

In-Silico Studies of Phytochemicals from *Allium sativum* with H5N1 Protein in Avian Influenza

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Abstract

Allium sativum or garlic is a medicinal plant that is reported for its applications in many biological activities, such as its antiviral, antidiabetic, and anti-inflammatory properties, etc. In this paper, the focus has been the identification of potential phytochemicals that can be extracted from *Allium sativum* that can act as a candidate for drug formation that possesses the capability to inhibit the H5N1 Avian Influenza, a type of highly pathogenic and zoonotic strain that poses a major threat to the public health sector. Using the tools provided in the in-silico studies, screening of the 145 phytochemicals from *Allium sativum* is done against the two major H5N1 proteins, that is, Hemagglutinin and Neuraminidase. The docking results provided along with the result that many compounds have stable interaction with a high binding affinity towards Neuraminidase and Hemagglutinin throughout the stimulation time. These results suggest that phytochemicals from *Allium sativum* can be promising candidates for the development of therapeutic agents against the H5N1 virus.

Keywords: Docking, H5N1, HPAI, garlic, Schrödinger, *Allium sativum*

INTRODUCTION

Avian influenza refers to the viral disease which is caused by a specific virus known to infect birds [1]. While it does not primarily present itself as a fatal disease in birds, this can very well become the reason for multiple outbreaks of viral epidemics with severe effects when altered in humans by its rapid evolution [2, 3]. It is a broad term that encompasses the various influenza-based conditioning caused due to the changing nature of its surface proteins [4, 5]. This virus should be considered as one of the most important and specific reasons for the rising number of cases and complications related to the poultry industry and other birds in the past years. As it would seem, many these deadly virus subtypes had similarly caused a dysfunction in the animals thus the negative consequences observed in the birds were evident as well.

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The conventional method to classify this virus broadly categorizes it into two distinct groups: HPAI or High Pathogenicity Avian Influenza and LPAI or Low Pathogenicity Avian Influenza [6]. The HPAI strains are those that tend to be activated and developed in most of the organ systems of the host and are associated with causing serious ailments, and hence, the reason for the increased fatality to morbidity ratio when compared to that of the other group, LPAI [3, 5]. LPAI strains are majorly those that are provoked in certain organs of the host with an optimum concentration of post-translational modifying enzymes, leading to mild to slightly severe effects in the host [3, 5]. Development of HPAI is

predominantly attributed to H5 and H7 subtypes, posing an even bigger threat to the poultry sector [2]. It then becomes fatal to the domesticated birds infected by such subtypes of the virus [2]. Strains of HPAI have attracted the attention of the public sector because of the history observed of their causing numerous zoonotic epidemics and outbreaks that threaten human lives [6–10].

Garlic (*Allium sativum*) is a spice that finds easy availability in many households of the Central and South Asian continents and has also been in use for many years as a food supplement and a traditional medicine to promote good health [11]. This is due to the organo-sulfur compounds (OSCs) present in it which have also been noted to reduce infections caused by the viruses, such as severe acute respiratory syndrome Corona Virus [11, 12]. These OSCs are known to be the most active bio-compounds in addition to being the compounds that give garlic its unique smell [11]. There are various OSCs in garlic with alliin being the predominant one that can further be converted into allicin with the help of the enzyme alliinase. Allicin, which is rather an unstable compound, has several in vitro decomposition products, namely diallyl sulfide, diallyl disulfide, diallyl trisulfide, and ajoene. It also contains non-sulfur-containing compounds, such as flavonoids, lectins, allixin, saponins, etc. Garlic content provides this commonly consumed food with rather beneficial properties, such as its antimicrobial activity on the MDR (Multi-Drug Resistant) bacterial species, making it an excellent antibiotic agent. In addition, it has been observed and considered beneficial in numerous experiments as well as in the in-silico study of compounds and proteins, its antibacterial, antifungal, antiparasitic, antioxidant, and anti-inflammatory activities [13].

Methodology

The primary purpose of this study is to find potential phytochemicals for the treatment and management of Avian influenza, H5N1 strain using an *in-silico* approach. The initial step of the process is to identify and prepare the target protein. In the present study, we have used H5N1 surface proteins, that is, hemagglutinin and neuraminidase proteins. We have used IMPPAT (Indian Medicinal Plants, Phytochemistry, and Therapeutics) database for the retrieval of all the phytoconstituents of garlic (*A. sativum*). The study was performed with a total of 145 compounds of *A. sativum*. We procured the structures of the H5 hemagglutinin and N1 neuraminidase through the PDB (Protein Data Bank) database with the PDB IDs being 2IBX (H5), 2HTY (N1), and 3CKZ (N1). We used multiple 3D structures for hemagglutinin and neuraminidase to apply the receptor grid on specified amino acid residues that are used as a control.

Protein Preparation

The protein was prepared using the protein preparation wizard module by Maestro Schrödinger. During protein preparation the water was eliminated, polar hydrogens were added and other problems like incomplete side chains, loops, residues, etc. were fixed. Hydrogens were minimized and the protein was further optimized and prepared for interaction studies [14, 15].

Ligand Selection and Preparation

A total of 145 phytochemicals of garlic were procured from IMPPAT database in 3D SDF file format. Ligand preparation was performed using LigPrep application in Schrödinger Maestro Suite (version 13.2.138). Further, an appropriate bond order for each structure was assigned and ionization states were generated [15]. Some of the compounds from garlic are illustrated in Figure 1.

Binding Site Preparation and Receptor Grid Generation

The binding site of the interaction of neuraminidase with garlic compounds was not known. Therefore, the binding site was predicted using SiteMap module of Schrödinger. The binding sites with a score >0.6 were selected for molecular interaction [16]. All the active site score for the protein targets is illustrated in Table 1.

Following this a receptor grid was further created using the SiteMap generated probable binding site locations on the protein of interest [16]. To contain the mass center of the ligand (to be docked

with the protein), a bounding box, that is, a grid box for each site with the dimensions $18 \times 18 \times 18 \text{ \AA}$ was defined using the Glide Receptor Grid Generation module of Maestro, Schrodinger [15] and it was centered on the mass center of each binding site (Site 1, 2, 3, 4, 5) at every grid formation. A bigger enclosing box with dimensions $22 \times 22 \times 22 \text{ \AA}$ was also defined to contain every particle (phytochemicals used) in the docked poses only [16].

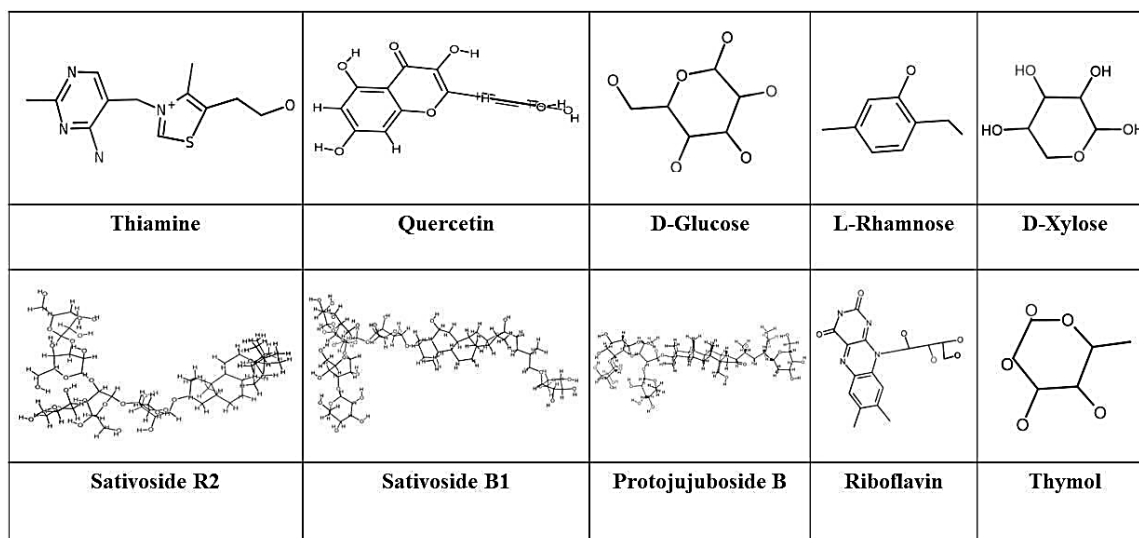


Figure 1. Major phytochemicals of *A. sativum* interacting with the target proteins.

Table 1. Illustrating Active Sites of the viral proteins of H5N1 subtype.

S.N.	Protein	SiteMap	Site Score	D-Score	Volume	Amino Acid	Residues
1.	2IBX	Site 5	1.088	1.099	624.603	Ile-149	Loop-150
2.	2HTY	Site 2	1.033	0.974	486.031	Gly-147	Asp-151
3.	3CKZ	Using amino acid residues to generate receptor grid.				Val-149	Arg-152

Molecular Docking Analysis

All 145 phytochemicals were docked against neuraminidase using the standard precision (SP)' mode of the Glide docking algorithm (grid-based ligand docking energetics) using the Schrödinger 2022-2 version [17]. The ligand docking module of glide was used to dock the ligands. Ligand docking was done using grid of the targeted site of protein [17]. Based on the docking score, the potential molecules were screened and identified.

Binding Free Energy Calculation

Post docking, we proceeded with MM-GBSA (Molecular Mechanics with Generalized Born and Surface Area solvation) refinement. The prime MM-GBSA is done to calculate the free energy of binding of the most promising screened ligands. The docked complexes of the best-docked poses for each ligand were subjected to MM-GBSA refinement in the Prime module [18].

For calculation of binding free energy by keeping a flexible residue distance of 5 \AA [18]. The binding energy was calculated by applying the formula:

$$\Delta G = \Sigma(\Delta G_{\text{Bind coulomb}} + \Delta G_{\text{Bind covalent}} + \Delta G_{\text{Bind H-bond}} + \Delta G_{\text{Bind Lipo}} + \Delta G_{\text{Bind packing}} + \Delta G_{\text{Bind}})$$

$$\text{Self cont} + \Delta G_{\text{Bind Solv GB}} + \Delta G_{\text{Bind vdw}}).$$

ADMET Analysis

ADME property analysis was done to ensure that the molecules we have screened out have a good ADME profile. ADME profiling is done to understand the chemical absorption, distribution,

metabolism, excretion, and toxicity of the drug candidate, which helps us to understand how compounds are processed by living organisms. We have performed ADME profile analysis, using QikProp module wherein we have calculated a lot of properties of the compound which indicated whether these ligands have good pharmacological profiles.

Results

It is crucial to find effective alternatives that can optimally bind with the neuraminidase's sialic acid and block its enzymatic activity, causing it to remain bound to the host cell surface and stop further colonization. For this purpose, after virtual screening, we have identified 5 garlic ligands that were used to find out their effect on H5 and N1, along with this we used 2 neuraminidase inhibitors (oseltamivir and zanamivir) to compare the efficiency of the phytochemicals of garlic with the already existing inhibitors. The molecules from garlic were more promising as compared to the known inhibitors.

Molecular Docking

The docking scores revealed that the interactions of the viral proteins, that is, H5 (2IBX) and N1 (2HTY and 3CKZ) proteins, with selected 145 compounds were quite promising. Out of 145, many compounds have shown appreciable interaction scores which lie in the range from -8.212 to -5.163 Kal/mol as illustrated in Tables 2, 3, and 4. The interaction of phytochemicals from garlic has shown better results than the already existing inhibitors indicating their better therapeutic potential towards the Target and subsequently on the disease itself. Out of 145 screened compounds we have selected 5 compounds that show potentially good scores, that is, sativoside R1, sativoside R2, protojubeside B, sativoside B1, and riboflavin (as top candidates) for H5 (PDB id- 2IBX); D-galacturonic acid, D-galactose, D-fructose, moupinamide, and sucrose (as top candidates) for N1 (PDB id- 2THY); and folic, D-xylose, D-galacturonic acid, and quercetin for N1 with selected residues grid (PDB id- 3CKZ); for further in-depth study of interactions. The tables below represent the database ID, ligand name, and Docking scores of the screened compounds out of 145 phytochemicals from garlic (*A. sativum*) along with the scores of already existing inhibitors of hemagglutinin and neuraminidase. The scores with values lesser than -5 indicate that the selected compounds show better potential than the existing inhibitors [17].

Protein–Ligand Interaction

While performing the in-depth analysis of the behavior of the interaction between the H5N1 surface proteins and the top ligands with the best docking scores. The interaction results of a ligand from each target protein taken are shown in Figures 2, 3, and 4.

- *2IBX*: We observed six common amino acids like GLU D:39, GLY D:155, ARG E:322, GLU E:25, GLU F:11, and GLY.

F:134 that are influencing the interactions. Overall, all the screened ligands are well placed in the binding site and have revealed some important amino acids that are involved in the interaction of almost all the compounds. The amino acids in the protein exhibit only one H-Donor type of hydrogen interaction with IMPHY011411 (Protojubeside B), whereas with IMPHY009693 (Sativoside R1) two types of interactions (H-donor and H-acceptor type interactions) were observed. It has been observed that the amino acids GLU D:39, ARG E:322, and GLY F:134 have been involved in the interaction in the form of a hydrogen bond with each ligand, making these three particularly essential amino acids across all the ligands having a better and stable interaction with the protein. These data suggest that their positioning might play a crucial role in the interaction of the protein and the ligands in the binding pocket.

- *2HTY*: We observed all amino acid ARG residues in the vicinity of the ligand to influence the interactions. In case of

IMPHY014919 (D-galacturonic acid), salt bridges are formed between the ARG residues and the target protein. ARG118 and ARG371 show single and double hydrogen bonds respectively at both the compounds taken, suggesting that these are the essential amino acids across all ligands having stable interaction with the protein.

- 3CKZ: GLN136, HIS144, ASN146, and SER153 of the ligand IMPHY015116 (D-xylose) are observed to have hydrogen bonds with the protein of interest, suggesting that these specific amino acids also influence the interaction of the ligand with the protein.

Table 2. Database ID, ligand name, docking scores, and glide energy of the screened compounds procured from garlic for the protein 2IBX.

Database ID d	Ligand Name Ligand Nam	Docking Scores	Glide Energy
IMPHY009693	Sativoside R1	-8.212	-77.547
IMPHY003241	Sativoside R2	-8.130	-72.378
IMPHY011411	Protojujuboside B	-7.534	-77.982
IMPHY002772	Sativoside B1	-7.404	-81.863
IMPHY000846	Riboflavin	-7.068	-49.594
IMPHY006310	Folic	-6.784	-49.523
IMPHY010848	Protoeruboside B	-6.638	-64.176
IMPHY003049	Hypromellose	-6.166	-63.381
IMPHY014919	D-galacturonic acid	-6.049	-35.181
IMPHY008936	alpha-guaiene	-5.853	-25.444
IMPHY005415	Degalactotigonin	-5.803	-52.001
IMPHY004202	F-gitonin	-5.686	-55.577
IMPHY010911	Eruboside B	-5.641	-57.281
IMPHY001218	Thymoquinone	-5.590	-24.097
IMPHY007598	Phenylacetic acid	-5.560	-22.312
IMPHY012053	Sucrose	-5.555	-46.530
IMPHY001246	Carvacrol	-5.542	-22.063
IMPHY011802	Ferulic acid	-5.505	-24.667
IMPHY001658	Thymol methyl ether	-5.504	-22.773
IMPHY015056	L-Rhamnose	-5.318	-30.942
IMPHY016837	1-Ethylquinolinium iodide	-5.295	-23.594
IMPHY003982	gamma-terpinene	-5.294	-19.025
IMPHY011643	alpha-terpinene	-5.292	-19.101
IMPHY006145	p-cymene	-5.255	-19.720
IMPHY012994	3-vinyl-1,2-dithiin	-5.253	-18.571
IMPHY009826	1-(11Z-icosenoyl)-2-(9Z,12Z-octadecadienoyl)-sn-glycero-3-phosphoethanolamine	-5.224	-51.174

Table 3. Database ID, ligand name, docking scores, and glide energy of the screened compounds procured from garlic for protein 2HTY.

Database ID	Ligand name	Docking scores	Glide energy
IMPHY014919	D-galacturonic acid	-6.124	-27.142
IMPHY012050	D-galactose	-5.670	-29.919
IMPHY014916	D-fructose	-5.249	-28.277
IMPHY011879	Moupinamide	-5.239	-42.756
IMPHY012053	Sucrose	-5.212	-47.442
IMPHY015056	L-rhamnose	-5.178	-28.529

Table 4. Database ID, ligand name, docking scores, and glide energy of the screened compounds procured from garlic for protein 3CKZ.

Database ID	Ligand name	Docking scores	Glide energy
IMPHY006310	Folic	-6.037	-55.559
IMPHY015116	D-xylose	-5.996	-29.405
IMPHY014919	D-galacturonic acid	-5.809	-30.082
IMPHY004619	Quercetin	-5.199	-40.703
IMPHY000005	Thiamine	-4.807	-27.770

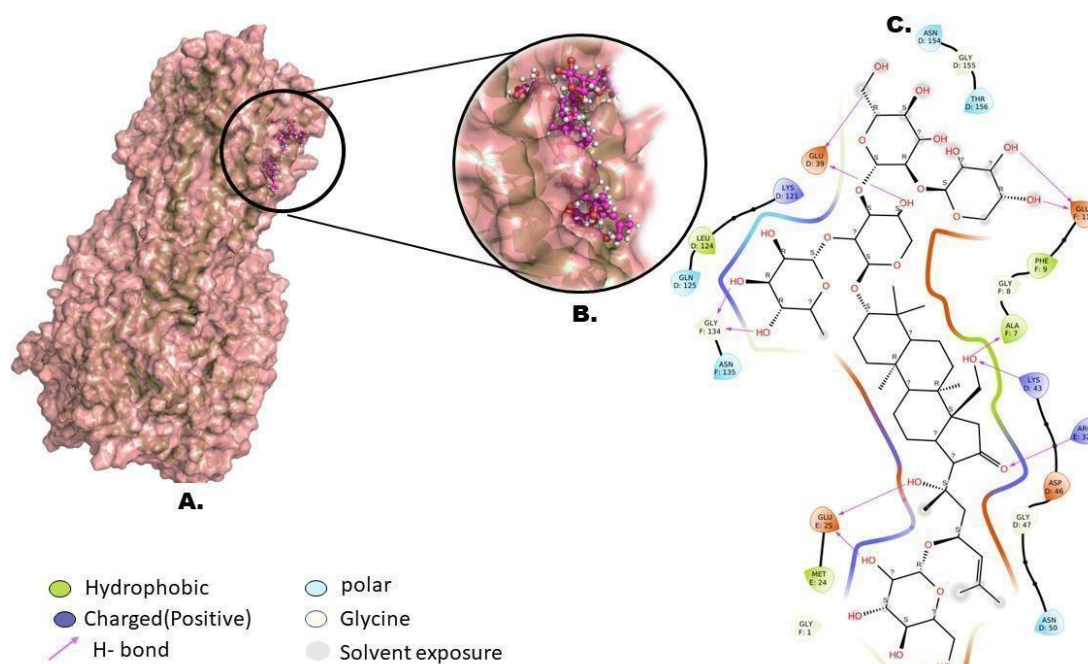


Figure 2. 3D representation of binding poses of ligand (IMPHY009693) with the receptor protein (2IBX). A. Represents binding pose of sativoside R1 with the active site of H5 hemagglutinin. B. Represents a detailed view of how sativoside R1 binds with the active site (Site 5) of H5 hemagglutinin. C. Represents the ligand interaction diagram of amino acids with the atoms, along with their nature and type as mentioned in Table 5.

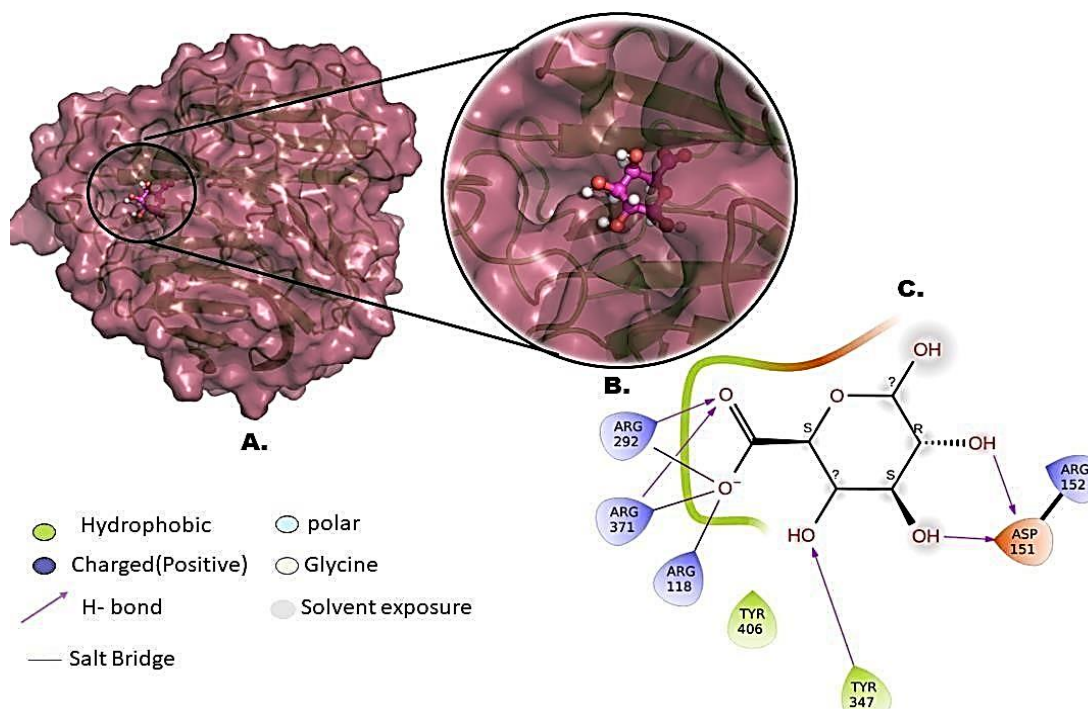


Figure 3. 3D representation of binding poses of ligand (IMPHY014919) with the receptor protein (2HTY). A. Represents binding pose of D-galacturonic acid with the active site of N1 neuraminidase. B. Represents a detailed view of how D-galacturonic acid binds with the active site (Site 3) of N1 neuraminidase. C. Represents the ligand interaction diagram of amino acids with the atoms, along with their nature and type as mentioned in Table 5.

Table 5. Database ID, ligand name, docking scores, and glide energy of the screened compounds procured from garlic for protein 3CKZ.

S.N.	Protein-PDB ID	Ligands	Docking Score	MMGBSA (kcal/mol)
1.	2IBX	IMPHY009693 (Sativoside R1)	-8.212	-97.52
		Interacting residues and type of interaction	Chain D: GLU D:39 (-ve, HB)*; LYS D:43 (+ve)*; ASP D:46 (-ve)*; GLY D:47*; ASN D:50 (Polar)*; PHE D:110 (HP); LYS D:121 (+ve); LEU D:124 (HP)*; GLN D:125 (Polar)*; ARG D:127* (+ve, HB); ASN D:154 (Polar)*; GLY D:155 (HB, HB)*; THR D:156 (Polar)*; ASP D:158 (-ve, HB)*. Chain E: THR E:22 (Polar); MET E:24 (HP); GLU E:25 (-ve); ARG E:322 (+ve, HB)*; PRO E:325 (HP)*. Chain F: GLY F:1; LEU F:2 (HP); ALA F:7 (HP); GLY F:8; PHE F:9 (HP)*; GLU F:11 (-ve)*; GLU F:105 (-ve); LEU F:108 (HP); ASH F:109 (-ve, HB); LEU F:133 (HP, HB) GLY F:134 (HB); ASN F:135 (Polar).	
2.	2IBX	IMPHY011411 (Protojubiloside B)	-7.534	-81.97
		Interacting residues and type of interaction	Chain D: GLU D:39 (-ve, HB, HB)*; LYS D:43 (+ve, HB)*; ASP D:46 (-ve)*; GLY D:47*; ASN D:50 (Polar)*; PHE D:110 (HP); LYS D:121 (+ve); LEU D:124 (HP); GLN D:125 (Polar); ASN D:154 (Polar); GLY D:155; THR D:156 (Polar). Chain E: MET E:24 (HP)*; GLU E:25 (-ve, HB, HB)*; ARG E:322 (+ve, HB)*. Chain F: GLY F:1; ALA F:7 (HP, HB)*; GLY F:8*; PHE F:9 (HP)*; GLU F:11 (-ve, HB, HB)*; GLY F:134 (HB, HB)*; ASN F:135 (Polar).	
3.	2HTY	IMPHY011879 (Moupinamide)	-6.124	-58.87
		Interacting residues and type of interaction	ARG118 (+ve, HB)*; GLN136 (Polar); GLY147*; VAL149 (HP, HB)*; LYS150 (+ve)*; ASP151 (-ve)*; TYR347 (HP); ASN369 (Polar); SER370 (Polar); ARG371 (+ve, HB, HB)*; TRP403 (HP); ILE427 (HP); ARG430 (+ve); PRO (HP); LYS432 (+ve); THR439 (Polar).	
4.	2HTY	IMPHY014919 (D-Galacturonic Acid)	-5.239	-20.13
		Interacting residues and type of interaction	ARG118 (+ve, SB)*; ASP151 (-ve, HB, HB)*; ARG152 (+ve)*; ARG292 (+ve, SB, HB)*; ARG371 (+ve, SB, HB)*; TYR406 (HP); TYR407 (HP, HB)*.	
5.	3CKZ	IMPHY015116 (D-Xylose)	-5.996	-11.99
		Interacting residues and type of interaction	GLN136 (Polar, HB, HB)*; GLY137; ALA138 (HP)*; HIS144 (Polar, HB); SER145 (Polar); ASN146 (Polar, HB)*; GLY147; THR148 (Polar); LYS150 (+ve); SER153 (Polar, HB)*; HIE155 (Polar); ARG156 (+ve)*.	

In the table, these symbols are used:

* = Important amino acids involved in interaction with protein.

- HB = Hydrogen bond +ve = Positively charged
- SB = Salt bridge
- HP = Hydrophobic -ve = Negatively charged

Table 6. Estimated molecular docking score (GLIDE XP), binding free energy (MMGBSA), binding site residues, nature, and type of interaction of ligands with the target protein.

Ligand ID	Ligand name	MM-GBSA dG bind
IMPHY009693	Sativoside R1	-97.52
IMPHY003241	Sativoside R2	-48.26
IMPHY011411	Protojubeside B	-81.97
IMPHY002772	Sativoside B1	-23.21
IMPHY000846	Riboflavin	-45.59

Table 7. MM-GBSA of compounds in the interaction of the phytochemicals with N1 (2HTY) protein.

Ligand ID	Ligand name	MM-GBSA dG bind
IMPHY014919	D-galacturonic acid	-20.13
IMPHY012050	D-galactose	-31.72
IMPHY014916	D-fructose	-33.89
IMPHY011879	Moupinamide	-58.87
IMPHY012053	Sucrose	-44.10

Table 8. MM-GBSA of compounds in the interaction of the phytochemicals with N1 (3CKZ) protein with specified amino acids.

Ligand ID	Ligand name	MM-GBSA dG bind
IMPHY006310	Folic	-10.67
IMPHY000005	Thiamine	-36.62
IMPHY014919	D-galacturonic acid	-4.35
IMPHY015116	D-xylose	-11.99
IMPHY004619	Quercetin	-26.59

Admet Analysis

The ADME analysis, performed by QikProp by Schrodinger, as represented in Tables 9, 10, and 11 provides multiple resulting properties of each ligand interacting appropriately with the target proteins. It displayed the molecular weight of the interested ligands (mol_MW); the Rule of 5, which displays the violated limits of the Lipinski's Rule (ligands that are considered to be drug-like satisfy the Rule of 5, with a maximum of 4 violations allowed, these are: mol_MW to be less than 500, QPlogPo/w to be less than 5, donorHB to be less than or equal to 5 and accptHB to be less than or equal to 10); the CNS where central nervous system activity is foreseen on a scale of -2 to +2, with negative values indicating an inactive status while a more positive value represents high CNS activity; the QPlogS where aqueous solubility (log S) is predicted on a range from -6.5 to 0.5; the QPlogBB where brain blood coefficient is predicted on a recommended range from -3.0 to 1.2; and the HOA or human oral absorption, where the HOA is predicted on a scale of 1, 2, or 3 indicating a low, mediocre or a high absorption orally [19]. These quantities have been enlisted in the table below. The data provided below suggest that the ligands have good or promising ADME properties and will provide an evaluation of safety and efficacy for the drug candidates to the drug developers.

Table 9. ADME parameters for the selected potential compound using QikProp (2IBX).

Ligand ID	mol MW	CNS	Rule of 5	QPlogS	QPlogBB	HOA scale
IMPHY000846	376.368	-2	0	-2.523	-3.01	2
IMPHY002772	1423.509	-2	3	1.868	-13.074	1
IMPHY003241	1197.327	-2	3	-2.084	-7.33	1
IMPHY009693	1377.484	-2	3	1.77	-9.583	1
IMPHY011411	1225.38	-2	3	-0.717	-7.848	1

Table 10. ADME parameters for the selected potential compound using QikProp (2HTY).

Ligand ID	mol MW	CNS	Rule of 5	QPlogS	QPlogBB	HOA scale
IMPHY011879	313.352	-2	0	-3.940	-1.429	3
IMPHY012050	180.157	-2	0	-1.041	-1.528	2
IMPHY012053	342.299	-2	2	-0.889	-2.761	1
IMPHY014916	180.157	-2	0	-0.807	-1.311	2
IMPHY014919	194.141	-2	0	-0.903	-1.846	2

Table 11. ADME parameters for the selected potential compound using QikProp (3CKZ).

Ligand ID	mol MW	CNS	Rule of 5	QPlogS	QPlogBB	HOA scale
IMPHY004619	302.24	-2	0	-2.83	-2.352	2
IMPHY006310	441.402	-2	2	-3.817	-5.129	1
IMPHY014919	194.141	-2	0	-0.903	-1.846	2
IMPHY015116	150.131	-1	0	-0.86	-0.992	2

CONCLUSIONS

In this paper, we focused on the H5N1 subtype of Avian Influenza, consisting of the major proteins H5 hemagglutinin and N1 neuraminidase proteins and their interactions with phytochemicals from *A. sativum*, a commonly consumed ingredient used in a vast population. Garlic was selected because of its beneficial properties, such as its antibacterial, antifungal, antiparasitic, antiviral, antioxidant, and anti-inflammatory properties. Furthermore, its antimicrobial activity on the MDR bacterial species, makes it an excellent antibiotic agent. We performed molecular docking analysis on the H5 and N1 viral proteins of the H5N1 virus strain by using various modules present in Maestro by Schrodinger like GLIDE, PRIME, QikProp, etc. to analyze the interaction of the procured phytochemicals from garlic with the target protein which can help to identify potential candidates for the formation of anti-H5N1 drug.

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