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IN SILICO DOCKING STUDIES OF SELECTIVE NATURAL INHIBITOR AGAINST PB1 PROTEIN OF INFLUENZA A (H1N1) VIRUS

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ABSTRACT

The influenza A (H1N1) virus, also termed as swine flu is a prominent source of disease and death since 2009. Currently there is a requirement to discover novel anti-viral drugs for incapacitating the increases. Conventionally, many herbal extracts of ginger, kalmegh, garlic, ajwain, menthe, tulsi, green tea and turmeric etc. has been used as progressive source of inhibition and cure of human influenza. The H1N1 polymerase protein composed of viral proteins PB1, PB2, and PA, accumulates with viral RNA and nucleoprotein (NP) to mediate transcription and replication of the viral genome. It is engaged amino acid sequence of H1N1 PB1 subjected to build the tertiary structure by modeler and validated by Ramachandran Plot, SAVES and ProSA server. Additionally, the build structure was subjected to dock with six natural compounds of herbs by Flexx software. Utmost of the selected compounds exposed more inhibitory activity against PB1 of H1N1 by binding. The current study furthermore shows the interaction of these selected compounds with residues of PB1 protein. Moreover, among six natural compounds, Curcumin found in turmeric detected to inhibit PB1(wild) protein of H1N1, this is intensely sustained by least binding energy (-13.0595) and Curcumin from turmeric, Caffeic acid and theaflavin from green tea was observed to inhibit PB1(mutated) protein of H1N1and it is strongly sustained by lowest binding energy (-17.8434, -15.6946 and -13.3391). In future, it might be give awareness to in vitro and in vivo evaluation study for Curcumin, Caffeic acid and theaflavin for further.

KEY WORDS

Influenza A Virus, molecular modeling, molecular docking, PB1.

INTRODUCTION

The H1N1 influenza virus arises due to the genetic recombination of genes from pig, human, and bird's H1N1 virus. Influenza A virus is a negative sense single-stranded RNA virus denotes to the *Orthomyxoviridae*, which causes respiratory infection in individuals. Genome of Influenza A virus carries eight segmented, and disease-causing proteins are revolved from viral mRNA Transcripted by the RNA-dependent RNA polymerase of influenza A virus. Polymerase complex of the influenza A virus is a heterotrimer comprising of PA (polymerase acidic protein), PB1 (polymerase basic protein 1), and PB2, and each element is essential intended for the virus reproduction (Neumann *et al*., 2004). The hemagglutinin (HA) and neuraminidase (NA) are the glycoproteins present in lipid membrane and it is unevenly spherical and is wrapped (Behera *et al*., 2012), which are vital for viral infection (Gallaher, 2009). There are several herb products commonly applied for cure of influenza infection, among them few natural compounds such as Aloin, Caffeic acid, Curcumin, Carvacrol, Theaflavin and Withaferin A have been designated for this study.

Aloe Vera is an herb which is used for ayurvedic, Homoeopathic and Allopathic types of medicine. Its leaves comprise several vitamins, minerals, enzymes,

amino acids, natural sugars and other active compounds with emollient, purgative, antimicrobial, anti inflammatory, anti-oxidant and superficial values for healthiness. Aloin is one of the anthraquinone produced by Aloe Vera, it is suitable for inhibition of various enveloped viruses such as herpes simplex, varicella zoster and influenza (Sydiskis *et al*., 1991). Antiviral activity against herpes simplex virus (DNA virus) and polio virus (RNA virus) observed in Caffeic acid. It also inhibits the multiplication of influenza A virus (Utsunomiya *et al*., 2014). Strong antioxidant with anti-inflammatory, anti-viral properties was observed in Curcumin and it is reported as an active compound (Winston *et al*., 2007). Carvacrol is an essential oil is used for the treatment of cold, cough, influenza, and asthma (Spickler AR ,2014). Nimbin, nimbinin, and nimbidin are neem oil it is very effective against swine flu (Gupta *et al*., 2015). Theaflavin and its derivatives has been shown antiviral activity and it is suitable for inhibiting flu reproduction (Narayanan *et al*., 2013 and Yiannakopoulou EC, 2012). Withaferin A (WA) has been shown wide range of pharmaceutical properties including anti-viral activity (Cai *et al*., 2015). In the present study, docking analysis were achieved to discover the atomic interaction and 6 selective active compounds such as aloin, Caffeic acid, Curcumin, Carvacrol, Nimbinin, Theaflavin and Withaferin A against H1N1 PB1 protein. It comprises modeling of PB1 protein structure using modeler followed by structure verification. Flexx software was used to study the molecular interaction between PB1 with selected natural compounds.

MATERIALS AND METHODS

Sequence retrieval: The H1N1 PB1 protein was used as the drug target. The PB1 protein sequence of H1N1 (ID: NP_040985.1) was downloaded from NCBI website (http://www.ncbi.nlm.nih.gov/).

Molecular Modelling: Molecular modelling of PB1 protein was performed using modelling server (Modeller). For modelling in Modeller, suitable templates were searched with BLAST-P against the PDB. Template was selected for modeling on the basis of query coverage and E-value. The model was generated for PB1 protein.

Mutate the modeled structure: The modeled structure was mutated at the 715th position (Valine was replaced with Serine) by using WHATIF Server web interface.

Molecular Docking: The selected compounds were obtained from PUBCHEM. Docking was performed to interpret the best binding pose of the test ligand in the active site of the receptor and also its binding affinity and conformation in the binding sites. The software used for docking was LeadIt. LeadIt modules help to investigate possible binding conformations of the receptor ligand complex using state of the art docking software – "FlexX". FlexX was the software to predict the protein ligand interaction. At first In LeadIt the docking option has to be selected. Protein of my interest was then uploaded. To select the receptor side there were two methods, the reference ligands and the selection of sphere to get the best score. A reference ligand may be defined as by clicking on the option and in sphere a spherical cut off of the protein was carried out using a reference ligand. In the next step, protonation states of the amino acids were selected. This was known as chemical ambiguities. It means the crystallographic unresolved region. These are highlighted in orange. An ambiguity affects the rotamers, alternate locations, protonation, H-torsion etc. These were adjusted manually by adjusting the torsion angle. After removing the chemical ambiguities, the 'finish' option was clicked. After that the ligands that were already merged were uploaded and the entries were confirmed by clicking 'ok'. After uploading both protein and ligands, the docking was performed by clicking on the option 'Apply & Dock'. After completion of the docking the scores were saved, and the docked poses were viewed. The interaction and the position of the ligand in the active site of the protein were viewed in 2-D using dock widget PoseView. Each pose is drawn with hydrogen and metal interaction. Hydrophobic interactions were given in green colour and hydrophilic bonds were given in red colour.

RESULTS AND DISCUSSION

Homology modeling of Wild strain

The structure of PB1 protein of H1N1 was predicted using Modeller since absence of experimentally determined structure of PB1protein. The sequence length of PB1 protein is 757. BLASTp was done for template search. One template was selected from BLASTp result (Table 1). The chosen template was 4WSB (Bat Influenza A polymerase with bound vRNA promoter) and it was used to predict the 3D structure of PB1protein. Based on the Molpdf score and DOPE score,

Model no 03 was selected for further studies. Structure verification was done for the build structure. The build structure analysis using Ramachandran plot has been shown that 97.2% residues were positioned in the most favorable region, in the generously allowed region 2.5% of residues were located however 0.3% the residues fell in the outlier region of the Ramachandran plot (Figure 1). The Z score value –8.86 was observed for build structure of PB1 it was fine within the series of intrinsic conformations of crystal structures were produced by ProSA-web server. From these results the build structure was suggested that the consistency of the proposed model.

Table 1: Shows summary of successfully produced models for PB1.

S.No	Model Filename	Molpdf	DOPE Score	GA341 Score
1	DEMO.B99990001.pdb	3822.19434	77858.79688	1.00000
2	DEMO.B99990002.pdb	3794.85181	77678.82812	1.00000
3	**DEMO.B99990003.pdb**	**3551.90259**	**77939.68750**	**1.00000**
4	DEMO.B99990004.pdb	3675.07617	77900.42188	1.00000
5	DEMO.B99990005.pdb	3666.36572	77738.89844	1.00000
6	DEMO.B99990006.pdb	3692.03613	78081.18750	1.00000
7	DEMO.B99990007.pdb	3717.45752	77936.70312	1.00000
8	DEMO.B99990008.pdb	3803.54028	78483.66406	1.00000
9	DEMO.B99990009.pdb	3928.35229	77852.42188	1.00000
10	DEMO.B99990010.pdb	3878.16479	78014.81250	1.00000

Figure 1: Shows Ramachandran plot of modeled structure of PB1(Wild)

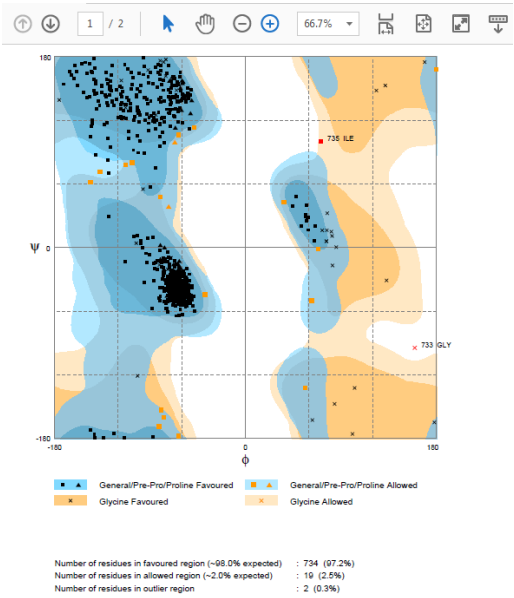


Table2: Shows Binding energy of Wild and Mutated PB1 protein with selected natural compounds.

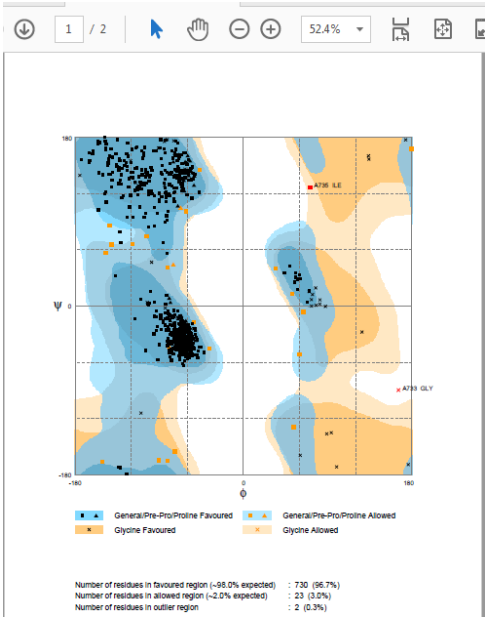
Name of the compound	Pubchem ID	Binding energy score of Wild PB1	Binding energy mutated PB1
Aloin	313325	-7.9414	-6.9113
Caffeic acid	689043	-3.3849	-15.6946
Curcumin	969516	-13.0595	-17.8434
Carvacrol	10364	-4.6548	-6.7777
Theaflavin	114777	3.2838	-13.3391
Withaferin A	265237	-1.4420	-9.5092

Mutant Structure-Mutating the modeled structure

The modeled structure was mutated at the 715th position (Valine was replaced with Serine) by using WHATIF Server web interface (http://swift.cmbi.ru.nl/servers/html/index.html). The mutated structure was then subjected to the structure verification. From the Structure validation analysis, the Ramachandran plot (Figure 2) shows number of

residues in favoured region (~98% expected) was 730 (96.7%), number of residues in allowed region (~2.0% expected) was 23 (3.0%), number of residues in outlier region was 2 (0.3%). ERRAT server prediction reports as overall quality factor was 94.332, VERIFY3D score was 72.39% and ProSA server score (Z Score) was -8.86. All of these outcomes recommended the reliability of the proposed model.

Figure 2: Shows Ramachandran plot of mutated structure of PB1



Preparation of ligand

The selected 6 natural compounds (Aloin, Caffeic acid, Curcumin, Carvacrol, Theaflavin and Withaferin A), stated in the literature and structures were retrieved form the pubchem database (Table 2).

Insilico Docking

Both natural compounds and wild strain of receptor molecule (H1N1 PB1) was prepared in Flexx software. The flexible docking was performed for the natural compounds and target protein was given as input and the flexible docking was performed. The negative and low value of bind indicates strong favorable bonds between protein and the natural compounds showing that the natural compound was in its best promising conformations. This in silico docking study, also observed that the natural compounds inhibit H1N1 PB1 of mutated and shows significant binding energy (Table 2). The molecular interaction with lowest binding energy compounds were shown (Figure3, 4 and 5). This insilico docking study exposed that most of the selected natural compounds has the ability to block the influenza infection. Most of the natural ligands were found to interact with H1N1 PB1 of wild and mutated strain with effective binding energy.

Figure 3: shows the interactions of Caffeic acid compound with Wild and mutated PB1.

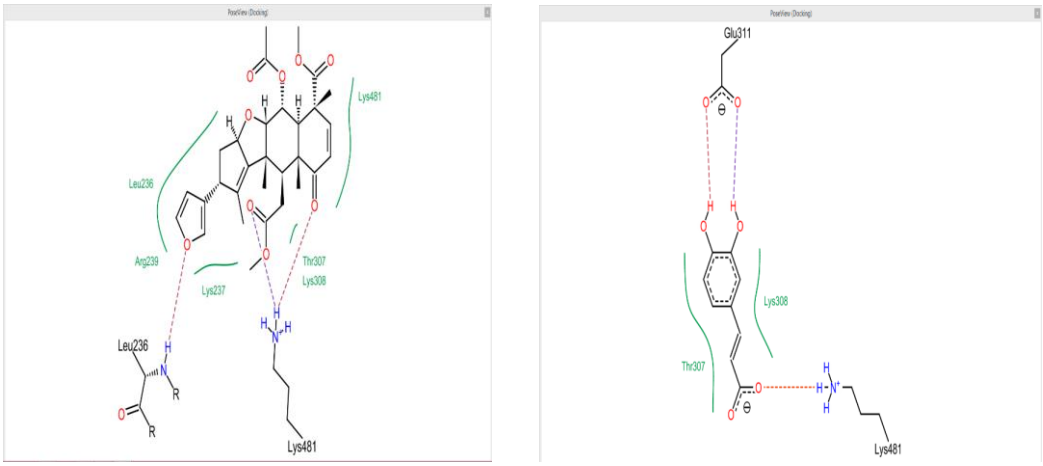


Figure 4: shows the interactions of Curcumin compound with Wild and mutated PB1.

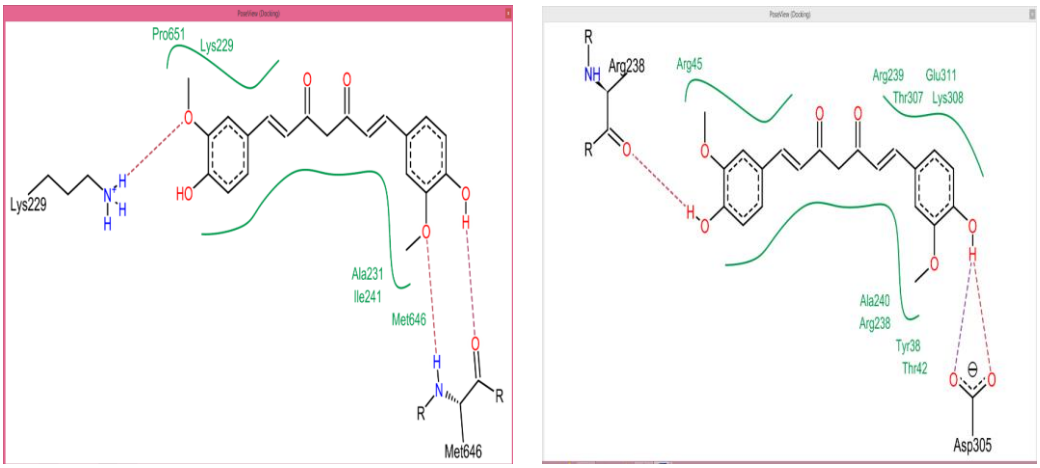
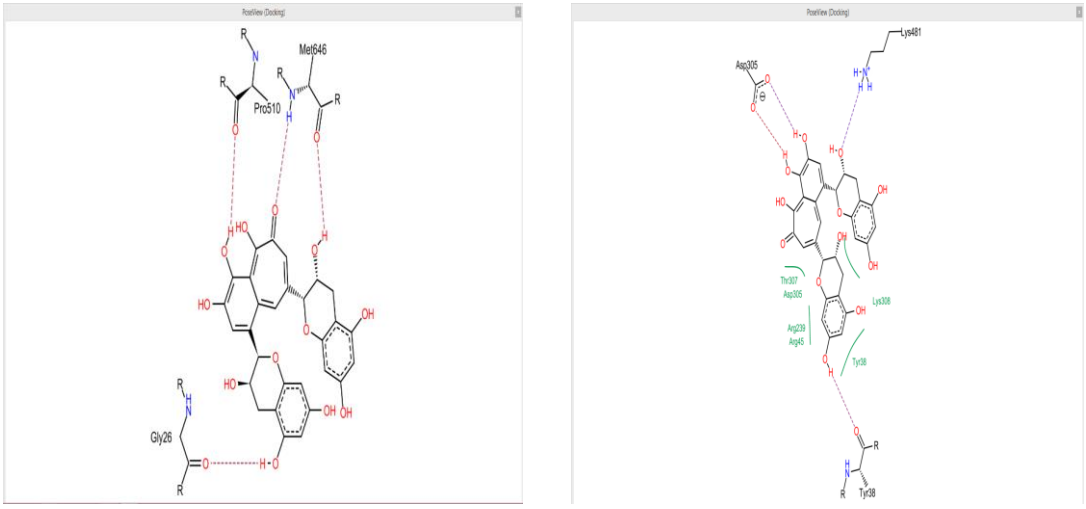


Figure 5: shows the interactions of Theaflavin compound with Wild and mutated PB1.



CONCLUSION

This present study concluded with variety of natural products is available with antiviral activity which was traditionally used to prevent or moderate the effect of viral disease. Many literatures have specified to use natural products of plant origin. Now a days the *in-silico* validation plays a vital role in the study of the antiviral activity natural products against viral proteins earlier in vitro and in vivo study. The PB1 protein of H1N1 is the major antigenic determinant; it is a suitable target for H1N1 study. In order to prevent or inhibit the viral infection, it is in need of identify or design new inhibitors against PB1 protein of H1N1. This study focused both wild and mutated PB1 protein of H1N1. The insilico docking study showed that the molecular interaction of PB1 (wild and mutated) protein of H1N1 with selected natural compounds.Out of all the selective medicinal compounds (Curcumin -17.8434, Caffeic acid -15.6946 and Theaflavin -13.3391) has been observed, the minimum binding energy values of the compounds suggested that can be subjected to in vitro and in vivo validation in future.

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