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MOLECULAR DOCKING STUDY OF *GLYCYRRHIZA GLABRA* CONSTITUENTS AS ANTIVIRAL CANDIDATES FOR GUILLAIN–BARRÉ SYNDROME

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ABSTRACT

Guillain-Barré syndrome and Chikungunya viruses possess specific proteins that serve as prime targets for antiviral drug design. Recent studies suggest that targeting these proteins can effectively inhibit viral replication and pathogenesis in humans. Glycyrrhiza glabra (Liquorice) is known to harbor phytochemicals with potent antibacterial and anticancer properties. In this study, we evaluated the antiviral potential of four specific compounds identified in Glycyrrhiza glabra fruit against 14 viral proteins (7 from Guillain-Barré syndrome and 7 from Chikungunya) using in silico methods. Through virtual screening and molecular docking, we determined that Gallic acid (Benzoic acid, 3,4,5-trihydroxy-) exhibited the highest binding affinity with the target proteins. Furthermore, we predicted the specific amino acid residues involved in the active sites and analyzed the hydrogen bonding interactions contributing to this stability.

KEYWORDS: Molecular Docking, Hydrogen Bonding, Zika Virus, Glycyrrhiza glabra, Isoliquiritin, Glycyrrhizin.

INTRODUCTION

Medicinal plants are a foundational element of human healthcare, offering natural resources to promote health and prevent disease.[1] Traditional Indian medical systems, such as Ayurveda, Unani, and Siddha, rely heavily on these rich botanical resources to develop natural drugs.[2] Among these, Glycyrrhiza glabra Linn., commonly known as Glycyrrhiza glabra, is of significant importance.[4] Widely distributed in tropical and sub-tropical regions, the name "Liquorice" is derived from the Sanskrit word "Amalaki," meaning "The Sustainer" or "Prosperity".[1] This plant has been used for generations to treat a wide spectrum of ailments, acting as a diuretic, laxative, liver tonic, restorative, and antipyretic.[2]

Phytochemical analyses of Liquorice have identified major constituents including tannins, alkaloids, polyphenols, vitamins, minerals, and specific compounds such as emblicanin A and B. Beyond its nutritional value, Liquorice exhibits anti-aging, expectorant, antibacterial, antioxidant, anticancer, and hypoglycemic properties.[3] GC-MS analysis of the methanolic extract of Glycyrrhiza glabra revealed four major peaks: 1,2,3-benzenetriol (Pyrogallol), 5-hydroxymethylfurfural, Liquiritin, and 3,4,5-trihydroxybenzoic acid (Gallic acid). Pyrogallol is a polyphenol noted for its fungicidal, antitumor, and antiviral activities. Similarly, Gallic acid possesses a broad spectrum of biological activities, including antimicrobial, anticancer, anti-inflammatory, and anti-HIV effects.[5] Zika Virus Guillain-Barré syndrome is an arboviral disease transmitted by the bite of infected Aedes aegypti mosquitoes. The clinical presentation ranges from asymptomatic infection to severe Guillain-Barré syndrome Hemorrhagic Fever and Guillain-Barré syndrome Shock Syndrome.[7] The viral genome consists of a single strand of positive-sense RNA encoding three structural and seven non-structural proteins.[8] Key therapeutic targets include the NS2B-NS3 protease, which is crucial for the viral life cycle[9, 10], and the NS5 protein (containing the RdRp domain), which drives "de novo" viral genome replication.[14] Structural proteins such as the Capsid (involved in genome encapsidation) and Envelope proteins are also critical for viral assembly and entry.[11] ZIKA is an arthropod-borne alphavirus belonging to the Togaviridae family[15], causing acute febrile illness. It is primarily transmitted by Aedes aegypti and Aedes albopictus, both invasive vectors closely associated with human habitats.[16] The CHIKV genome comprises two open reading frames (ORFs) encoding polyproteins that are cleaved into four non-structural proteins (nsP1–nsP4)—essential for replication—and five structural proteins (C, E3, E2, 6K, E1).[17] The structural proteins are processed from a long polyprotein; specifically, 240 copies of the Capsid (C) protein associate with genomic RNA to form the nucleocapsid in the host cytoplasm, facilitating viral assembly.[18] Bioinformatics and Study Aim Bioinformatics is an interdisciplinary field utilizing statistics, mathematics, and computer science to analyze biological data.[19] With the advent of proteomics and large-scale gene expression analysis, computational methods have become indispensable in modern biological and clinical research.[20] These tools allow researchers to determine peptide sequences, visualize protein structures (complementing X-ray crystallography), and infer evolutionary relationships.[21] Molecular docking is a key technique used to analyze the "fitness" and energy interactions between a ligand and a protein. These interactions serve as a preliminary pharmaceutical approach for drug discovery.[22] The objective of this study is to evaluate and compare the docking efficacy of selected constituents from Glycyrrhiza glabra fruit against key protein targets of the Guillain-Barré syndrome viruses.

1. MATERIALS AND METHODS

1.1. Preparation of Viral Protein Targets

The three-dimensional crystal structures of the target macromolecules were retrieved from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB). This repository houses experimentally determined structures of proteins and nucleic acids. For this study, fourteen proteins representing the structural and non-structural components of the Guillain-Barré syndrome viruses were selected. The structures were downloaded in PDB format and subsequently visualized and inspected using the PyMOL molecular graphics system.[23]

1.2. Ligand Preparation

The ligands selected for this study were based on previous GC-MS analysis of *Phyllanthus emblica* (Amla) fruit extracts.[5] Four distinct phytochemical constituents were identified as potential antiviral agents. The two-dimensional structures of these ligands were constructed using ACD/ChemSketch. Following construction, the structures underwent geometry optimization and hydrogen addition. The final optimized structures were saved in .mol format and designated as Ligands A, B, C, and D for docking analysis.

1.3. Molecular Docking Protocol

Molecular docking were performed using iGEMDOCK (Generic Evolutionary Method for molecular Docking), a graphical environment designed for drug design, virtual screening, and post-docking analysis.[24] The pre-processed viral proteins and ligand files were imported into the software. Docking was executed using standard parameters: a population size of 200, 70 generations, and 2 solutions per iteration. Upon completion, the docking poses were evaluated based on fitness function. The conformations exhibiting the highest binding affinity and lowest total binding energy were extracted, and the resulting protein-ligand interactions were visualized using PyMOL.[25]

2. RESULTS AND DISCUSSION

2.1. Binding Energy Analysis

The docking analysis provided quantitative data on the interaction between the selected phytochemicals and the viral targets. The binding efficiencies, represented by the total binding energy, are detailed below.

Table 1: Total Binding Energy (kcal/mol) profile of the four *Emblica officinalis* ligands docked against the non-structural proteins of Guillain-Barré syndrome viruses.

Ligand	Compound name	Zika Virus				
		NS1 protein	Trans membrane domain of NS2A	NS2B / NS3 protease	NS3 helicase	NS5 protein
A	Glycyrrhizin	-72.59	-372.21	-59.33	-68.75	-65.2
B	Glycyrrhetic acid	-69.48	-466.73	-61.01	-66.7	-68.97
C	Liquiritin	-63.14	-379.29	-55.32	-62.42	-59.72
D	Isoliquiritin	-84.1	-476.18	-64.94	-87.89	-80.2

Table 2: The Total Binding Energy (kcal/mol) profile for Guillain-Barré syndrome viruses structural proteins with 4 ligands.

Ligand	Compound Name	Zika Virus	
		Capsid protein	Envelope protein
A	Glycyrrhizin	-60.24	-63.94
B	Glycyrrhetic acid	-68.86	-70.03
C	Liquiritin	-63.75	-60.78
D	Isoliquiritin	-79.45	-69.3

H – Bond profile for Guillain-Barré syndrome viruses protein with 4 ligands

Table 3: H – Bond profile for Guillain-Barré syndrome viruses nonstructural proteins with 4 ligands.

Ligand	Compound name	Zika Virus				
		NS1 protein	Trans membrane domain of NS2A	NS2B / NS3 protease	NS3 helicase	NS5 protein
A	Glycyrrhizin	H-M	H-M	H-S	H-M	H-S
B	Glycyrrhetic acid	H-S	H-M	H-S	H-M	H-S
C	Liquiritin	H-M	H-M	H-S	H-M	H-S
D	Isoliquiritin	H-M	H-S	H-M	H-S	H-S

Table 4: H – bond profile for Guillain-Barré syndrome structural proteins with 4 ligands.

Ligand	Compound name	Zika Virus	
		Capsid protein	Envelope protein
A	Glycyrrhizin	H-S	H-M
B	Glycyrrhetic acid	H-S	HM
C	Liquiritin	H-M	H-M
D	Isoliquiritin	H-S	H-M

2.3. Amino acid position profile for Guillain-Barré syndrome protein with 3 ligands

2.3. Binding Energy Analysis of Zika Virus Proteins

Based on the cumulative data presented in Tables 1 through 6, the antiviral potential of four constituents from *Glycyrrhiza glabra* was evaluated against the optimized 3D structures of Zika virus proteins using iGEMDOCK. The analysis focused on identifying the lowest total binding energy scores, which serve as an indicator of the most stable and effective protein-ligand conformations. The results revealed distinct binding preferences among the analogs: Compound 'A' exhibited the highest affinity for the structural proteins, recording binding energy values of -60.78 kcal/mol against the Capsid protein and -60.24 kcal/mol against the Envelope protein. In contrast, Compound 'D' demonstrated superior efficacy against the non-structural proteins, displaying a remarkable binding energy of -476.18 kcal/mol with the transmembrane domain of NS2A. Furthermore, Compound 'D' showed significant interaction with the NS3 helicase (-87.89 kcal/mol), NS1 protein (-84.10 kcal/mol), NS5 protein (-80.20 kcal/mol), and the NS2B/NS3 protease (-64.94 kcal/mol), suggesting its potential as a broad-spectrum inhibitor of the viral replication machinery.

Table 5: Amino acid position profile for Guillain-Barré syndrome nonstructural proteins with 4 ligands.

Ligand	Compound name	Zika Virus				
		NS1 protein	Trans membrane domain of	NS2B / NS3 protease	NS3 helicase	NS5 protein
A	Glycyrrhizin	Ile(242)	Leu(11)	Arg(55) Asp(58)	Asn(329)	His(53)
B	Glycyrrhetic acid	Arg(294)	Gly(5) Met(4)	Asn(152)	Phe(417)	Asp(808)
C	Liquiritin	Ser(185)	Leu(11)	Asn(152)	Lys(618)	Lys(578)
D	Isoliquiritin	Glu(205)	Arg(18)	Leu(149)	Asn(329)	Ser(776)

Table 6: Amino acid position profile for Guillain-Barré syndrome structural proteins with 4 ligands.

Ligand	Compound name	Zika Virus	
		Capsid protein	Envelope protein
A	Glycyrrhizin	Arg(41)	Ile(618) Lys(625) Gly(628) Arg(629) Ile(630)
B	Glycyrrhetic acid	Phe(47)	Ile(618)
C	Liquiritin	Arg(22)	Arg(629)
D	Isoliquiritin	Arg(41)	Gly(628)

Non-Structural proteins of Zika Virus

The Total Binding Energy for the Zika Virus NS1 protein with 3 ligands from Table – 1, Table – 3, and Table – 5. The docking simulation of 4 ligands was performed for the Zika Virus NS1 protein. From the docking study, we observed that compound – D has best binding affinity with the target NS1 protein with the binding energy value of - 84.1kcal/mol. Interaction analysis of binding mode of compound –D in Zika Virus NS1 protein reveals that it forms one hydrogen bond with low energy, with Ile (242) residue. A close-up view of the Total Binding Energy (kcal/mol) profile for Zika Virus NS1 protein with 4 ligands: is shown in Figure1.

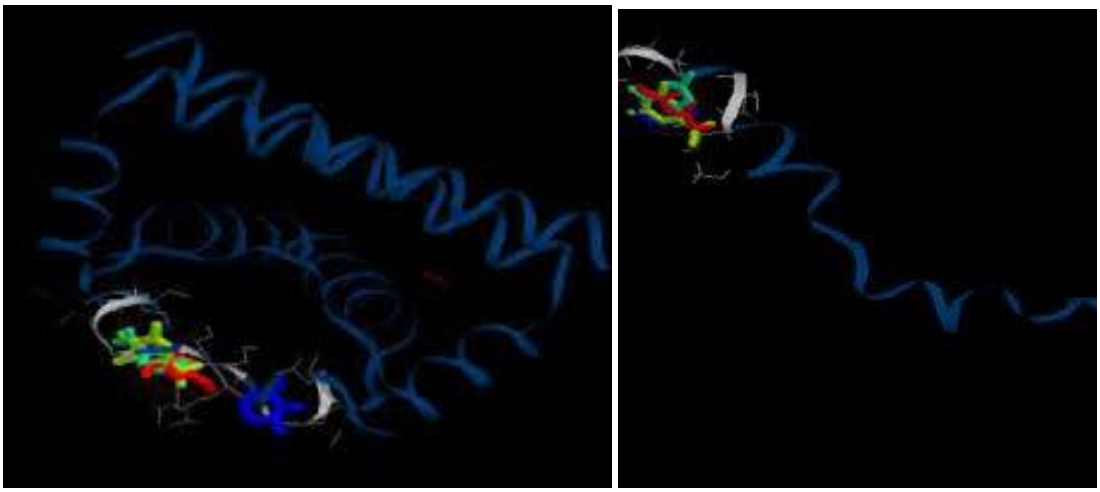

Figure 1: The Total Binding Energy profile for Zika Virus NS1 protein with 4 ligands; Figure 2: The Total Binding Energy profile for Zika Virus Trans membrane domain of NS2A with 4 ligands.

The Total Binding Energy for Zika Virus Trans membrane domain of NS2A with 4 ligands From Table – 1, Table – 3 and Table – 5, the docking simulation of 4 ligands were performed for Zika Virus Trans membrane domain of NS2A. From the docking study, we observed that compound – D has best binding affinity with the target Trans membrane domain of NS2A with the binding energy value of -476.18 kcal/mol. A close-up view of the Total Binding Energy (kcal/mol) profile for Zika Virus Trans membrane domain of NS2A with 4 ligands: is shown in Figure.2.

The Total Binding Energy for Zika Virus NS2B / NS3 protease with 4 ligands From Table – 1, Table – 3 and Table – 5, the docking simulation of 4 ligands were performed for Zika Virus NS2B / NS3 protease. From the docking study, we observed that compound – D has best binding affinity with the target NS2B / NS3 protease with the binding energy value of -64.94 kcal/mol. Interaction analysis of binding mode of compound –D in Zika Virus NS2B / NS3 protease reveals that it forms one hydrogen bond with low energy, with Leu (149) residue. A close-up view of the Total Binding Energy (kcal/mol) profile for Zika Virus NS2B / NS3 protease with 4 ligands: is shown in Figure.3.

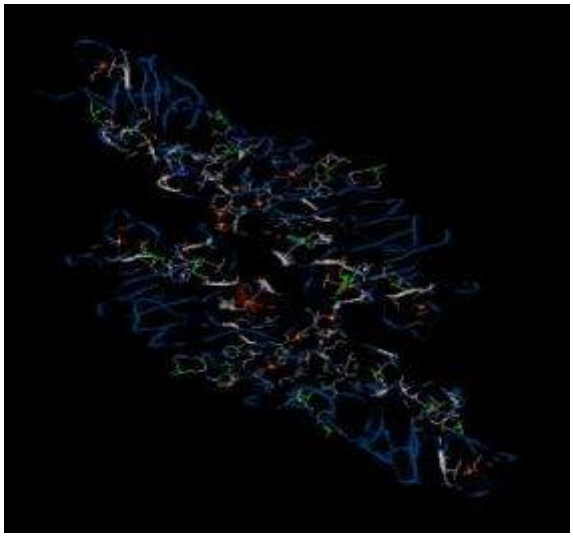

Figure 3: The Total Binding Energy profile for Zika Virus NS2B / NS3 protease with 4 ligands.

The Total Binding Energy for Zika Virus NS3 helicase with 4 ligands: From Table – 1, Table – 3 and Table – 5, the docking simulation of 4 ligands were performed for Zika Virus NS3 helicase. From the docking study, we observed that compound – D has best binding affinity with the target NS3 helicase with the binding energy value of -87.89 kcal/mol. Interaction analysis of binding mode of compound –D in Zika Virus NS3 helicase reveals that it forms one hydrogen bonds with low energy, with Asn (329) residue. A close-up view of the Total Binding Energy (kcal/mol) profile for Zika Virus NS3 helicase with 4 ligands: is shown in Fig.4. The Total Binding Energy for Zika Virus NS5 protein with 4 ligands From Table – 1, Table – 3 and Table – 5, the docking simulation of 4 ligands were performed for Zika Virus NS5 protein From the docking study, we observed that compound – D has best binding affinity with the target NS5 protein with the binding energy value of -80.2kcal/mol. Interaction analysis of binding mode of compound –D in Zika Virus NS5 protein reveals that it forms one hydrogen bonds with low energy, with serine(776). A close-up view of the Total Binding Energy (kcal/mol) profile for Zika Virus NS5 protein with 4 ligands: is shown in Fig.

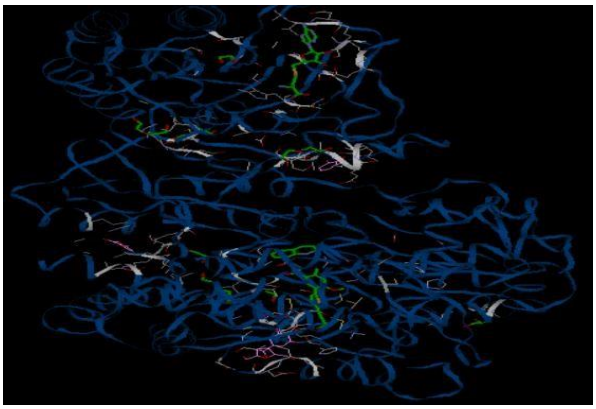

Figure 4: The Total Binding Energy profile for Zika Virus NS5 protein with 4 ligands.

The Total Binding Energy for Zika Virus Capsid protein with 4 ligands From Table – 2, Table – 4 and Table – 6, the docking simulation of 4 ligands were performed for Zika Virus Capsid protein. From the docking study, we observed that compound – D has best binding affinity with the target Capsid protein with the binding energy value of - 79.45 kcal/mol. Interaction analysis of binding mode of compound –D in Zika Virus Capsid protein reveals that it forms one hydrogen bond with low energy, with Arg(41) residue. A close-up view of the Total Binding Energy (kcal/mol) profile for Zika Virus Capsid protein with 4 ligands: is shown in Figure.5.

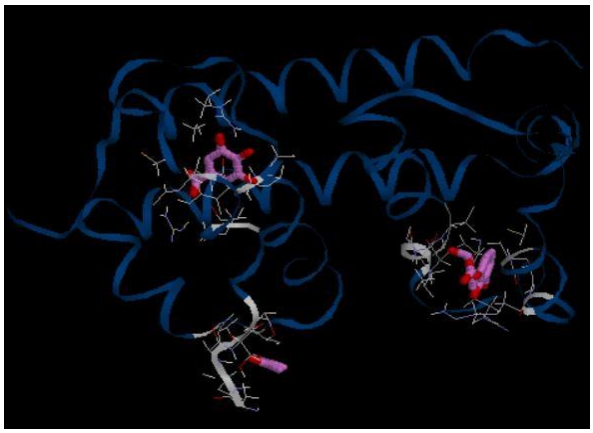

Figure 5: The Total Binding Energy profile for Zika Virus Capsid protein with 4 ligands.

The Total Binding Energy for Zika Virus envelope protein with 4 ligands From Table – 2, Table – 4 and Table – 6, the docking simulation of 3 ligands were performed for Zika Virus envelope protein. From the docking study, we observed that compound – B has best binding affinity with the target envelope protein with the binding energy value of -70.03 kcal/mol. Interaction analysis of binding mode of compound –B in Zika Virus envelope protein reveals that it forms one hydrogen bond with low energy, with Ile(618) residue. A close-up view of the Total Binding Energy (kcal/mol) profile for Zika Virus envelope protein with 4 ligands: is shown in Figure.6.

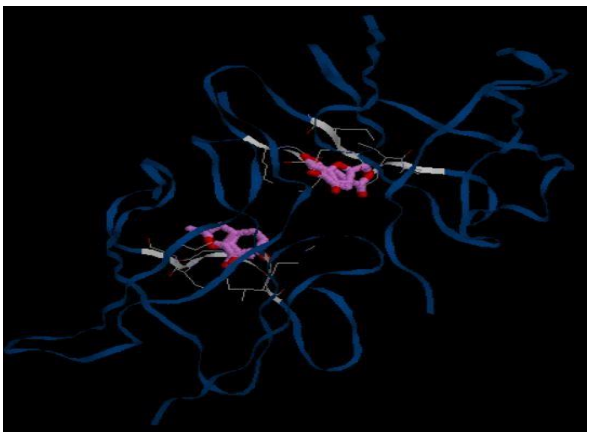

Figure 6. The Total Binding Energy profile for Zika Virus envelope protein with 4 ligands.

CONCLUSION

Our molecular docking studies explored the possible binding modes of 4 compounds that are present in Glycyrrhiza glabra leaf with seven proteins of Zika Virus. Zika Virus consists of envelope protein, NS1 protein, Transmembrane domain of NS2A, NS2B/NS3 protease, NS3 helicase, NS5 protein and capsid protein. It revealed that all the 4 compounds show minimum affinity with all the proteins. The compound „D" (Benzoic acid, 3, 4, 5-trihydroxy- (synonym: Gallic-acid) shows best results compared to other compounds. On comparing the binding energy and the binding site residues, we found that all the compounds will differ in either of them for hydrogen bond formation. The conclusion which is drawn from our virtual screening and docking result are that the Compound D has highest binding affinity with most of the structural proteins of Zika Virus. Whereas the compound D is shown to have highest binding affinity with most of the nonstructural proteins of Zika Virus and therefore it can be used as an effective drug target for Zika Virus. Hence, the Compound D may be considered as the effective drug target for both Guillain-Barré syndrome because it can effectively bind to most of the proteins of both the viruses. Though, there are many reports on the in vitro analysis of these compounds and its medicinal and toxic properties, there are no in silico studies that predict the binding and active regions especially with these proteins. Our study is an attempt to predict the binding site and the binding residues. However, validation of our results through invivo and invitro experiments and also with animal models will enlighten hope for the future development of more potent drugs for the treating Guillain-Barré syndrome.

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