by calculating Z score and probability density function based normal distribution curve analysis. For the Gemdock results, the statistical analysis showed that the compound Geraniin produced low Z scores (chain A: -3.012, chain B: -2.337 as compared to other compounds (Figure. 5 a, b). This evidently concludes that the compound Geraniin effectively binds without any conformational change to the 2UUQ enzyme, thereby resulting in low fitness value. On the other hand, Z score analysis for Patchdock results has showed high positive Z score values (chain A: 3.103, chain B: 3.399) for Geraniin (Figure. 6 a, b). This shows the distinctive features of compound Geraniin compared to the other drug candidates. Further, it indicates that the affinity for the compound Geraniin is unique from the mean of the population of other drug compounds. Simultaneously, the individual probability was calculated for each Z score and a normal distribution analysis was performed. Figure. 7 and 8 shows the distribution of Z scores for all the compounds with respect to

their probability values (P), which neatly followed a bell shaped normal distribution curve. In case of Gemdock results, the compound Geraniin has exhibited lower Z scores with the appropriate criteria of low P value < 0.05 for both A and B chains of 3F0T (chain A: -3.012, P value 0.005, chain B: -2.337, P value 0.01) (Figure. 7 a, b) Likewise, the P value for

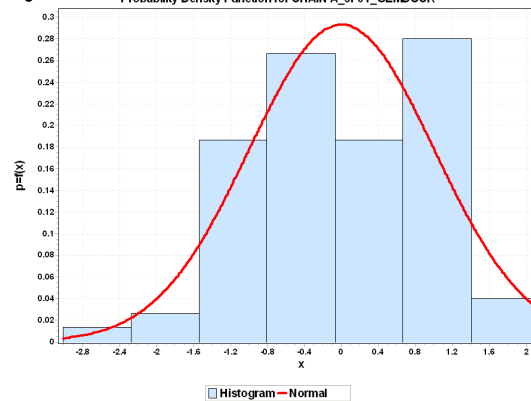Figure. 7a — Probability Density Function for CHAIN A_3F0T_GEMDOCK

Figure. 7b — Probability Density Function for CHAIN B_3F0T_GEMDOCK
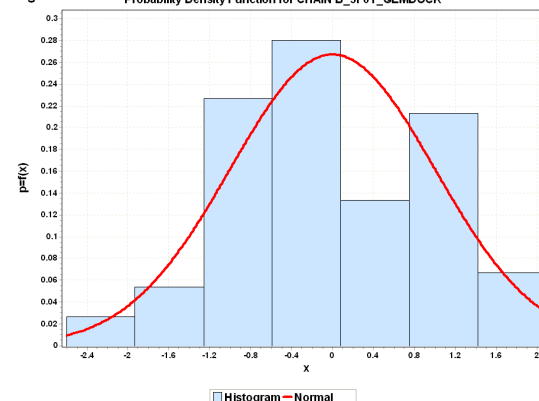
Fig. 7: This represents the normal distribution analysis of the Z scores with respect to their probabilities of distribution of the compounds fitness values via Gemdockv2.0 software. The P values distribute on the Y axis and the Z score is on X axis. (a) For chain A, the compound Geraniin shows Z score -3.012, P value 0.005, and (b) For chain B, Geraniin Z score is -2.337, P value 0.01. The normal distribution analysis was performed by EasyFit5.4 professional software.

Fig. 8a — Probability Density Function — CHAIN A_3F0T_PATCHDOCK

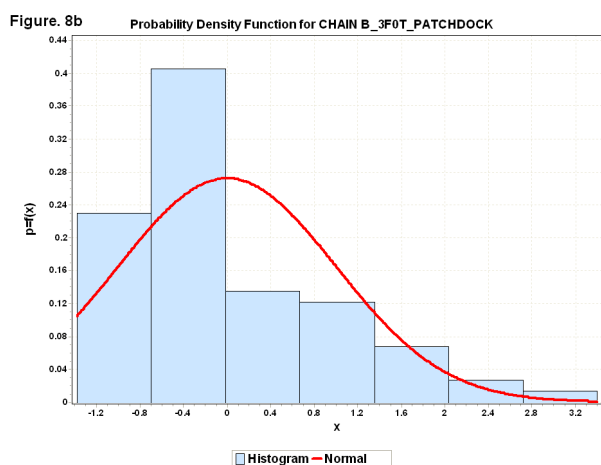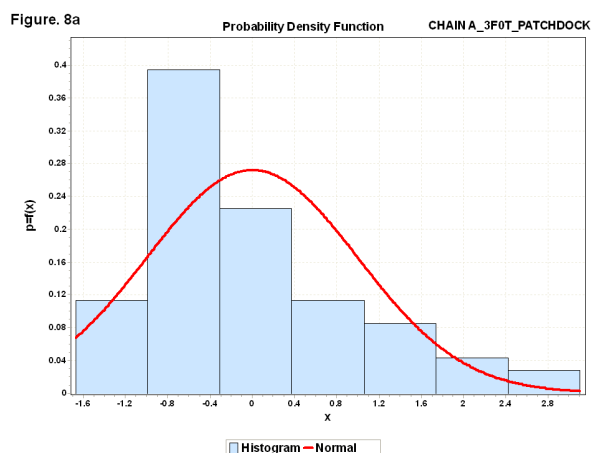Fig. 8b — Probability Density Function for CHAIN B_3F0T_PATCHDOCK

Fig. 8: This represents the normal distribution analysis of the Z scores with respect to their probabilities of distribution of the compounds docking score via Patchdock software. The P values distributed on the Y axis and the Z score is on X axis. (a) For chain A, the compound Geraniin shows Z score 3.103, P value 0.03, and (b) For chain B, Geraniin Z score is 3.399, P value 0.01. The normal distribution analysis was performed by EasyFit5.4 professional software.
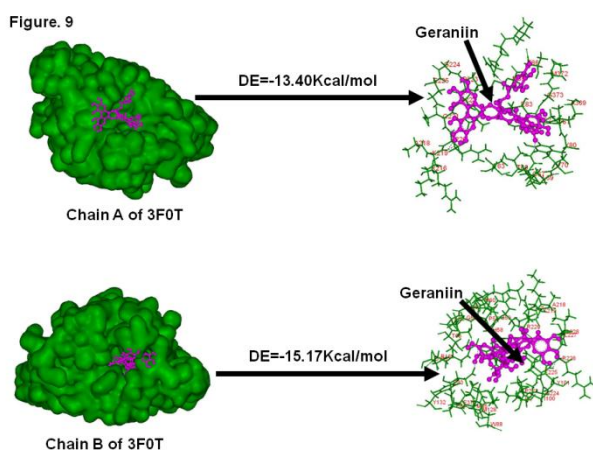

Fig. 9. The refined docking of Geraniin with both chains of HASV-1 thymidine kinase by Autodock4 software: The

Geraniin (Magenta) binds at the cavity of chain A (green) with docking energy of -13.40Kcal/mol and at chain B with docking energy of -15.17Kcal/mol.

Patchdock results also exhibhited lower P values for Geraniin (chain A: 3.103, P value 0.03, chain B: 3.399, P value 0.01) (Figure. 8 a, b) as compared to other drug compounds. This clearly shows that the Geraniin compound agrees to the hypothesis of 95% confidence interval and indicates that there is less chance of getting similar affinity by any other compound for both the chains of 3F0T as showed by Geraniin.Significantly, we identified the following residues surrounding the Geraniin compound with in 6 Å radius: H58, G59, M60, G61, K62, T63, T64, T66, Q67, L69, V70, A71, G73, E83, W88, M85, E95, I97, Y101, D162, K219, R220, R222, P223, E225. These residues appropriately matched with the functional site residues that predicted by various computational servers like PINTS, Q-Site finder and PROFUNC. Simultaneously, refined docking was performed through Autodock4 by considering the predicted sites as grid centers. We found that the Geraniin compound bound the enzyme with the lowest docking energy of -13.40 Kcal/mol for chain A and -15.17 Kcal/mol for chain B. On the other hand, Acyclovir bound the chain A of the enzyme with energy of -7.48 Kcal/mol and -7.96 Kcal/mol for chain B. In addition, Geraniin has shown appropriate hydrogen bonds with the residues: RA89, QA67, KA219, EB83, KB62, RB163, EB225, KB219 (Figure. 9).

5. DISCUSSION

We propose a computational molecular docking strategy for finding natural alternative medicine against Herpes simplex virus type-1. Our approach combines both functional genomics study and molecular docking analysis.. In this work, we have selected HSV-1 thymidine kinase as our target because of itplays a significant role in DNA synthesis and cell division. We predicted its functional site using the prediction servers such as PINTS, Q-Site finder and PROFUNC. These servers predicted several amino acids residues but we have reported the consensus amino acids residues only. Furthermore, these residues were validated using molecular docking approach such as Patchdock, Gemdock and Autodock4 computational docking tools. The compounds for docking were selected from the literature which reported antiviral plant metabolites and the well known anti-herpes drug compounds like Acyclovir. Based on docking analysis, we found that the plant metabolite Geraniin has shown higher affinity for thymidine kinase enzyme as compared to other compounds. Interestingly, our statistical analysis also proved that Geraniin compound is unique comparedto the population of drug candidates by showing higher affinity. The Z score analysis of both Patchdock and Gemdock have shown that the Geraniin compound produces lower Z scores for both the chains A & B of thymidine kinase enzyme as compared to other compounds. Moreover, the probability of theZ scores obtained have satisfied the 95% confidence interval statistically for both the cases of chain A & B of thymidine kinase enzyme. This clearly shows that the Geraniin compound, known to be tannin that is found in medicinal plant *Phyllanthus urinaria (*Euphorbiaceae) can be used as an alternative potent anti herpes drug.

6. CONCLUSION

Our molecular docking study confirms that the natural antiviral plant metabolite Geraniin has more affinity for the thymidine kinase enzyme of HSV-1 as compared to existing anti herpes drug Acyclovir. The statistical results are also in good agreement for Geraniin to be a potential alternative medicine against herpes disease. The natural origin of the Geraniin compound makes it more cost effective with no side effects as compared to the commercially available anti herpes drug Acyclovir, which is more expensive and causing side effects in the human body. Therefore, we conclude that the Geraniin compound has the potential to act as an effective drug against HSV-1.

7. ACKOWLEDGEMENT

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