RESEARCH PAPER

Screening of Phytochemicals to Treat Kyasanur Forest Disease Virus (KFDV) Using Computational Approach

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ABSTRACT

Kyasanur Forest Disease (KFD) is a tick-borne viral hemorrhagic fever caused by the Kyasanur Forest Disease Virus (KFDV), which is also known as monkey fever. The virus's primary vector is Haemaphysalis spinigera, which is a type of hard tick. The virus spreads to humans through either direct contact with hard ticks or through contact with dead monkeys (it does not spread from human to human). The virus causes sudden fever, severe muscle pain, vomiting, and bleeding. To treat this virus, no antiviral drugs are available; the only treatment is to stabilize the patient throughout the acute infection period, which is phase-1. During phase-1, the patient suffers severe haemorrhagic signs, and during phase-2, the patient suffers neurological effects and inflammation in the brain and the surrounding membranes. So, to discover a suitable drug to treat KFD, we collect data about FDA-approved phytochemicals and use in silico methods like molecular docking in PyRx to identify the possible phytochemicals which have the ability to suppress the effects caused by the virus by finding the binding affinity between the viral protein and the phytochemical. Then, the toxicity of the phytochemical is predicted using ProTox-3.0 (Banerjee et al., 2024).

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INTRODUCTION

Kyasanur forest disease in an tick bone viral hemorrhagic fever which was initially identified in Western Ghats of southern India [Karnataka] at an forest named Kyasanur Forest in 1957. This virus is classified under Flaviviridae family, The motality rate 2-10%. The primary vector of this virus is hard tricks (Haemaphysalis spinigera) which will spread the virus to other animals like monkey and other mammals. When the hard tricks bit the monkey the monkey gets infected and which will act as an amplifier, because the virus will start to replicate in high concentration inside the amplifying host (or) primary host which is monkey, even though there are other mammals like shrews it does not amplify as effective as monkey. The virus will spread to humans when the humans get in contact with the infected dead monkey. Humans are known as dead end host because the virus does not spread from human to human (Bohra et al., 2025; Pandey et al., 2025).The virus has different phases, primary phase and secondary phase which are known as which are known as incubation phases. During primary phase the virus cause high fever, chills, headache and sevier mucle pain. After this phase patents will recover but 10-20% may begins the second phase after one week gap. Second phase is neurological phase which can cause trumas, neck stiffness, vision difficulty and some time brain inflammation. To treat this virus, there is no approved antiviral drug available. So the treatment is to support the patent by keeping them stable [BP, platlets count,etc,]. So to identify capable drug which will has ability to suppress the virus, computational techniques like molecular docking and molecular dynamic simulation are used (Kaushal et al., 2025; Srilekha & Kandi, 2024).

The drug which are used as ligand during the molecular docking is phytochemicals and organic molecules like secondary metabolites obtained from microorganism, which are FDA approved drugs, has anti oxidands, antimicrobial anti fungal property. The viral protein NS3 helicase is an enzyme that helps in the replication process of viral RNA, which is also has higher affinity to the drugs hence it used as an receptor (Zhang et al., 2024).

.METHODOLOGY

RECEPTOR PREPARATION

Fig 1 : NS3 helicase

The receptor as been identified as NS3 helicase which is an viral protein responsible for the replication process of RNA molecule present in the virus. The receptor has been identified and downloaded in legacy PDB format from PDB(Protein Data Bank), the PDB ID is 7V4R, then the receptor has been prepared using Discovery studio. The unwanted molecules like water molecules and hetatoms has removed and saved as modified receptor (Kandagalla et al., 2023).

LIGAND PREPRATION

The ligand is phytochemicals which are approved by FDA(Food and Drug Administration), which was listed using internet sources and AI. More then 200 ligand has been downloaded and saved as one file. The ligand should be downloaded in 3D-SDF format, to run molecular docking. There are other internet source like zinc database which has data set of multiple ligand in SDF format which is easy to access.

MOLECULAR DOCKING

The receptor and ligands has been chosen and prepared, to perform molecular docking the platform used here is PyRx. The modified receptor is loaded which is in legacy PDB format, then converted as macromolecule. Then the ligand file has been uploaded in openbable which is in SDF format, the ligand file is comprised of more then 200, all the energy are minimized then the ligand are converted to SDF format. Normally the active site of the receptor should be selected before docking is started but the technique used here is blind docking which doesn't required to select the active site, the site where docking takes place is selected which covers entire protein because it is blind docking. After blind docking top 10 ligand has been identified.

	Ligand ID	Binding A	RMSD UB	RMSD LB
1	MODIFIED_8223_uff_E=1020.51	-10.7	0	0
2	MODIFIED_8223_uff_E=1020.51	-10.5	9.776	3.044
3	MODIFIED_60838_uff_E=695.77	-10.2	0	0
4	MODIFIED_10206_uff_E=1064.03	-9.9	0	0
5	MODIFIED_10621_uff_E=589.56	-9.8	0	0
6	MODIFIED_11049407_uff_E=945.72	-9.8	0	0
7	MODIFIED_107876_uff_E=535.16	-9.8	0	0
8	MODIFIED_8223_uff_E=1020.51	-9.8	5.067	2.283
9	MODIFIED_5281613_uff_E=720.81	-9.7	0	0
10	MODIFIED_442428_uff_E=615.92	-9.7	0	0

Fig 2: docking result of 200 ligands

Then the best ligand which has lower binding affinity is chosen, the pubchem ID is 8223 the name is ergotamine. Then to cross verify this I repeated the docking process with the best ligand. The result obtained is the below figure.

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Ligand	Binding Affinity	rmsd/ub	rmsd/lb
7V4R-modified_8223_uff_E=1020.51	-11.1	0	0
7V4R-modified_8223_uff_E=1020.51	-10.7	2.501	1.902
7V4R-modified_8223_uff_E=1020.51	-10.2	3.552	2.498
7V4R-modified_8223_uff_E=1020.51	-9.9	9.15	3.091
7V4R-modified_8223_uff_E=1020.51	-9.5	8.969	2.836
7V4R-modified_8223_uff_E=1020.51	-9.1	9.256	3.262
7V4R-modified_8223_uff_E=1020.51	-8.7	5.121	3.663
7V4R-modified_8223_uff_E=1020.51	-8.6	8.558	5.512

Fig 3: docking result of ergotamine

The best ligand and receptor interection has been studied (Achappa et al., 2025).

MOLECULAR DYNAMIC SIMULATION

GROMACS (version 2024.3) was used to carry out the molecular dynamics simulations of the protein/ligand system. The protein was parameterized with the CHARMM36 force field, while GAFF2 was used to generate the ligand parameters (Lindorff-Larsen et al., 2010). The protein-ligand complexes were then placed into periodic cubic boxes containing solvent water molecules and were neutrally charged by adding counter ions to create a system with physiological ionic strength. Electrostatic Long-range interactions were treated using the Particle Mesh Ewald (PME) method. The van der Waals interactions were computed using force-switch cutoff methods. In order to constrain the hydrogen bonds in the production simulation, the LINCS algorithm was used and a 2 fs integration time step was implemented. In preparation for production runs, each system underwent a steepest-descent energy minimization procedure to eliminate any negative contacts. The final system was equilibrated via 2 steps: (1) a 1 ns NVT ensemble simulation (temperature-controlled by the velocity-rescale thermostat) followed by (2) a 2 ns NPT ensemble simulation (pressure-controlled with the Parrinello–

Rahman barostat) (Lin et al., 2019). Upon completion of the equilibration steps, 100 ns of production simulations were conducted under NPT conditions without restraint. In order to carry out the trajectory analysis, common GROMACS tools were utilized. When measuring, we measured the root mean square deviation and root mean square fluctuation of a mediating protein agent, its radius of gyration, the number and stability of hydrogen bonds, the solvent-accessible surface area, and the center of mass distribution relative distance (Archana et al., 2021; Wang et al., 2010).

RESULT

Molecular Docking

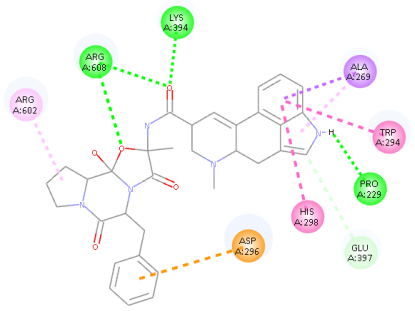

Fig 4: 2D representation of bonding

Using the Discovery Studio software program, the binding affinity from the molecular docking studies between the drug ergotamine (PubChem ID: 8223) and the KFDV NS3H protein, which is in a complex with inorganic phosphate (PDB ID: 7V4R), was assessed. The analysis showed a very strong binding affinity based on a docking score of -11.1 kcal/mol indicating that ergotamine binds very tightly to the target. The 2D interaction diagram shows that ergotamine is very well stabilized within the binding pocket due to the formation of numerous stabilizing contacts between the ligand and protein residues. A number of polar interactions between LYS A:394, ARG A:668, and ARG A:662 through the formation of hydrogen bonds contributed significantly to anchor the ligand. Furthermore, hydrophobic (e.g. ALA A:263 and TRP A:294) and π–π stacking (e.g. PRO A:229 and HIS A:238) interactions also contributed significantly to the van der Waals stabilization of the ligand. An electrostatic interaction was observed with ASP A:296 while additional polar contacts occurred with GLU A:397 and also provided further evidence to support that the ligand is very specific for this target.

Hydrogen Bond Formation

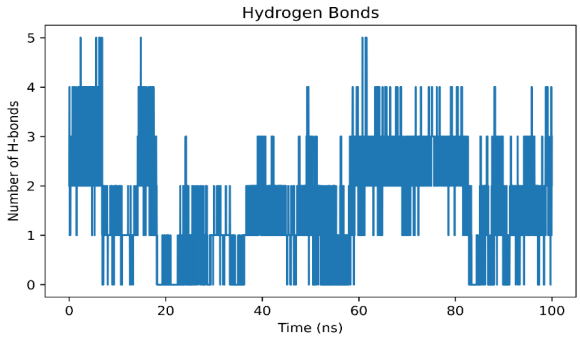

Fig 5: graphical representation of hydrogen bond formation

This graph illustrates how many hydrogen bonds were created between ergotamine and the KFDV NS3H protein during the 100 ns simulation. After some fluctuations during an initial period of equilibration, the number of hydrogen bonds reached a stable value (around 2-3 per frame) from approximately 60 ns until the end of the simulation, with occasional spikes up to 4-5 bonds as shown on the graph. This result suggests that the polar interactions measured in the docking study were significantly maintained throughout the production run of the simulation and thus contributed to the strong anchored binding of the ligand in the binding site.

Root Mean Square Deviation of Ligand with Respect to the Protein

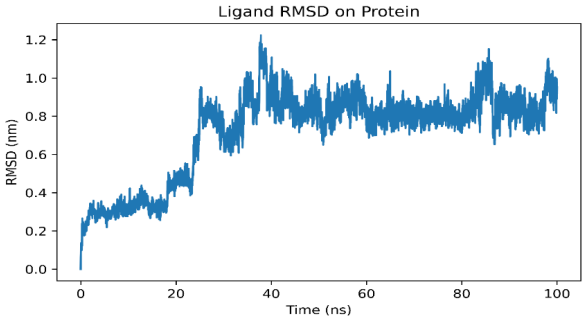

Fig 6: graphical representation of RMSD

The following figure shows the root mean square deviation (RMSD) of the ergotamine ligand in relation to

its protein backbone. The RMSD begins at 0 and gradually increases to about 0.8 nm during the first 25 ns of the trajectory due to the ergotamine ligand adjusting its position. After that point, the RMSD plateaus between about 0.7-1.0 nm, with only small fluctuations during the remainder of the trajectory. There was no evidence of large jumps or continuous upward trend in the RMSD, suggesting that the ergotamine ligand remained bound within the active site of the protein throughout the entire trajectory without significant dissociation or positional movement

Protein Ligand Interaction Energy

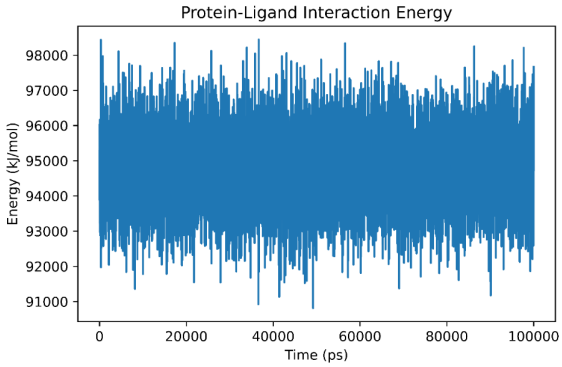

Fig 7: graphical representation of Protein Ligand Interaction Energy

The energies associated with protein-ligand interactions have been plotted throughout the entire 100 ns (100,000 ps) simulation. The energy of the system remained favorably low and very little variation occurred over the course of the study, with an average energy of approximately 96,000 kJ/mol, without any evidence of progressive movement or abrupt changes in the values of energy. In fact, many small spikes can be observed in the energy measurements, but none of the spikes exceeded a narrow band indicating the stability of all types of non-bonded interactions (e.g., van der Waals, electrostatic, and hydrogen bonding) existing between ergotamine and the target protein KFDV NS3H. The radius of gyration (Rg) of the KFDV NS3H protein as a function of simulation time is shown as a graph. The Rg values varied only slightly between 2.24 and 2.34nm, and averaged approximately 2.28nm. Rg is an indication of the compactness and stability of the shape of the protein throughout the course of the 100 ns simulation. The conjugation of the two molecules does not appear to cause global unfolding or expansion of the three-dimensional structure of KFDV NS3H.

RMSF per Residue

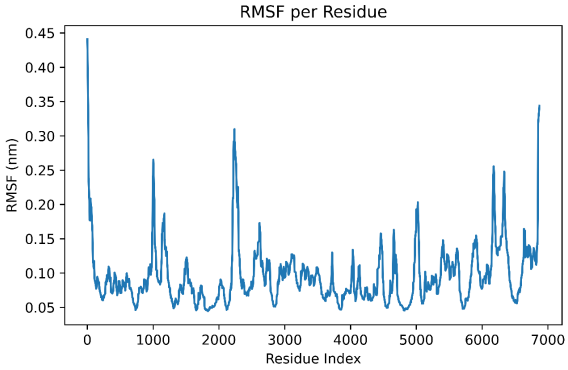

Fig 8: graphical representation of RMSF per Residue

Residue Root Mean Square Fluctuation (RMSF) values are used to highlight the local flexibility along a given protein sequence. The RMSF values for most residues are low (less than 0.15 nm), indicating that the majority of these residues are rigidly held within their respective 3D structures (i.e., local areas of protein rigidity) in their core regions as well as their ligand-bound states. Residues that exhibit relatively high RMSF values are predominantly found in the N-terminal region (residues ~ 1–50), a number of additional flexible loop structures, and at the extreme end of the C-terminal region (~ residue 7000). Active site residues, which are directly involved in the binding of ergotamine, exhibit minimal fluctuations in their RMSF values, suggesting that interactions between ligand and protein are stable over time.

CONCLUSION

There are currently no approved antiviral therapies for Kyasanur Forest Disease (KFD), and treatment is restricted to supporting the patient through the acute stage of infection. We conducted a study employing in silico computational methodologies to screen a library of 200 FDA approved phytochemicals against the KFD virus (KFDV) NS3 helicase (PDB-ID:7V4R), an important enzyme that is essential for the replication of viral RNA, as there are many patients that have been infected with KFDV by the time they receive medical care. Using a molecular blind docking analysis with PyRx, we found that the phytochemical ergotamine (PubChem ID:8223) was the most favorable candidate for binding to KFDV NS3 helicase, with a binding affinity score of -11.1 kcal/mol. Ergotamine was shown to be tightly secured in the target's binding pocket through many hydrogen bonds, hydrophobic interactions

and electrostatic contacts. Additionally, the structural integrity of the ergotamine-KFDV NS3 helicase complex was validated through the performance of a 100 ns molecular dynamics simulation, using GROMACS. The results from the molecular dynamics simulation indicated that the interaction between ergotamine and KFDV NS3 helicase was both stable and strong based on several key metrics. Specifically, the ergotamine ligand remained in the active site without substantial dissociation and exhibited RMSD values that stabilized between 0.7 nm and 1.0 nm..Throughout the production process, each frame was formed with 2-3 hydrogen bonds formed via stabilizing polar interactions. The KFDV NS3H protein-ligand interaction energy value was consistently low and stable (average: ~96,000 kJ/mol) without significant books in value. Additionally, the overall three-dimensional structure of the KFDV NS3H was observed to be very compact and the activity site residues were measured to be highly rigid (RMSF minimal). Therefore, this study's computationally-derived results provide substantial support for the conclusion that ergotamine can stably bind with high efficacy to KFDV NS3; therefore, it is a candidate drug for the inhibiting of KFDV replication and provides strong evidence to guide the future development of strategies to treat KFDV.

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