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Inhibitory potential of natural plant products against influenza virus

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Abstract

H5N1 being highly pathogenic avian influenza virus that causes severerespiratory infection causing avian flu spreads rapidly among the birds' population. The virus is epizootic in nature but in the last decade several cases have been reported in humans as well. In this review, we have used five different plant products to find their inhibitory potential against 3s12 viral influenza protein. We have used Autodock software for performing molecular docking on 3s12 protein present in H5N1 influenza virus. The docking results were analysed and visualized using PyMol visualization tool and then binding conformation of the protein -ligand complex is studied. We used ADME analysis to find the Lycorine which is a toxic alkaloid extracted from Lycoris radiata plant has the highest binding energy –7.72 Kcal/mol with 3s12 haemagglutinin protein making it a better inhibitor of the protein as seen in molecular docking performed. The binding of lycorine at the active site of 3s12 gives the best drug value.

Introduction

Influenza A virus (IAV), that causes flu, is a widely known virus among the population as it is responsible for public health ailments every year. Birds living in aquatic habitat are a primary source of infection but it also infects a majority of other birds and animal species. Due to this property, the virus can jump species occasionally and lead to the pandemic experienced by people from time around(1). H5N1 influenza virus have tendency to infect wide range of mammalian and avian hosts having greater effects on poultry farm(Wang et al., 2018). Two strains of influenza virus namely H5N1 and H7N9 are said to have shown potential to infect and spread in humans. H5N1 avian influenza virus is highly pathogenic (HP). In the year 1997, the country of Hongkong experienced deaths of 6 individuals from the 18 approved cases of H5N1 virus infection(1). On 21st July of 2021 the first case of human infected from H5N1 was reportedHaryana, India(WHO, 2021). Till date, 239 total cases have been reportedfrom the different laboratories of the world with 134 deaths (WHO, 2022).Influenza A virus (IAV) is able to show mutation rapidly, the reason being its widespread RNA which makes it difficult to develop a new vaccine that is both effective and cost-effective. The timely development can further help prevent potential virus outbreaks. The main cause of changing in flu virus is antigenic drift and antigenic shift and due to this virus becomes more vulnerable than its traditional design of existence(CDC, 2021). To the present day, two variations of antiviral drugs has been given clearance against influenza. One includes inhibitors for M2 (ion) channel that prevents the virus entering the host cytoplasm(De Clercq, 2006; Hu et al., 2017). Examples includeperamivir, zanamivir and oseltamivir. Second being polymerase acidic endonuclease inhibitor including marboxil and baloxavir. However, it has been noticed from the available data that a high number of the presently available IAV strains are resistant to M2 inhibitors, these strains are expected to develop resistance towards neuraminidase NA inhibitors, that might limit the usage of those licensed drugs because these drugs reduce the virus fitness leading to mutation in the strain.

Influenza virus's replication in the host takes place in the nucleus of the host. This unique RNA feature gives H5N1 advantage of accessing the processes being performed in the nucleus during replication. But it further enhances complex cellular traffic which is essential for the virus to start a successful infection. Since the influenza virus has segmented RNA, it leads to increased traffic in cell as every viral part needs to traverse through cellular barriers at assembly and entry instances (Dou et al., 2018). Entry of the virus in the cell is under the jurisdiction of hemagglutinin (HA) which creates an initial complex of 3 HA1 and 3 HA2subunits. Every HA 2 contains a fusion peptide (FP), transmembrane domain and soluble ectodomain (SE). HA 1 prepares for binding with sialic acids, the

next step followed is the virus endocytosis process, followed by dissociation of HA 1, rearrangement of HA 2 structure and pH reduction forming a final complex of soluble ectodomain hairpin. Reduction of pH leads to HA 2-mediated viral particle or fusion of endosome (membrane). Fusion peptide is considered a different helix hairpin(Ranaweera et al., 2018). After HA 1 binds to the receptor, IAV undergoes endocytosis. Increasing level of acidity and pH alterations results in conformation changes that cannot be undone in the endosome. As a result, the FP moves towards the periphery of endosomal membrane. This leads to the formation of complex of the virus and endosome membranes. The virus is given entry into the cell via endocytosis process and 8 RNA proteins containing distinct viral genome. They are internalized via endocytosis, and the eight viral ribonucleoproteins (vRNPs), which constitute the segmented viral genome, are deployed in the cytoplasm. The vRNPs then enter the nucleus through nucleus pore complex followed by the transcription into mRNA. Among these mRNAs, two are spliced alternatively. The viral mRNA thus produced is transferred to cytoplasm for translation. vRNPs are the basis of replication of the virus. vRNPs are converted into cRNPs (complementary ribonucleoproteins), that are again reverted back to vRNPs. These vRNPs are moved out of nucleus and into the cytoplasm. Here in combination with viral proteins, these assembled the virus budding from the host.

H5N1 virus, which is transferred from wild aquatic species to chickens and cross species to infect various species, contains the target protein 3s12. In this article, we focused on this protein and a group of five phytochemicals that have the potential to prevent viral entrance into the host and suppress viral expression.

Materials and Method

In this study, we have collected pools of data using various databases such as Protein Data Bank (PDB), Pub Tator, PubMed Central and European PubMed Central. The data about the structure of protein was obtained from PDB and downloaded as pdb files. The structures of ligands were taken from PubChem. Various tools and software used are listed in Table 1. Most of them were open-source software. Every protein and ligand were studied on their residues level and then the drug score was calculated. The methodology used is listed as follows:

1. **Protein/Molecule**: - The first step is to identify the protein of interest which can be done by reading research and review papers on the topic of interest. After, the protein is selected, its structure is downloaded from Protein Data Bank (PDB) in the pdb format. This file contains data about the atomic level of interaction of the protein and the atomic co-ordinates.
2. **Ligands**: After the ligands are finalized, their structures are downloaded from PubChem database in the SDF format. Since AutoDock accepts .pdf file, open babel was to convert SDF file into PDB file.
3. **Docking**: Protein and ligand are prepared in the AutoDock software. Protein is prepared by remove water, adding polar hydrogen, adding kolman charges and removing any heteroatoms present. After the protein and ligand are prepared, the grid formation is done by setting the grid on the coordinates of the active sites and area surrounding it if not sure. After the grid is formed and grid parameters are set, the output is saved as .gpf file. The next step is to run auto grid. The process produces maps and glg file which are used to then perform docking. The docking parameters are set and the algorithm selected for docking is genetic algorithm. Then the output is saved as .dpf file. The next step followed is running autodock. The file generated from this process is dlg file which contains the final outcome as well as the conformations of the ligand with protein and many other information.
4. **Analysis**: The dlg file is analyzed in the autodock and the various possible conformations and their different parameters like binding energy, inhibition constant, and others are studied. The best conformations are visualized using Pymol, LigPlot, Protein+. Protein Ligand Interaction Profiler also known as PLIP is used for visualization purpose. The hydrophobic interactions, hydrophilic interactions, salt bridges and pie-stack interaction are studied. Further analysis and visualization are done using PyMol. PyMol helps study protein- ligand binding interactions.

5. **ADME Analysis**: ADME analysis of the ligands was done that showed the various characteristics of the drug from it topological surface area to its patient alert, bioavailability score, skin permeation and GI absorption. The ligands were further checked for their activity and their synthetic accessibility before the docking process.

Results and Discussion

HA is responsible for binding of the virus to cell surface receptors, and it mediates liberation of the viral genome into the cytoplasm through membrane fusion. The current study is based on the analysis of phytochemicals exhibiting tendency to inhibit the HA protein, thereby inhibiting the penetration of virus into the cell.

In the present study, 5 ligands were shortlisted from a pool of reported ligands with antiviral abilities. These include Andrographolide, Biochanin-A, Lycorine, 1,3-Dicaffeoylquinic acid, and Chlorogenic acid.

a) **Andrographolide**

Andrographolide is a labdane diterpenoid produced by the *Andrographis paniculata* plant that has a wider range of applications in therapeutic studies involving anti-inflammatory and anti-platelet aggregation activities as well as potential antineoplastic properties. The molecular weight of andrographolide is 350.4 and its counts for H-bond donor is 3 and acceptor is 5 and have 25 heavy atom count. The anti-inflammatory effect of andrographolide seems to have a connection with the nitric oxide (NO) inhibition. Andrographolide might start the nitric oxide or cyclic GMP pathway. It inhibits the activity of PLC gamma2 kinase (phospholipase C gamma 2)/PKC (protein kinase C) along with phosphoinositide 3 kinase (PI3K)/protein kinase B (AKT)- mitogen activated protein kinase (MAPK) signalling pathways in activated platelets to inhibit platelet aggregation and has anti-neoplastic activity.

b) Biochanin–A

Biochanin-A belongs to the class of 7-hydroxyisoflavones, that is introduced with OH (hydroxy) group at 5 positions along with a methoxy at 4 positions. As a phytoestrogen, it has immense advantages in cancer prophylaxis diet plan. Biochanin-A plays inhibitor, antineoplastic agent and as a tyrosine kinase inhibitor. Biochanin-A is a conjugate acid of a Biochanin-A (1-). Its molecular weight is 284.26 and counts of H- bond donor is 2 and acceptor is 5.

c) Chlorogenic Acid

Chlorogenic acid, a polyphone is an ester of caffeic acid and quinic. It is mainly extracted from coffee and black tea and have the potential to be a chemo preservative and antioxidant. Its molecular weight is 354.31 and count for H-bond donor is 6 and H-bond acceptor is 9. In order to avoid the inhibition of DNA damage and give protection against carcinogenesis induction, chlorogenic acid produces free radicals. It also enhances the working of the immune system and increases macrophage activation, cytotoxic T-lymphocyte proliferation, Natural Killer (NK) cells. It also inhibits the activity of matrix metalloproteinases.

d) 1,3 Dicaffeoylquinic Acid

It is a natural product found in *Arnica Montana, Arnica chamissonis*, and other species. The molecular weight of this molecule is 516.4. It's count for H-bond donor is 7 and H-bond acceptor is 12. It is said to have antiviral properties and is used as anti-toxin.

e) Lycorine

Lycorine is indolizidine alkaloid which has 3,12-didehydrogalanthan molecule substitutions at 2 positions by OH (hydroxy group) and at 9 and 12 by methylenedioxy. molecular weight of lycorine is 287.31 and count for H-bond donor is 2 and H-bond acceptor is 5. It plays a vital role an antimalarial, anticoronaviral agent and protein synthesis inhibitor.

The pharmacokinetics and other properties of the selected ligands are studied with the SwissADME software. This tool helps to calculate various features of the ligand. Topological surface area is used to predict the molecular structure of a drug molecule (ligand) using Lorentz equation(Sawano et al., 2020). Lipophilicity was yet another propertyobserved via ADME analysis. It is indicative of the solubility of the drug in polar and nonpolar solvents in the patients and its metabolism. Lipophilicity is seen to improve the off-target binding (promiscuity). When the drug experiences higher off- target binding, the risk associated with drug increases as its toxicity soars. After screening sets of compound data, it is observed that drugs with Log P value >3 have more chances to cause toxicity (Stephens et al., 2018).

Solubility is the process in which solute dissolves in the solvent forming a homogeneous mixture. It is one of the most important benchmarks which can be used to achieve the required amount of drug shown in methodical circulation about the desirable (anticipated) response. Low aqueous solubility is another hurdle witnessed while developing the formulae of new drugs and for generic development(Savjani et al., 2012).Researches depicted that how a drug moves in the body and how it functions and is removed out of the body. This is also known as Pharmacokinetics. What type of response a person will show to a drug depends on certain factors like how the body reacts to the drug, and the action of the drug on the site designated for drug action? The intensity of the response depends on the various physical, chemical and structural properties of the drug. However, the intensity, speed of onset and duration of the response mainly depend on parameters such as how much dosage of drug is taken and the rate of its action referencing to the administration site keeping in notice the action site of drug, as well as rate at which drug is eliminated from the patient(Waller et al., 2018). Majority of the drugs intake by patients are absorbed by the blood from the gastrointestinal tract through two ways. It can be by active absorption or passive absorption. A few other pathways are facilitated transport, pinocytosis and via pores present in the membranes. Passive diffusion method solely depends on the phytochemical (drug) movement across the membrane to the blood from a higher concentration to a lower concentration. The speed of the movement depends on the area required by the drug, its size, permeability of membrane, concentration gradient and solubility of the lipid. Epithelial cells present under the membrane allows the entry of molecules soluble in lipids but is somewhat permeable to other substances (Prescott, 1974). Bioavailability (BA) score is the parameter that calculates the absorption rate of the drug when taken orally. If the “rule of five” condition is satisfied by a drug while calculating the score then the BA score of 0.55 is considered effective while taking drug orally. Prodrugs with the BA score of 0.55 are considered to show better response when intake is done orally (Mazumder et al., 2021). A process that determines the level of easiness required for a drug synthesizing is called Synthetic Accessibility. If we can have a better and efficient method for finding the synthetic accessibility, the method can provide a major breakthrough in drug discovery process(Baba et al., 2018).

In the analysis, it was found that these selected phytochemicals have better affinity towards the 3s12 protein. They have the potential to inhibit the virus that can be proven by their binding energies and ADME analysis. Theselected inhibitors may work at full potential to block the activities of HA protein of H5N1 IAV. Amongst the five ligands tested, lycorine was predicted to be the best phytochemical in our selection range. Lycorine showed the best binding affinity with HA and as it shows better binding energy requirements than any other ligand taken. The ADME analysis also depicted that under the selected ligands, lycorine showed the optimum drug behaviour in many aspects.

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Table 1:Tools and softwares used

Molecular Task		**Tools & Software used**	**Accessibility**
Preparation of protein	Crystal Structure PDB	PDBe	Freely online
		RCSB	Freely online
	Visualization of protein	PyMOL	Educationalversion
	Protonation of protein	AutoDock Tool-1.5.6	Freely online
	Minimization energy	AutoDock Tool-1.5.6	Freely online
1. Preparation of ligand	Structure downloaded	PubChem	Freely online
	Converting format of file	Open Babel	Freely online
	Detecting root	AutoDock Tool-1.5.6	Freely online
2. Docking	Predicting binding site	Literatures	----------
	Protocol of docking	AutoDock Tool-1.5.6	Freely online

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Table 2: Selected ligands molecular docking analysis

Ligand name	Inhibition constant	Amino acids (Intermolecularinteraction)	Intermolecular interactions distance (Å)	Binding energy of ligand
Andrographolide	21.96 µM	1. Hydrophobic Interactions ILE TRP TRP ASN LEU 2. Hydrogen Bonds LYS ASN ASN 3. Salt Bridges LYS	1.Hydrophobic Interactions 3.92 3.84 3.32 3.06 3.07 2.Hydrogen Bonds 2.93 1.72 2.49 3. Salt Bridges 5.26	-6.36
Biochanin-A	138.01 µM	1. Hydrophobic Interactions TYR HIS VAL LEU 2. Hydrogen Bonds HIS ASP ARG	1.Hydrophobic Interactions 3.59 3.26 2.96 3.94 2. HydrogenBonds 2.81 2.21 2.33	-5.27
Chlorogenic Acid	160.14 µM	1. Hydrophobic Interactions ASP LEU VAL 2. Hydrogen Bonds GLU GLU ASN ASN ARG VAL 3. Salt Bridges ARG	1. Hydrophobic Interactions 3.29 3.23 3.93 2. Hydrogen Bonds 2.27 1.73 1.79 2.11 3.01 1.63 3. Salt Bridges 4.73	-5.18
1,3-Dicaffeoylquinic Acid	3.63 µM	1. Hydrophobic Interactions ILE ILE GLU TYR THR	1. Hydrophobic Interactions 3.52 3.38 3.42 3.34 3.07	-3.33

		2. Hydrogen Bonds GLU GLU GLY TYR TYR HIS ASP LYS ASN	2. HydrogenBonds 2.31 2.35 2.95 2.85 2.04 2.96 2.13 2.90 2.20	
Lycorine	3.64 µM	1. Hydrophobic Interaction ILE TYR HIS VAL LYS LEU ARG 2. Hydrogen Bonds ILE TYR TYR ASP 3. PI-Cation Interactions TYR 4. Salt Bridges ASP	1. Hydrophobic Interaction 3.51 3.74 3.81 3.47 3.86 3.87 3.24 2. Hydrogen Bonds 2.71 2.29 2.06 2.41 3. PI-CationInteractions 3.99 4 Salt Bridges 4.17	-7.42

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Table 3: ADME analysis of selected ligands

Ligands	Water Solubility	Lipophilicity (iLOGP)	Druglikeness	Pharmacokinetics	Topological surface area	Medicinal chemistry
Andrographolide	Soluble	2.45	Bioavailability score = 0.55	High GI absorption,-6.9 cm/s skin permeation	86.99	Synthetic accessibility is 5.06
Biochanin-A	Moderately Soluble	2.55	Bioavailability score = 0.55	High GI absorption,-5.91 cm/s skin permeation	79.90	Synthetic accessibility is 2.89
Chlorogenic acid	Soluble	0.96	Bioavailability score = 0.11	Low GI absorption, -8.76 cm/s skin permeation	164.75	Synthetic accessibility is 4.19
Lycorine	Soluble	2.06	Bioavailability score = 0.55	High GI absorption,-8.07 cm/s skin permeation	62.16	Synthetic accessibility is 4.20

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Legends to Figures

Figure1: Flow chart on docking analysis

Figure 2: Docking results

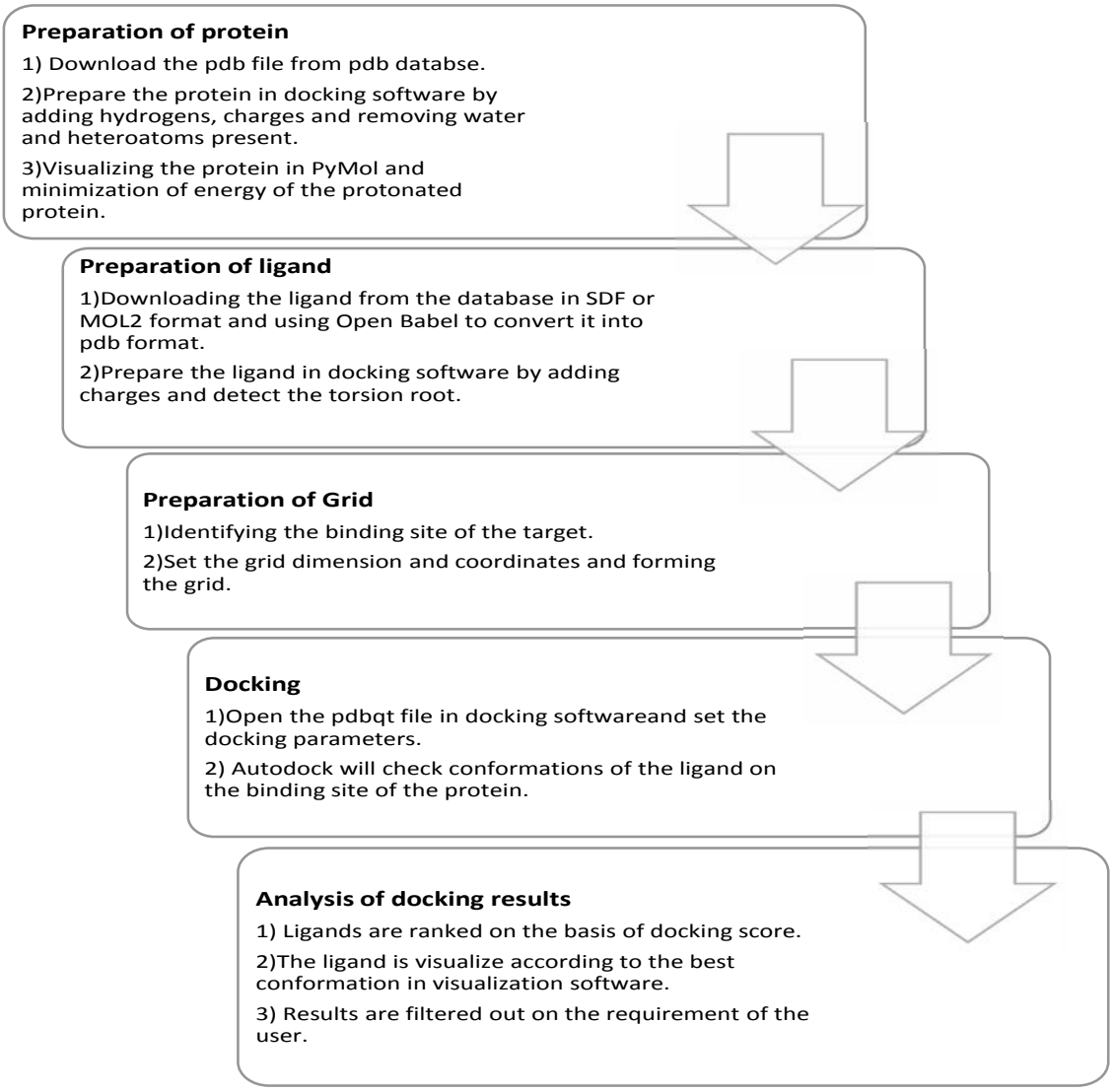

Figure 1. Flowchart of docking analysis

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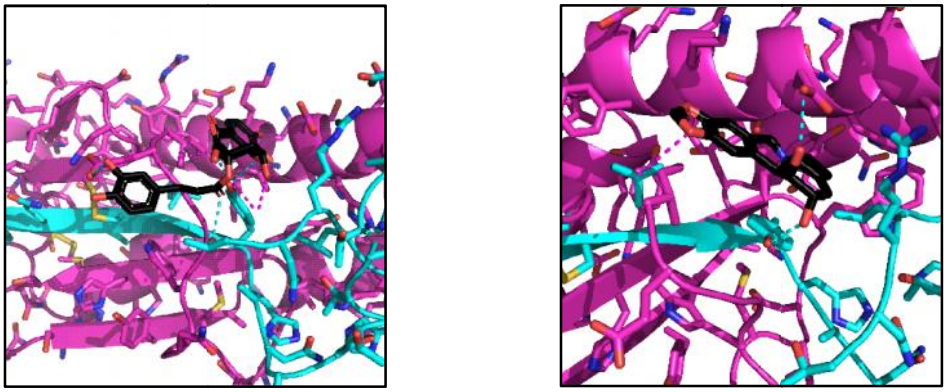
a) Andrographolide b) Biochanin – A c) Chlorogenic acid



d)1,3 Dicaffeoylquinic acid e) Lycorine

Figure 2. Docking results

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