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Zika Virus NS5 Protein novel Inhibitors from *Limonium sinense* phytochemicals using Glide: In silico Approach

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Article History

Abstract

Zika virus infection causes significant congenital disabilities, in addition to microcephaly while an excited mother is infected during pregnancy. Mosquito vectors are the main spreaders of the zika virus which includes *Aedes albopictus* and *Aedes aegypti*. presently clear-cut and definite treatment for the zika virus is not yet available. engrossing in silico approach present study determines the active fighter constituents from aboriginal antiviral herbs to regulate the zika virus. The Lipinski rule filter was used for the Phytoconstituents to determine their molecular interactions and pharmacokinetic studies. NS5 polymerase protein (PDB ID; 5U04) and ligand interactions were determined using Schrodinger Maestro software version 12.7. The outcome displayed that Quercetin, Moupinamide, Epigallocatechin gallate, and Myricetin have sharpened synergism with the asparte active site of NS5 RdRps with docking score (-6.087, -5.838, -5.812, -5.418 Kcal/mol). Analysing the pharmacokinetic study hydrogen bonds with 2.5 Å for target Aspartate amino acid have prime activity. the present study propounds that Quercetin can be used as an inhibitor of the Zika virus.

Keywords: Zika virus, NS5 polymerase, Limonium sinense, molecular docking.

Introduction

In 1947, the outbreak of Zika virus (ZIKV) in Zika Forest in Uganda. Alera et al., 2015 was reported. Derived from a group of *Aedes africanus* mosquitoes and *Sentinel rhesus* macaque monkey the mosquito-borne arbovirus parasite survives (Hermann L et al., 2015). *Aedes aegypti* and *Aedes albopictus* are the most

common spreading agents of ZIKV (Bollati and Baccarelli. 2010). On February 2016 an international public health emergency concern was declared by WHO. Following this by the end of the year ZIKV was reported worldwide in more than 80 countries (4). Headache, joint pain, rashes, swollen lymph nodes, myalgia, retroorbital ache, conjunctivitis, mild fever (37.8ºC – 38.5ºC) And cutaneous maculopapular rush are the most common symptoms of ZIKV. Due to Influenza-like non- specific symptoms the ZIKV can be either diagnosed or misdiagnosed (Hermann Let al., 2015; Priya S et al., 2018 Gourinat AC et al., 2015). ZIKV infections are associated with discrete congenital disorders, inclusive of microcephaly, fetal growth restriction, and in adults Guillain-Barre syndrome (Pattnaik et al., 2018). ZIKV is diagnosed by RT-PCR and ELISA techniques to detect biologically confirmed virus RNA in serum (Sharma P et al., 2020). As reported in a serological study in Nigeria, 40% of the citified population transmitted neutralising antibodies to the Zika virus (Diallo D et al., 2011). However, the Last decay has rapidly spread and emerged in Europe, Asia and America (Aliota MT et al., 2017). ZIKV nature umbrella has 11,000 nucleotides with a single positive-strand RNA genome. Genus Flavivirus belongs to the *Flaviviridae* family. It is coincident with Dengue virus (DENV), yellow fever virus (YFV), West Nile virus (WNV), and Japanese encephalitis virus (JEV) (Ramharack P et al., 2017). Precursor membrane (prM) protein, capsid (C) protein, and envelope (E) protein are the three structural proteins in flaviviral genome encoding. Seven Non-Structural proteins include NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. NS5 performs genome replication (Duan W et al., 2017)**.** An architecture of typical right-hand shape is the structure of NS5 ZIKV RdRp flaviviruses, including three subdomains: fingers, thumb and palm. Flaviviral RdRps, such as the hepatitis C virus (HCV) record highly conserved aspartates as active sites (Wu J et al., 2015). It is a vital target for antiviral drug development. Vaccines or drugs are still unavailable to prevent or treat this viral disease (Shan et al., 2016). In this study, Kinship from the family Plumbaginaceae the medicinal plant *Limonium sinense* is distributed in China and Taiwan. Traditionally the herb medicates to treat haemorrhages, enhance blood circulation, piles, hepatitis, diarrhoea, antiviral, bronchitis, and other disorders. *L. sinense* flavonols, flavonol glycosides, flavonol glycoside gallates, flavones, flavanones, and flavan-3-ols are the major active constituents (Chaung et al., 2003) The present *in silico* study scrutinises the binding efficiency of active constituents of the *Limonium sinense* plant against the non-structural NS5 polymerase protein using the maestro module. However, the literature survey has not reported any *in-silico* studies on active compounds of *Limonium sinense*, which act against the protein from the Zika virus.

Materials and Methods

In the study, ten phytoconstituents from the *Limonium sinense* medicinal plant are selected, as given in Table 1. The selected active candidates are potential inhibitors against viral infections such as HCV, and Type-1 Herpes simplex virus (Hsu WC et al., 2015 and Lin LC et al., 2000). The 2D structure of phytoconstituents downloads from the database PubChem in SDF (structure data file) format.

Ligand preparation

The ligand preparation was done by Ligprep module version 3.6 for all ligands. For each input structure, the Ligprep module constructs a low-energy, single, three-dimensional structure with the ionisation states, number of conformations, and tautomers for the types of functional groups present or molecular weight. The Optimised Potentials for Liquid Simulations (OPLS3e) enhanced force field produced for each structure with low energy up to ten ligand conformations. (Madhavi Sastry G et al., 2013)

Protein preparation

From Protein Data Bank (PDB ID: 5U04) the Zika virus crystal structure of NS5 RdRp, with a resolution of 3.0 Å, was retrieved. For viral RNA synthesis, a polymerase and RNA capping performed by methyltransferase is NS5 protein (Zhao B et al., 2017). In Protein Preparation Wizard of Maestro 10.4 crystal structure (NS5) is imported. The preprocessed protons of protein fixes bond orders, and then review and modify that not found cocrystal in A chain of Zikv (Yadav R et al., 2021). At the junction of the template and central canal, the RNA-dependent RNA polymerase domain containing the NTP channel will merge. From motifs C and A three aspartates (Asp 535, Asp 665 and Asp 666) are localised that coordinate divalent metal ions for nucleotide polymerisation (Ng KK et al., 2008). Then in refining, optimising protonation states and hydrogen-bond networks and performing in NS5 protein. After H-bond optimisation, the entire structure relaxes using the Imperf module of Impact and the OPLS_2005 force field. The options explored for protein minimisation included hydrogen only or all-atom with a termination criterion based on the heavy atoms' root-mean-square deviation relative to their initial location. (Madhavi Sastry G et al., 2013)

Grid-based molecular docking

In this study to analyse *L. sinense* active compounds interactions with ZIKV NS5 polymerase, molecular docking performs Ligand Docking with grid-based (Glide) version 12.7. ZIKV NS5 polymerase binds strongly in complex with two IDX-184 and MK-0608 inhibitors with ZIKV RdRp in GTP (Elfiky et al., 2018). Most flaviviral RdRps reported aspartates highly conserved active site, along with active site residues (Asp 538, Asp 665 and Asp 666), marks as constraints for the docking (Malet H et al., 2008). Coordinates of grid box with X=20.66, Y=68.15, and Z=96.43 generated at the source IDX-184 and MK-0608 inhibitor in the centroid. For the selected ligand molecule generated grid (5U04) is suitable for docking. Here the ligand flexibly docks using the 'extra precision glide docking (Glide XP), as per the generated grid. Generating active poses suiting active sites of the compounds is a speciality of XP docking. (Friesner et al., 2006).

ADME properties

The Qikprop tool (Schrodinger model) analysed the Drug-likeness properties of all the selected ligands. Based on Lipinski's and Veber's Rule, choose the phytoconstituents. (Geetha et al., 2022)

Results and Discussion

Medicament and therapy for Zika virus infection are unknown in the pharmaceutical industry worldwide (Singh P et al., 2019). Many kinds of research were happening on other viruses, except Zika. Initiatives are taken to prevent Zika virus infection in pregnant women and newborn babies by the researchers and the government. The study was initiated with active site residues (aspartates) to identify effective lead compounds rather than the protein cocrystal structure, drug, or pathway due to no proper or poor availability of literature. This study explores lead compounds that act on the non-structural RNA-dependent RNA polymerase domain protein (NS5) of the ZIKA virus. Virus replication is prevented, by a decrease in the affinity of the virus with the host cell.

ADME study

A total of ten selected phytoconstituents from *L. sinense*. They are listed in Table 1. Engaging Qikprop ADME physico-chemical properties prediction of Phytoconstituents were evaluated. The consideration to analyse the Absorption, Distribution, Metabolism and Excretion properties are values of Rule of Five (RO5), molecular weight (MW), Hydrogen Bond Donor, Hydrophobic component of the SASA (FOSA), Hydrogen Bond Acceptor, and polarizability (QPpolrz). Quercetin-3-O-Alpha-L-Rhamnopyranoside**,** Isomericitrin and Myricetin 3-Rhamnoside contains some violations. The remaining obeyed the Lipinski rule of five and have low molecular weight and low molecular weight, as shown in Table 2

Docking study

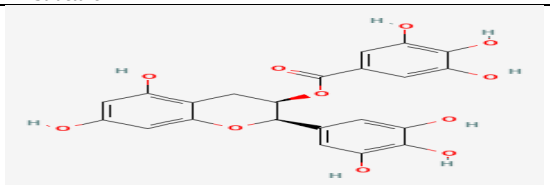
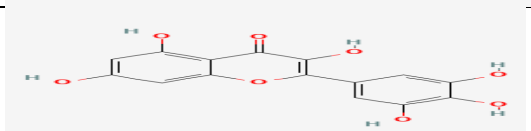
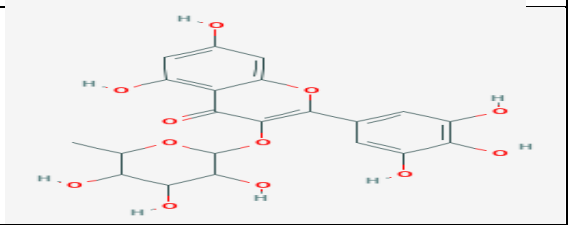
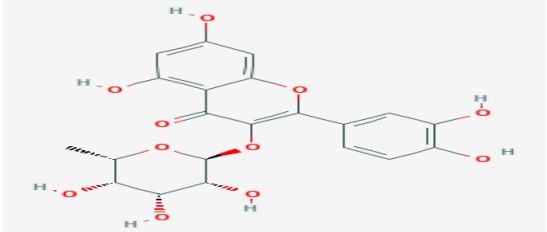
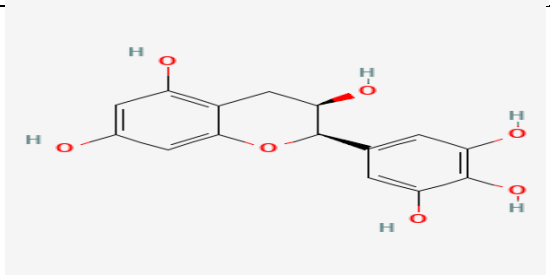
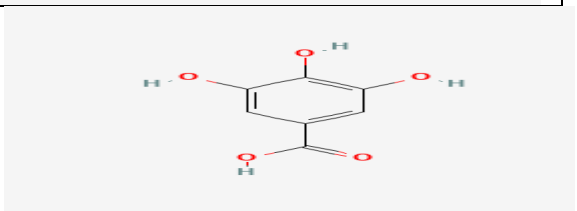
In ZIKV, non-structural NS5 the C-terminal domain is essential for the synthesis of viral genome. It encodes with NS5-RdRP. The ZIKV NS5 protein architecture comprises three fields: fingers, thumb, and palm parallel right-handed, the latter of which represents an active site contains the highly conserved aspartate in most of the reported flaviviral RdRps, as well as hepatitis C virus (HCV). In this work, in silico study, the binding affinity was performed between filtered L. sinense Ligands molecules and the active site of NS5 protein PDB(5U04) ZIKV by Schrodinger Maestro software for molecular docking analysis. Docking score Kcal/mol), Binding energy, Amino acids Residues, Hydrophobic interaction (π-π staking), H-bond distance (Å) and number of H-bonds as shown in Table 3. From these results, the best binding affinity to ZIKV polymerase is observed in the docked ligand quercetin. The docking score is -6.087 Kcal/mol, generating three H- Bonds bound with amino acids GLN 620, ALA 533, and ASP 665. Figure 1 explored 2D and 3D structures. The Moupinamide Docking score is -5.838 Kcal/mol. Hydrophobic interaction appeared with LYS 688. It binds ARG 623, GLN 620, and ASP 665 with 3 H- bonds. Figure 2 demonstrates 2D and 3D structures of complex Moupinamide and ZIKV NS5 protein. Epigallocatechin Gallate docking score is -5.812Kcal/mol. 2D and 3D designs are shown in Figure 3. It binds with six amino acids SER 699, GLN 620, ASP 665, ALA 533, ASP 666, and ASP 666. It has glide energy higher than to standard drug sofosbuvir. Figure 4 exhibited 2D and 3D Structures. Myricetin docking score is -5.418 Kcal/mol binds with three amino acids ALA 533, GLN 620, and ASP 665. Epigallocatechin docking score is -5.085 Kcal/mol. Residues are LYS 688, GLN 620, and ASN 616 complex with ZIKV NS5 protein by three H- bonds shown in Figure 5. The above-discussed five compounds have higher binding affinity than compared Standard drugs. Sofosbuvir

docking score is -4.628 Kcal/mol. All the compounds have more H-bonds bound with amino acid residues. In particular, these are all interpreted with target active site aspartates (ASP 665, ASP 666 in motif C) except Epigallocatechin. Hence the hydrogen bond between the NS5 Polymerase proteins and amino acid residues is less than 3Å distance. From these results, we suggested that all five phytoconstituents are effective for ZIKV infection. Quercetin showed a higher affinity than standard, assuring nucleotide inhibitors against the ZIKV RdRp.

Conclusion

The current study finds the best interacting active constituents to inhibit the ZIKV NS5 polymerase. Sofosbuvir suggests Zika virus-infected patients due to a similar structure of HCV, NS5 RdRps protein. We have screened ten compounds from the antiviral plant *Limonium sinense*. The docking score for ten complexes ranged from -6.087 to -4.039 kcal/mol. Five compounds are selected based on scoring and other physical parameters. Compared to the reference drug. Quercetin showed a high binding affinity against NS5 polymerase(5U04). Analysing the results most suitable attachment inhibitor is quercetin hence it can be utilised as a lead compound to control the Zika virus. Therefore, it is predicted to be fit for human consumption.

Table 1: Name of the ligands, Molecular Formula, 2D structure and the PubChem ID of *Limonium sinense*

S.no	phytocompound Name	PubChem Id	Molecular Formula	2D Structure
1	Epigallocatechin Gallate	65064	$C_{22}H_{18}O_{11}$	
2	Myricetin	5281672	$C_{15}H_{10}O_8$	
3	Myricetin 3-Rhamnoside	5352000	$C_{21}H_{20}O_{12}$	
4	Quercetin-3-O-Alpha-L-Rhamnopyranoside	6325794	$C_{21}H_{20}O_{11}$	
5	Epigallocatechin	72277	$C_{15}H_{14}O_7$	
6	Gallic Acid	370	$C_7H_6O_5$	

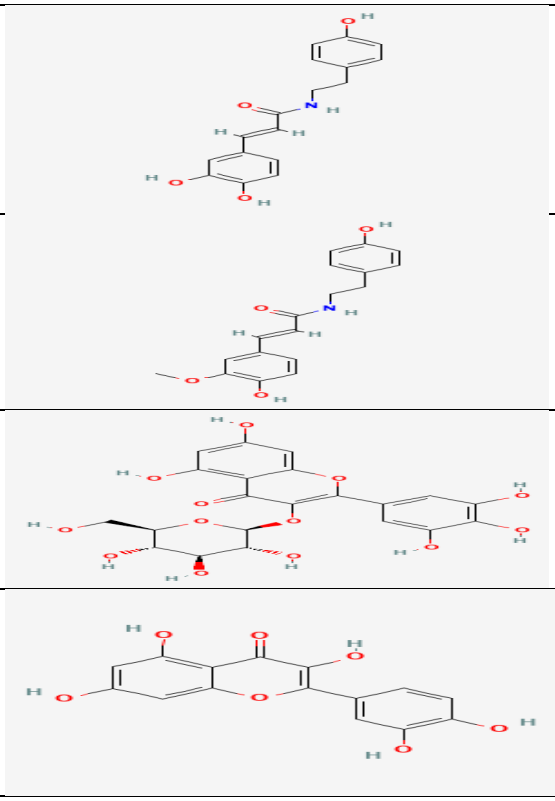
7	N-Caffeoyltyramine	9994897	$C_{17}H_{17}NO_4$	
8	Moupinamide	5280537	$C_{18}H_{19}NO_4$	
9	Isomericitrin	5318606	$C_{21}H_{20}O_{13}$	
10	Quercetin	5280343	$C_{15}H_{10}O_7$	

Table 2: ADME Profile for *Limonium sinense* phytoconstituents.

s.no	phytocompound name	Molecular Weight ≤ 500	HBD ≤ 5	HBA ≤ 10	Log P ≤ 5	Lipinski	No.of Rotatable bonds ≤ 10	PSA ≤140A°	SASA	QPpolrz
1	N-Caffeoyltyramine	299.326	4	4.75	1.968	0	9	105.231	604.542	31.384
2	Quercetin-3-O-Alpha-L-Rhamnopyranoside	448.382	6	12.05	-0.497	2	9	194.879	655.737	38.323
3	Myricetin 3-Rhamnoside	464.382	7	12.8	-1.151	2	10	218.861	638.044	37.067
4	Isomericitrin	480.381	8	14.5	-1.896	2	12	238.772	655.627	37.229
5	Myricetin	318.239	5	6	-0.299	1	6	164.839	530.521	27.427
6	Moupinamide	313.352	3	4.75	2.842	0	9	91.055	634.112	33.605
7	Quercetin	302.24	4	5.25	0.367	0	5	143.331	519.281	27.548
8	Epigallocatechin	306.271	6	6.2	-0.187	1	6	137.479	521.159	27.36
9	Epigallocatechin Gallate	458.378	8	8.75	-0.251	2	10	212.473	697.041	39.756
10	Gallic Acid	170.121	4	4.25	-0.578	0	4	116.091	343.199	13.251
11	Sofosbuvir	529.458	3	14.9	1.252	2	10	173.74	745.287	47.611

Table 3: Molecular Docking Results of *Limonium sinense* selected phytoconstituents.

S. no	phytocompound name	Docking score Kcal/mol	No. Of H-bond	Amino acids Residues	H-bond distance A^0	Glide Energy kcal/mol	Hydrophobic interaction
1	Quercetin	-6.087	3	GLN 620 ALA 533 ASP 665	2.02 2.32 1.59	-39.939	--
2	Moupinamide	-5.838	3	ARG 623 GLN 620 ASP 665	2.74 1.84 1.69	-44.981	LYS 688
3	Epigallocatechin Gallate	-5.812	6	SER 699 GLN 620 ASP 665 ALA 533 ASP 666 ASP 666	2.37 2.52 1.71 2.03 2.02 1.67	-56.13	---
4	Myricetin	-5.418	3	ALA 533 GLN 620 ASP 665	1.94 2.08 1.76	-37.395	---
5	Epigallocatechin	-5.085	3	LYS 688 GLN 620 ASN 616	1.82 2.73 2.04	-38.914	ARG 623
6	**Sofosbuvir**	**-4.628**	**2**	**ALA 533** **SER 712**	**2.33** **1.80**	**-51.854**	---

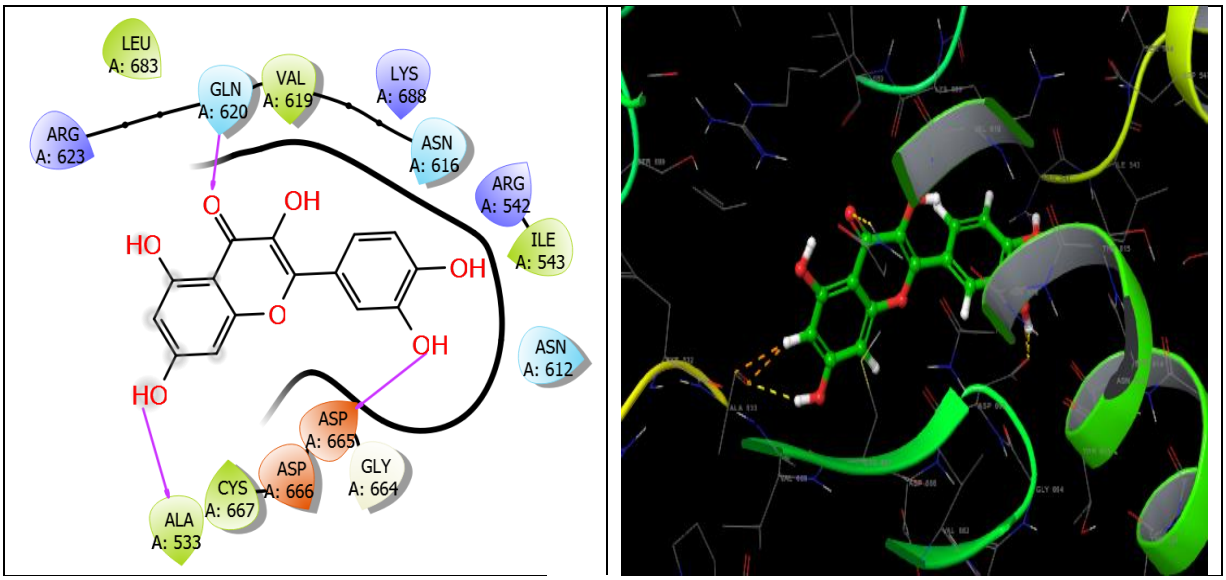


Figure 1: 2D and 3D Docking interactions of **Quercetin** with NS5 polymerase ZIKV protein

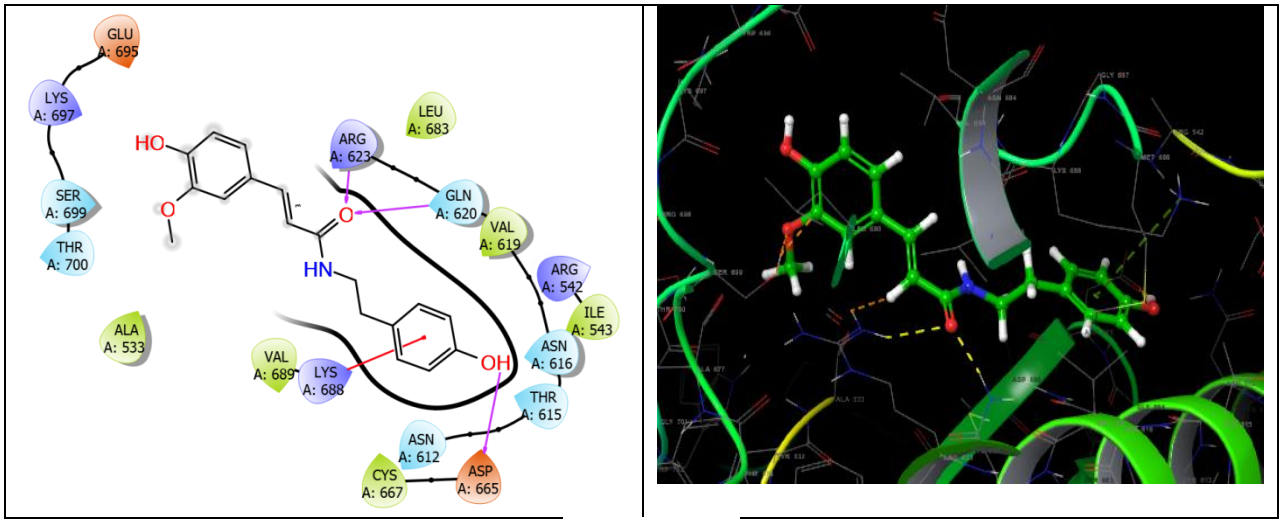


Figure 2: 2D and 3D Docking interactions of **Moupinamide** with NS5 polymerase ZIKV protein

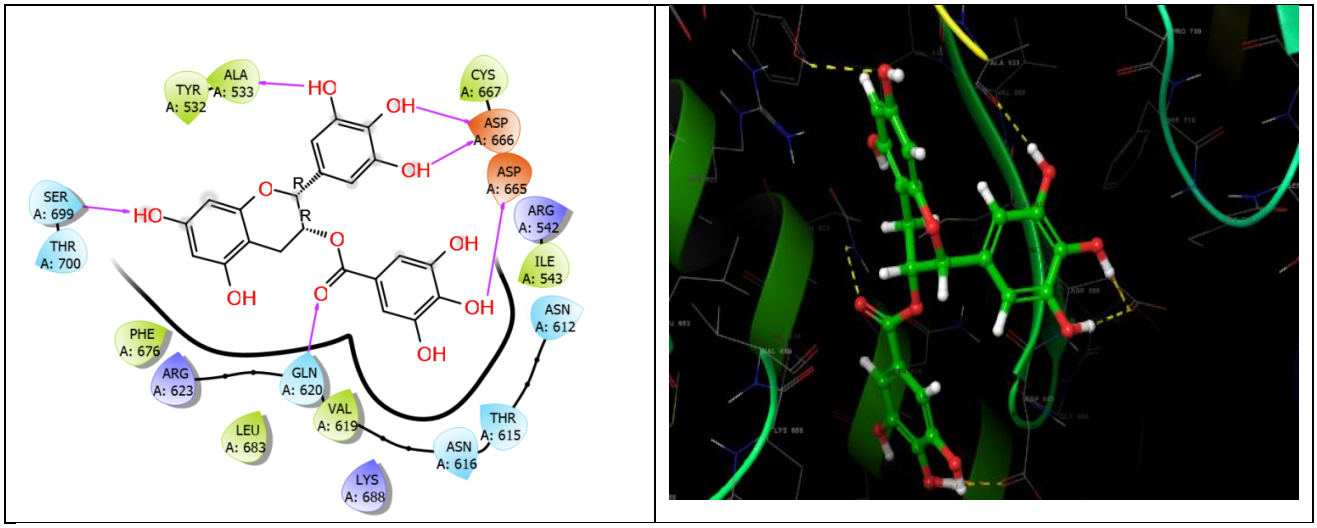


Figure 3: 2D and 3D Docking interactions of **Epigallocatechin Gallate** with NS5 polymerase ZIKV protein

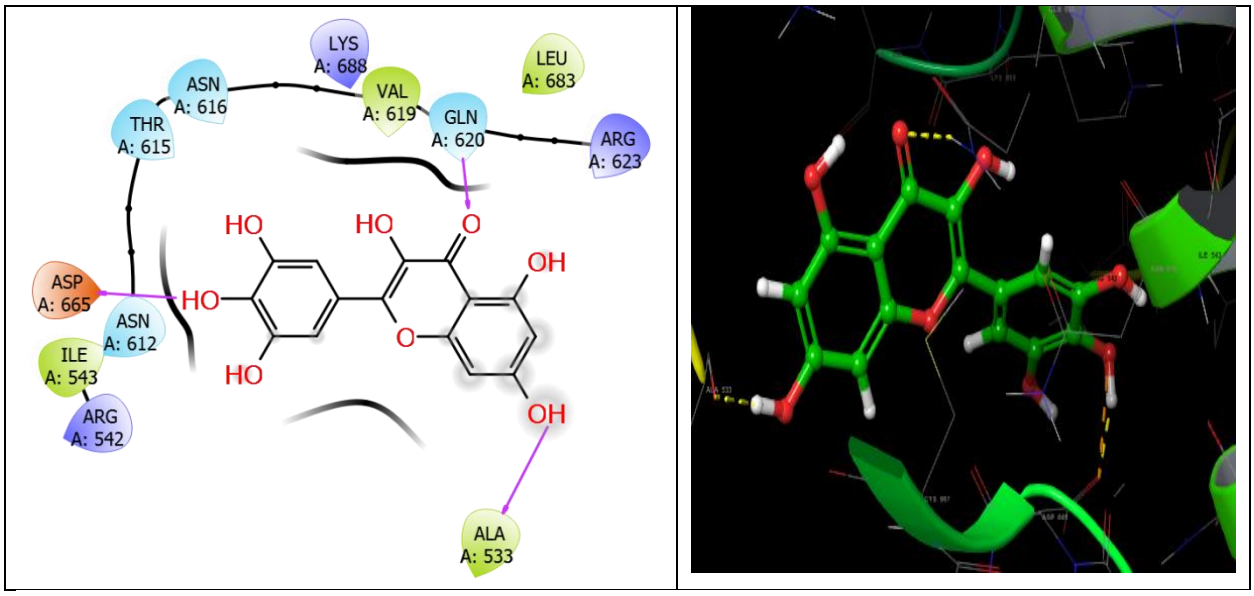


Figure 4: 2D and 3D Docking interactions of Myricetin with NS5 polymerase ZIKV protein

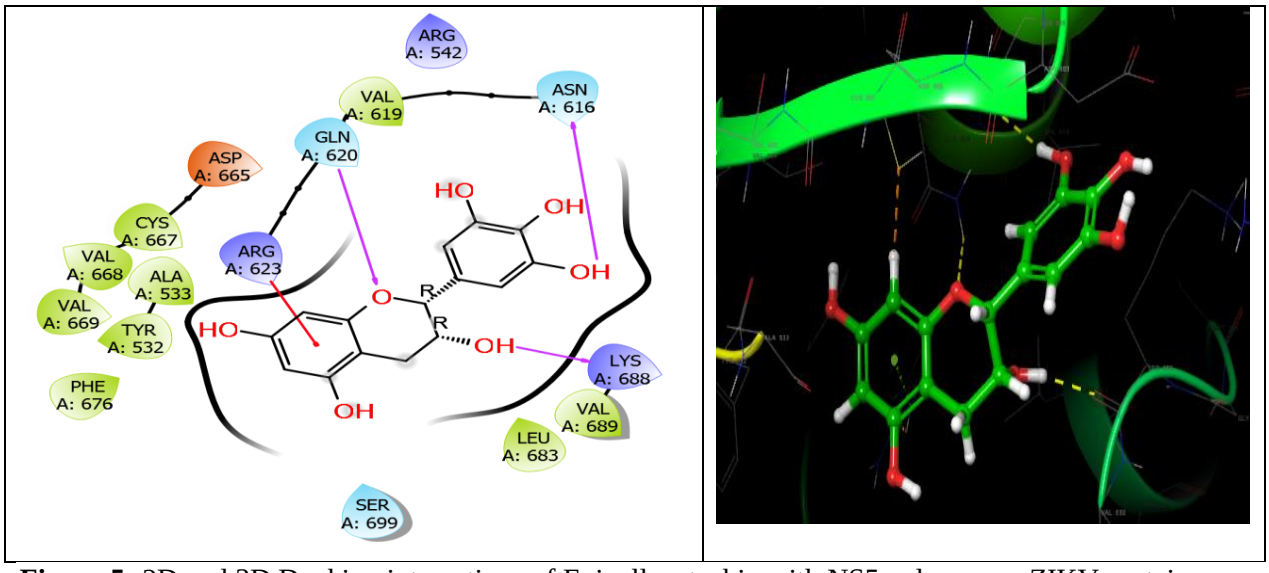


Figure 5: 2D and 3D Docking interactions of Epigallocatechin with NS5 polymerase ZIKV protein

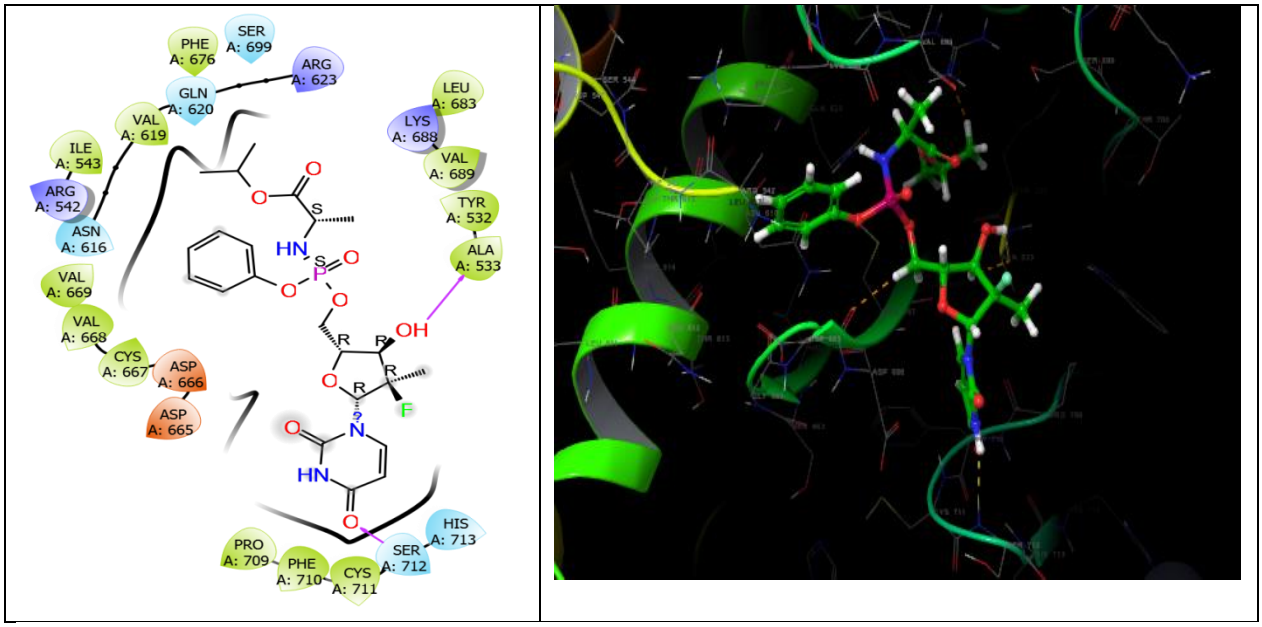


Figure 5: 2D and 3D Docking interactions of Sofosbuvir with NS5 polymerase ZIKV protein

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