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Research Article

IN-SILICO EVALUATION OF GLYCYRRHIZA GLABRA DERIVATIVES AS POTENTIAL ANTIVIRAL AGENTS AGAINST INFLUENZA

Dr. Vijayalakshmi N [1], Nagendra Babu Battula[2*], Veena K[3], Levaku Pranathi[4]

[1]Assistant Professor. (A), JNTUA-Oil Technological & Pharmaceutical Research Institute, Anantapur, Andhra Pradesh, India-515001.
[2]Assistant Professor. (A), JNTUA-Oil Technological & Pharmaceutical Research Institute, Anantapur, Andhra Pradesh, India-515001
[3]Assistant Professor. (A), JNTUA-Oil Technological & Pharmaceutical Research Institute, Anantapur, Andhra Pradesh, India-515001
[4]Assistant Professor. (A), JNTUA-Oil Technological & Pharmaceutical Research Institute, Anantapur, Andhra Pradesh, India-515001

ABSTRACT
Influenza is a significant respiratory disease and seasonal outbreaks of the disease have a serious impact of morbidity and mortality across the globe. Although vaccines and antiviral drugs such as oseltamivir are available, the virus rapidly mutates making it difficult to treat it. The antiviral activity of natural products derived by *Glycyrrhiza glabra* (licorice), i.e. Liquiritigenin, Glabridin and Oseltamivir, was evaluated on the basis of in-silico molecular docking towards MORC3 CW domain Influenza NS1 peptide (PDB ID: 6O5W) in the present research. It was found that Docking simulations show that Liquiritigenin had the most favorable binding affinity with lowest MolDock score of -65.27, then the next is Glabridin and Oseltamivir. These findings imply that Liquiritigenin and Glabridin can be considered as promising antiviruses to be developed in the future. The results affirm the possible role of *Glycyrrhiza glabra* extracts as an adjunct to influenza, which can be further experimentalized.

Keywords: - Influenza**,** *Glycyrrhiza glabra***,** Molecular docking**,** Liquiritigenin and Antiviral agents.

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INTRODUCTION

Influenza Disease

The flu otherwise referred to as influenza is a very contagious respiratory disease which is caused by influenza viruses. It occurs mostly in the nose and the throat and in rare cases in the lungs and causes a number of symptoms including fever, cough, sore throat, muscle ache, fatigue and a headache. The illness may be mild to severe and others may cause complications such as pneumonia, hospitalization and even death [1,2]. The influenza viruses are categorized based on type A, type B, type C and type D with type A and B causing seasonal flu outbreaks. The influenza is transmitted primarily by respiratory drops as a person who is infected sneezes, talks or coughs. One can also transmit it through contact with surfaces that are infected with the virus and then touching the face. It is recommended to prevent it by having an annual vaccination, yet the flu is a major health issue of concern on the global level. Besides vaccination, antiviral drugs can also be applied in treating flu but due to the development of antiviral resistance and the high rate of the virus evolvement, it is quite difficult to treat the illness

Corresponding Author: **Nagendra Babu Battula** Email: nagendrababu29@gmail.com

successfully [3]. Thus, a renewed interest in natural remedies and therapeutic approaches based on plants that have solutions to the fight against influenza infections is increasing, which include such substances as *Glycyrrhiza glabra* (licorice) and their therapeutic potential is observed [4].

Glycyrrhiza glabra

One such plant is *Glycyrrhiza glabra*, also referred to as licorice that has been in use in traditional medicine over thousands of years. Its root includes various bioactive compounds which are therapeutically active such as glycyrrhizin, flavonoids, saponins and polysaccharides. *Glycyrrhiza glabra* is also extensively known to possess anti-inflammatory, antiviral, antioxidant and immunomodulatory. Traditional medicine systems such as Ayurveda and Traditional Chinese Medicine have employed it in the treatment of numerous ailments such as digestive disorders, respiratory and skin disorders. Active compounds found in the plant, particularly glycyrrhizin have been reported to have potent anti-inflammatory and antiviral effects and it can be used in the treatment of infections, including the flu and the common cold and even herpes. Licorice is also reputed to have the effect of calming sore throats, coughing as well as boosting the general immunity. Modern studies have validated most of these traditional applications and have shown that it has the potential in the treatment of such ailments as hepatitis and even cancer. Nevertheless, *Glycyrrhiza glabra* has side effects such as high blood pressure and electrolyte imbalance when used in a long-term or in high doses and this fact makes its intake and dosage control necessary [5-10].

Anti-Influenza Effect of *Glycyrrhiza glabra*

The *Glycyrrhiza glabra* or licorice has shown to possess great anti-viral effects especially against influenza virus. Glycyrrhizin is a triterpenoid saponin, which is found in the root of the plant and is known to prevent the replication of various viruses such as influenza. Studies have shown that glycyrrhizin has anti-influenza activities that are based on interference with the process of entry and replication of viruses. It is suspected to interfere with the capability of the viral envelope to fuse to host cell membranes and thus the viruses are unable to enter the host cells and infect. Also, glycyrrhizin has been revealed to have immune-enhancing properties, which activate interferons, a protein with critical roles in the body defense against viral infections [11-13]. The anti-inflammatory effects of licorice also alleviate the effects of the influenza disease including fever and body aches by regulating the inflammatory response. The *Glycyrrhiza glabra* contains other compounds including flavonoids and polysaccharides that play a role in its antiviral effects because of their effect on improving immune functioning and preventing virus replication. Because of its safety and efficacy, licorice has become a prospective natural adjunct to the traditional influenza medication and can be used as an alternative or complement to prevent and/or treat the flu [14-17].

***Glycyrrhiza glabra* Phytoconstituents**

Glycyrrhiza glabra contains numerous phytoconstituents that have been attributed to their numerous therapeutic uses. The most significant and non-investigated licorice compound is glycyrrhizin, a saponin with effective anti-inflammatory, antiviral and immunomodulatory effects. The major active constituent is regarded to be glycyrrhizin which is a key ingredient in most of the therapeutic applications of licorice, including treatment of the influenza. Besides glycyrrhizin, flavonoids, including liquiritin, isoliquiritin and glycyrrhizin, have been found in *Glycyrrhiza glabra*, which adds antioxidant as well as anti-inflammatory properties. It has been reported that these flavonoids scavenge free radicals, protect cells against oxidative damage and regulate immune responses. Saponins are also found in licorice and they have been found to possess anti-inflammatory and antitumor effects. The licorice root polysaccharides too are immune boosters, which improve the resistance of the body to infections. Others are coumarins, triterpenoid and astragalosides, which add to the wide-spectrum of therapy of the plant. These phytochemicals are synergists, giving rise to diverse health effects such as the lowering of inflammation, fighting off infections and the body in general [18,19]. *Glycyrrhiza glabra* is a significant plant in the traditional and contemporary medicinal practices due to the diversity of these bioactive compounds.

MATERIALS AND METHODS

Protein Preparation

The molecular docking experiment was triggered by first preparing the target protein structure. The Protein Data Bank (PDB) was accessed to get Crystal structure of MORC3 CW combined with viral Influenza NS1 peptide; PDB ID:M 6O5W (target protein, a potential virulence factor) that is relevant in host cell adhesion and biofilm formation. The protein is a very good drug discovery target. The protein was imported into the software (Molegro Virtual Docker MVD software) through the File Import Molecule Protein functions. A number of refining and optimization processes were performed to allow the docking simulation to be accurate. Ligands that were co-crystallized, non-essential water molecules, irrelevant heteroatoms and protein chains which may cause interference were removed. The element polar hydrogen atoms were added to the structure to have the proper charge distribution and geometry by the Repair → Add Missing Hydrogens tool. Automatic allocation of atom types and bond orders was done to achieve structural consistency. The PV and E profile of the largest cavity

with the highest volume was determined in order to determine the possible ligand binding zones. This site was regarded to be most biologically significant in the interaction with antifungal phytocompounds and the site was selected in the Detect Cavities tool to dock.

Ligand Preparation

Liquiritigenin, Glabridin and Oseltamivir are the selected ligands because they are reported to be extremely active in antifungal action especially plants such as *Glycyrrhiza glabra*. The chemical structure of these bioactive compounds was downloaded via the PubChem database as 2D or 3D structures and then transformed to the standard chemical file formats such as. mol or .sdf to be compatible with the docking software. The minimization of energy of the ligands was performed by Chem3D software with a MM2 or MMFF94 force field. The process of bond angle optimization, length optimization and steric clash minimization in the form of energy minimization optimized 2D structures to the correct 3D structure. Then the ligands were imported into Molegro Virtual Docker (MVD) through File Import Molecule Ligand. Hydrogen atoms missing were reintroduced and valency problems were addressed so as to provide the appropriate molecular geometry and compatibility to docking simulations. As a standard reference agent, Oseltamivir, which is an antifungal agent, was employed to compare the docking performance and the binding affinity of the natural ligands.

Molecular Import and Binding Site

The ligands and the energy-minimized working protein structure (6O5W) were transferred to the Molegro Virtual Docker (MVD) workspace where the docking simulations were performed successfully. The docking was physiologically relevant as preparation steps were done. To start with, the protonation of both the protein and the ligands was kept constant at a physiological pH of 7.4 to ensure that the electrostatic interactions and the hydrogen bonding patterns were preserved during the docking process. Corrections of hydrogenation and charge were done to give appropriate behavior of the molecules and structural integrity in the docking environment. Defining the binding site of the target protein was done by docking wizard tool of MVD selected protein define binding site. This played a very important role in ensuring that the docking algorithm targeted biologically interesting areas. Coordinate points were set (X, Y, Z) and a reasonable radius of 8-12 A was chosen which would cover the main cavity of the protein and the active site was manually positioned. The identification of cavity and similarity to the chosen region was the basis upon which the docking simulation was focused to ensure that the right interface of interaction was simulated.

Docking Setup

To start a new docking project, Molegro Virtual Docker (MVD) was opened and in the menu, docking > Start Docking Wizard > Create New Docking Job. In the simulation, MolDock SE (Simplex Evolution) algorithm was selected because it is very efficient in the fact that it explores the ligand conformational space and finds the best binding poses with high degree of accuracy. A number of critical parameters were established with the view of having an effective and accurate docking process. The primary scoring functions applied were the MolDock Score and the Re-Rank Score which measured binding affinities in terms of non-bonded interactions, steric complementarity and energy components. The population size used was 50 and a maximum of 1500 docking runs were carried out to a total of 20 independent docking runs, to ensure that enough ligand conformations were explored. A 100 cutoff selection criterion was used to pick the best docking poses and the top 10 poses of each ligand were stored to be further analyzed. Also, docking constraints were employed in order to give preference to the interactions with the known active site residues which are involved in the ligand binding process. These parameters were set in such a way as to have a good balance of the calculation speed and the extensive coverage of the conformational spaces to give dependable and biologically meaningful docking information.

Interaction Analysis and Docking

After the docking simulations, Molegro Virtual Docker (MVD) has generated a few binding poses per ligand sorted by MolDock Scores with lowest values indicating the strongest binding affinities predicted. Each pose was analyzed with the help of the View Ligand Interactions tool, which provided the visual model of the main interactions between the ligand and the active site of the 6O5W protein or between the ligand and individual amino acid residues: hydrogen bonds, hydrophobic contacts and electrostatic interactions. Other essential parameters were studied as well such as the bond length, bond angle and identification of specific interacting residues. The stability and specificity of ligand binding was computed by calculating the energy contributions of all forces including van der Waals interactions, hydrogen bonding, torsional strain and electrostatics. The docking accuracy was compared to the natural orientation of the ligand in the binding site to assess the most optimum docked conformations and to assure that the structural relevant is not irrelevant. All the data such as 2D interaction charts, binding energy curves and structural data of the docked complexes were exported in standard format such as: mol2, pdb and high resolution image files to facilitate documentation, presentation and publication purposes [20-25].

RESULTS AND DISCUSSION

The docking of the ligands Liquiritigenin, Glabridin and Oseltamivir to the crystal structure of the MORC3 CW domain fused with the viral Influenza NS1 peptide (PDB ID: 6O5W) was conducted in the present study using Molegro Virtual Docker (MVD). The objective was to measure the binding affinity of these ligands to the protein target and make a comparison of the docking scores in order to be able to judge if these ligands are good inhibitors of the influenza virus or not. The findings in this paper consist of MolDock scores, Rerank scores and the hydrogen bond interactions of each ligand. Also, 2D, 3D and secondary views of the docking poses of the docking of each ligand with the protein structure are given to depict the modes of interaction and binding specificities. These results indicate the relative docking efficacy of the ligands, especially that of the ligand binding affinity and strength of interaction, as reflected in the MolDock and Rerank scores. The results are further described in detail below beginning with a table that summarizes the ranking of the ligands according to the performance of their docking.

Table 1: Ranking of Ligands and poses against Crystal structure of MORC3 CW domain fused with viral Influenza NS1 peptide based on MolDock score
Protein: 6O5W

Ligand	Species Name	MolDock Score	Rerank Score	H Bond
114829	Liquiritigenin	-65.27	-53.62	-6.25
124052	Glabridin	-56.07	-36.47	-8.22
65028	Oseltamivir (Standard)	-53.79	-21.41	-0.99

To examine the binding affinity of three ligands (Liquiritigenin, Glabridin and Oseltamivir) to the crystal structure of the MORC3 CW domain in the fused form with viral Influenza NS1 peptide (PDB ID: 6O5W), the molecular docking analysis was carried out. To evaluate the binding potential of these ligands, the docking scores, rerank scores and hydrogen bond interactions were taken. The ranking of the ligands according to the MolDock score, rerank score and hydrogen bond interactions are presented in Table 1. Liquiritigenin had the best MolDock score of -65.27 that is much lower in value (a higher binding affinity) than Glabridin (-56.07) and Oseltamivir (-53.79) among the ligands. These were further confirmed by the rerank scores with Liquiritigenin having the strongest binding potential (-53.62) succeeded by Glabridin (-36.47) and Oseltamivir (-21.41). The highest amount of hydrogen bonds was observed to form with the target protein between Glabridin (-8.22) and Liquiritigenin (-6.25) and Oseltamivir (-0.99). The ability of Glabridin to form stronger hydrogen bond means that binding interactions are more stable than the rest of the ligands.

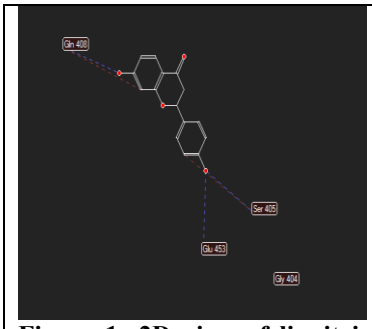


Figure 1: 2D view of liquitrigenin Vs 6O5W

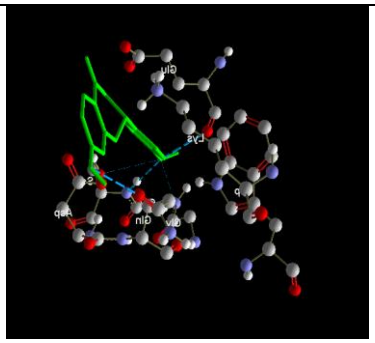


Figure 2: 3D view of liquitrigenin Vs 6O5W

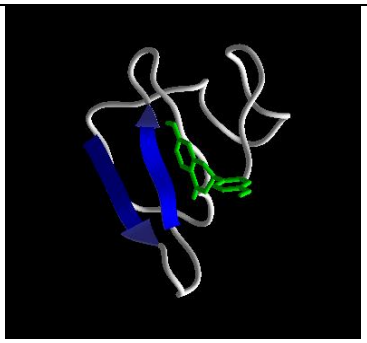


Figure 3: Secondary view of liquitrigenin Vs 6O5W

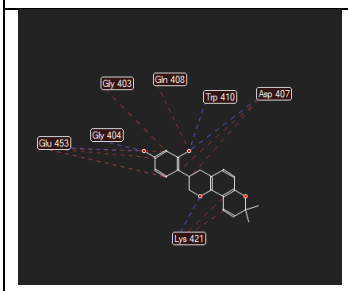


Figure 4: 2D view of Glabridin Vs 6O5W

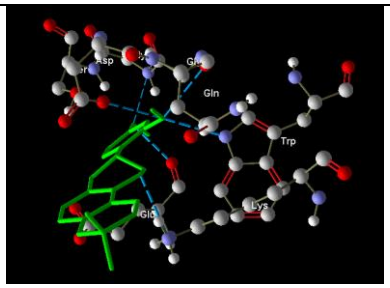
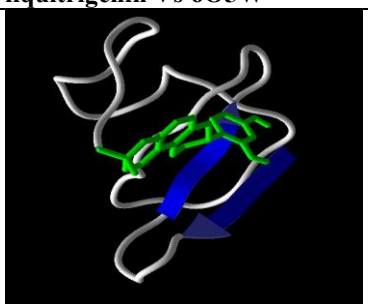


Figure 5: 3D view of Glabridin Vs 6O5W



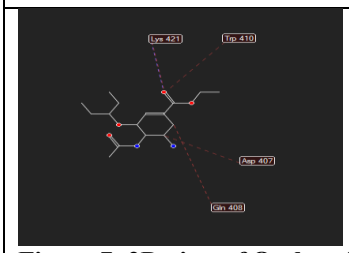


Figure 7: 2D view of Oseltamivir Vs 6O5W

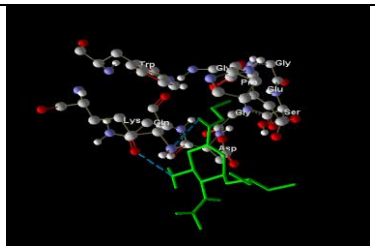


Figure 8: 3D view of Oseltamivir Vs 6O5W

Figure 6: Secondary view of Glabridin Vs 6O5W

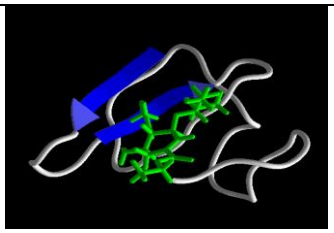


Figure 9: Secondary view of Oseltamivir Vs 6O5W

The docking results have been graphically presented in figures 1-9. The 2D views of the ligand-protein interactions are depicted in figure 1, 4 and 7 and they reveal the significant hydrogen bonds and hydrophobic interactions between the ligands and the active site of 6O5W. The 3D views of the ligand binding to the protein were illustrated in Figure 2, 5 and 8 giving visual evidence of the binding poses. Lastly, Figures 3, 6 and 9 show secondary structural views, which ensure that the ligands fit in the active site of the protein. The molecular docking findings suggest Liquiritigenin has the most preferable binding affinity and interaction with the target protein followed by Glabridin and Oseltamivir. These findings indicate that Liquiritigenin can be a good prospective study to develop as an antiviral against Influenza. These compounds have strong hydrogen bonding and binding energy which has given them a promising future as a good inhibitor of influenza virus and further in vitro and in vivo research is needed.

DISCUSSION

Based on the study, the binding affinity of three ligands, which are Liquiritigenin, Glabridin and Oseltamivir, was tested in-silico against the crystal structure of the MORC3 CW domain fused with viral Influenza NS1 peptide (PDB ID: 6O5W). This was aimed at determining their potential as antiviral agent against Influenza in terms of docking performance, strength of interaction and stability of the ligand-protein complexes.

Liquiritigenin had the best binding affinity as it had a MolDock score of -65.27, significantly less than that of Glabridin (-56.07) and Oseltamivir (-53.79). This implies that Liquiritigenin exhibits a better affinity with the target protein than the other ligands. The present finding is in line with the past researches that have underscored the antiviral properties of glycyrrhizin and its derivatives, particularly against respiratory viruses such as influenza [1,3]. This is further supported by the rerank score of Liquiritigenin (-53.62), which shows that it has a good binding potential and stability in the docking process.

In spite of the fact that Liquiritigenin demonstrated the best binding affinity, Glabridin established the greatest number of hydrogen bonds (-8.22) with the protein, they may have better interactions with the protein in terms of hydrogen bonding. The high number of hydrogen bond interactions of Glabridin follows the findings of other studies that licorice flavonoids are essential in antiviral effects of *Glycyrrhiza glabra* [2]. Furthermore, the existence of saponins, e.g., Liquiritigenin and flavonoids in licorice has been well reported in synergistic effects in regulating the immune responses as well as preventing viral replication [9,12].

When compared to Oseltamivir, a known antiviral drug, it is possible to conclude that both Liquiritigenin and Glabridin have significant potential as natural substitutes or supplements of traditional antiviral treatment. These compounds could be useful in the development of antiviral drugs and therefore the docking analysis indicates that they can be used as effective inhibitors of influenza. The docking matches make good contributions to the binding potential of Liquiritigenin, Glabridin and Oseltamivir on the influenza virus. Although Liquiritigenin had the best docking score, Glabridin had a good hydrogen bonding pattern thus making it a good option to be further investigated. The results correspond with other in-silico and in-vitro studies that highlight the significance of bioactive compounds of *Glycyrrhiza glabra* as possible antiviral agents [14,15]. Experimental validation needs to be done in future research to identify the efficacy and safety of these natural compounds against Influenzand related viral infections.

CONCLUSION

This research indicates the potential of Liquiritigenin and Glabridin that are derivatives of *Glycyrrhiza glabra* as effective antivirals against Influenza. Molecular docking of the compounds in-silico revealed that they possessed desirable binding affinities with the target protein, MORC3 CW domain fused with Influenza NS1 peptide (PDB ID: 6O5W) with Liquiritagenin displaying the strongest interaction. The results correspond to the other literature that shows the antiviral and immune-modulating effects of *Glycyrrhiza glabra* compounds. These bioactive compounds especially Liquiritigenin may be used as potential alternatives or

supplement to the traditional antiviral drugs and could offer alternative means of drug discovery. Also, the possibility of Glabridin to make stable hydrogen bonds with the target protein implies its potential in increasing the stability of the interactions with ligands. On the whole, this research paper is an initial step toward more experimental research, such as in vivo and in vitro, to establish the therapeutic effect of these compounds against the Influenzand other viral infections. Considering the current difficulties in the treatment of Influenzand the intensive ability of the viral organism to develop resistance, natural substances of medicinal plants such as *Glycyrrhiza glabra* can be a good source of the development of new antiviral medications.

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