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Potential of phytochemicals from Silybum marianum against Hemagglutinin from Human Influenza A virus (pdm09 strain): An in-silico drug discovery analysis

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ABSTRACT

Influenza viruses contain single-stranded ribonucleic acid, having negative polarity and use RNA polymerase which lacks proofreading. This characteristic has led to severe influenza pandemics, as antigenic



shifts allow them to resist currently available vaccines. H1N1 is one of the influenza viruses that has come forward as a severe warning to human lives. Phytochemicals have been used to cure several diseases as they have high potentials to eliminate various diseases and infections in human beings. Herein, the potential of phytochemicals from a plant named *Silybum marianum* was analyzed against the hemagglutinin protein of Human Influenza A virus (pdm09 strain) opting computer-aided drug discovery protocols. Based upon the analysed properties including pharmacological properties, pharmacokinetics, binding affinities, stability in binding, and reactivity of compounds against receptor, 4 phytochemicals were identified and virtually screened out of 79 docked compounds, as those compounds showed efficient and exceptional results against targeted receptor. The results indicate that these compounds can be considered potential inhibitory candidates for hemagglutinin protein of Human Influenza A virus (pdm09 strain), after *in vitro* and *in vivo* validations.

Keywords: H1N1, Silybum Marianum, Molecular Docking, DFT, Molecular Dynamics

INTRODUCTION

Influenza viruses are the members of family Orthomyxoviridae, and are consisted of negatively sensed singlestranded RNA which encodes two glycoproteins named; Hemagglutinin (HA) and Neuraminidase (NA), one Acidic polymerase protein (PA), two Polymerase basic proteins PB1 and

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PB2, two Nonstructural proteins NS1 and NS2; NS2 known as NEP and two Matrix proteins M1 and M2.¹ Out of these proteins, two viral glycoproteins HA and NA are significant, because of their involvement in host cell attachment and host cell egress, respectively. Neuraminidase (NA) consists of one larger domain made by anti-parallel beta-sheets that are responsible for the establishment of propeller-like structure in its head region. In order to satisfy its form, a total of eight disulphide bonds contribute. While the active site of NA is bordered by twelve flexible loops and is located at the amino-terminal area of the central but parallel strand. It facilitates the movement of the virus

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through mucus and helps it to reach and infect epithelial cells of the host. $^{\rm 2,3}$

On the other hand, Hemagglutinin is a major viral antigen, which induces a neutralizing antibody response. It is homotrimer, comprising of monomer, divided into two subunits; HA1, a receptor binding part and HA2, a membrane anchoring part, both of them are tied by a single disulphide bond. The binding of HA with receptors containing $\alpha 2$, 6-linked sialic acid residues on the host cell surface, initiates viral entry via receptor-mediated endocytosis.⁴ The higher replication rates but the poor proofreading ability of RNA polymerase leads to the generation of progeny viruses having mutated genome. Moreover, the favourable environmental conditions further support the proliferation of these viruses and help them to become the predominant population.⁵ Total of four pandemics such as Spanish flu of 1918, Asian flu of 195, Hong Kong flu of 1968 and swine flu of 2009, had been reported to occur globally due to the deadly group of these microorganisms.¹

A broad class of anti-influenza drugs was Neuraminidase inhibitors; oseltamivir phosphate (Tamiflu) and M2 ion channel blockers; amantadine and rimantadine. Unfortunately, the capacity to develop resistance without dropping the transmissibility was displayed by some viruses. However, for the treatment of influenza infection, the development of certain therapeutic monoclonal antibodies (mAbs) and small molecules is under process.⁶

Phytochemicals are the chemical compounds commonly found in fruits, vegetables, nuts, legumes, and grains. Various infectious and non-infectious ailments have been reported to be treated by the use of medicinal plants. These medicinal plants are a rich source of phytochemicals and almost 25% of commonly used medicines are reported to be isolated from medicinal plants. In this modern age, where the greater number of infectious diseases and other problems has been challenging human health, medicinal plants can provide a way to the discovery of various anti-infectious drugs ⁷. Due to the limited availability of antiviral resources the continuous development of novel anti-influenza molecules is required ⁸. This can be done through the adoption of various procedures and strategies such as the introduction of an improved existing drug, selection and validation of new molecular targets, identification of inhibitors of various steps involved in virus's replication cycle to prevent the establishment, identification of agents involved in the modulation of antiviral defence responses and manipulation of synergistic effects of various compounds to act upon different molecular targets ^{9,10}.

The purpose of this study was to study the inhibitory potential of phytochemicals against Hemagglutinin protein of influenza virus A (H1N1)'s pdm09 strain, as this strain is least targeted in literature. The set of phytochemicals belonged to Silvbum marianum and comprised a total of 90 phytochemicals. The compounds were analysed pharmacologically and were subjected to molecular docking analysis against targeted receptor. The screened phytochemicals were also compared with experimentally validated inhibitors. The stability in the binding of phytochemicals and their reactivity was also analyzed against the Hemagglutinin.

EXPERIMENTAL PROCEDURES

The study targeted the Hemagglutinin protein from H1N1's pdm09 strain. Thus, to effectively meet the objectives of the study, a series of steps were performed based on computational drug discovery approaches. The flowchart for methodology is provided in Figure 1.

Collection of phytochemicals and protein structure retrieval

The targeted receptor i.e. Hemagglutinin protein from H1N1's pdm09 strain was retrieved as a 3D crystal structure from RCSB Protein Data Bank with PDB ID: 4LXV (https://www.rcsb.org/structure/4LXV). As the study was based on phytochemicals from Silybum marianum, therefore, using plant name as a keyword, literature was searched and names for phytochemicals were searched. Mainly, the targeted databases were MAPS and MPD3,^{36,37} and also these compounds have been reported previously in a study by Rasool et al.³⁸ This resulted in a library of 90 phytochemicals, which all belonged to Silvbum marianum. For setting control and threshold, compounds were searched and a compound named Oximacro® was selected. which is obtained from cranberry extract.³⁹ It was found to have three major components; Proanthocyanidin A, Proanthocyanidin A2 and Proanthocyanidin B2-di-gallate. As human influenza A H1N1 viruses have a higher mutation rate, the more genetic assortment has been seen, which may lead to developing more resistance against Oximacro®.39



Figure 1. Flowchart of methodology

Initial screening of phytochemicals

The pharmacological and pharmacokinetic properties based on parameters of ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) were evaluated with the help of the SwissADME webserver⁴⁰ and PreADMET webserver,⁴¹ as reported in ref.⁴²⁻⁴⁶. SwissADME server was utilized for the determination of ADME properties of the phytochemicals, while PreADMET was utilized to assess the druglikeness features and toxicity level of the drug. The structural files (.SDF) of the 90 phytochemicals were utilized for the prediction purposes.

Molecular docking

To analyze the binding mode of phytochemicals, molecular docking of the compounds was carried out. The docking study illustrated the interaction of the compounds with the particular targeted protein. In this study, Hemagglutinin protein was the targeted protein while the phytochemicals were used as ligands. The AutoDock Tools v1.5.7 and AutoDock Vina ⁴⁷⁻⁴⁹ were utilized to perform the docking of targeted protein and a set of ligands. The binding energies, as well as the inhibitory constant K_i (μ M) of these compounds, were calculated. For calculation of K_i, equation 1 was used.

$$\dot{K}i = \frac{\Delta G}{e^{R \times T}} \tag{1}$$

A grid box was generated and x, y, z dimensions were specified for the ligand-protein interaction within the grid box. This grid comprised of $26 \times 24 \times 30$ Å dimensions. The grid was centred at the binding site of the protein as the binding site was reported in crystallization study of the protein ⁵⁰.

Molecular dynamics simulation

Besides molecular docking, binding of potential phytochemicals with the protein and the stability of these complexes was further estimated through MD Simulation. MD simulation is a widely used computational method. This method provides a way of evaluating the protein-ligand stability, investigation of their major conformational changes as well as rescoring of resultant complexes in terms of their binding affinities. However, the analysis of only those complexes was done that showed higher binding affinities. MD simulations were carried out through Groningen Machine for Chemical Simulations (GROMACS) v 5.0 at different temperatures ⁵¹. Through analysis, Root means square deviation (RMSD) scores were computed to observe changes between the initial state of complex and after MD simulation of the complex. Similarly, the graphs for the radius of gyrations (Rg) were plotted to observe fluctuations and compactness of structure.

DFT Analysis

In order to analyze the effectiveness, efficiency and reactivity properties of screened phytochemicals, DFT based analysis was carried out through the use of the ORCA program. ORCA provides the facility of computing quantum-based, molecular orbital descriptors i.e. HOMO (highest occupied molecular orbit) and LUMO (lowest unoccupied molecular orbit) energies and the calculation of ΔE (band energy gap) by use of expression ELUMO – EHOMO. In order to prepare input files, a chemical analyzer tool "Avogadro" was used. A hybrid exchangecorrelation functional which is a combination of Hartree-Fock exchange functional, and known as B3YLP exchange-correlation functional, was employed to carry out these DFT based calculations. $^{\rm 52}$

RESULTS

Screening of phytochemicals based upon ADMET analysis In this study, the phytochemicals library comprised 90 Silybum Initially, compounds from marianum. all phytochemicals were subjected to pharmacological analysis by using ADMET analysis. Based on this analysis, a screening criterion was set to filter out safe, drug-like compounds for human administration. The criteria for screening phytochemicals were: violations from Lipinski's rule = zero; soluble = yes; absorption in gastrointestinal (GI) tract = efficient or moderate; blood-brain barrier (BBB) permeability = no; and Toxicity = nontoxic. Based on this filter, 79 compounds out of 90 were screened for their suitable ADMET properties.

Molecular docking analysis for screened compounds against the receptor

The biological target of the present study was Hemagglutinin protein from H1N1's pdm09 strain, thus it was considered as a receptor for molecular docking, while the ligands were the 79 screened compounds from ADMET analysis phase. To validate the effectiveness of screened compounds, a previously-reported biologically-validated compound named Oximacro was selected and comparison was made with compounds screened in the present study. The Oximacro® is obtained from cranberry extract and three major components; Proanthocyanidin Α, Proanthocyanidin A2 and Proanthocyanidin B2-di-gallate. These 3 compounds were docked with the targeted receptor and among these 3 compounds, the Proanthocyanidin B2-di-gallate showed binding affinity of -8.6 kcal/mol, which was highest as compared to Proanthocyanidin A and Proanthocyanidin A2 (Fig. 1(E)). Thus, in this study, the value of binding affinity expressed by Proanthocyanidin B2-di-gallate was considered as the threshold value for screening promising compounds.

When interactions with the protein were made, all phytochemicals showed different affinities. However, four phytochemicals showed promising binding affinities, which were more or equal to the threshold value i.e. -8.6 kcal/mol. These four phytochemicals were JacarelhyperolB, Theaflavinegallate, JacarelhyperolA and OroxinB, (Figure 3) with a binding affinity of -8.9kcal/mol, -8.8 kcal/mol, -8.6kcal/mol. and -8.6 kcal/mol, respectively (Figure 2).



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Figure 2: Phytochemicals showing promising results against targeted receptor (a) JacarelhyperolB with a binding affinity of -8.9kcal/mol (b) Theaflavinegallate with a binding affinity of -8.8 kcal/mol (c) OroxinB with a binding affinity of -8.6 kcal/mol (d) JacarelhyperolA with a binding affinity of -8.6kcal/mol (e) Proanthocyanidin B2-di-gallate (control) with a binding affinity of -8.6 kcal/mol.

JacarelhyperolB, with the binding of -8.9kcal/mol and K_i value of 0.294 μ M, formed a different number of conventional hydrogen bonds with ARG₁₀₂, ASP₅₆, ASP₈₆, THR₈₉, and ASP₉₄ (Fig. 1(A)). Theaflavinegallate showed the binding affinity of -8.8 kcal/mol with the Ki value of 0.349 μ M and formed conventional hydrogen bonds with PHE₁₄, LYS₁₄₆ and ASP₁₄₇. Furthermore, it formed Pi-alkyl bond with ARG₁₁₃, unfavourable donor-donor interactions with THR₇₂, carbon-hydrogen bonds with GLY₆₃ PHE₂₅₅, GLU₆₈, Pi-cation interactions with HIS₁₃₈, and Pi-stack interactions with TRP₆₀ (Fig. 1(B)).

OroxinB with Ki value of 0.489 μ M and binding affinity -8.6kcal/mol, formed conventional hydrogen bonds with SER₁₄₃, GLU₆₈ and LYS₁₄₆. Furthermore, carbon-hydrogen bond and Pidonor hydrogen bond with TRP₆₀, THR₇₂, PHE₁₄₄, and Pi alkyl interaction with ARG₁₁₃ and LYS₁₄₆ were also observed (Fig. 1(C)). JacarelhyperoIA with Ki value of 0.489 μ M and binding affinity of -8.6kcal/mol, formed a conventional hydrogen bond with ASP₆₄, and Pi-cations and anions with GLU₁₁₂, ARG₁₁₃ and LYS₁₄₆. LYS₁₄₆ also made Pi-alkyl interactions with ligand and formation of Pi-sigma was also observed (Fig. 1(D)).

Compactness of complexes through MD simulation

Compactness and stability of phytochemical-ligand complexes were analyzed by monitoring the radius of gyration (Rg) and RMSD scores were computed to observe changes between the initial state of complex and after MD simulation of the complex through MD simulation. Each complex was subjected to 20ns MD simulation, at different temperatures, as illustrated in Fig. 4.

The graphs depicted few fluctuations and Rg value for each complex ranged between 0.8 Å -2.0 Å in all cases, which was

even better than the Rg of control i.e. < 2.94 Å. RMSD values reported in Table 1, depicted high stability as these values ranged between 0.55Å- 2.87 Å.

 Table 1: Average RMSD values for complexes of compounds passing threshold at temperature 300K, 325K, 340K and 350K

Complex of Hemagglutinin and phytochemicals	Average	e RMS	D at	various	
	temperatures (å)				
	300K	325K	340K	350K	
JacarelhyperolB	0.55	0.77	1.25	1.36	
Theaflavinegallate	0.59	1.04	1.29	1.61	
OroxinB	1.20	1.70	1.89	1.96	
JacarelhyperolA	1.65	1.88	2.62	2.87	
ProanthocyanidinB2-di- gallate (Control)	1.70	1.93	2.72	2.94	



Jacarelhyperol B



Oroxin B



Figure 3. Chemical structure of main compounds used in this study.

RMSD values of experimental complexes were even better than the control complex i-e the complex of HR1 and Proanthocyanidin B2-di-gallate. However, JacarelhyperolB complex at different temperatures (Table 1) highlighted a promising potential as compared to the other screened phytochemicals, because the average RMSD values of its complex at different temperatures, were less than the RMSD values for other complexes.



Rg graph of Hemaglutanin-JacarelhyperolB

Figure 4: Rg graph of control and experimental ligands with the targeted receptor (a) JacarelhyperolB (b) Theaflavinegallate (c) OroxinB (d) JacarelhyperolA (e) Proanthocyanidin B2-di-gallate (control)

DFT analysis and reactivity of screened complexes

Besides molecular docking and MD simulations, the reactivity of these phytochemicals was analyzed in terms of band energy gap and molecular orbital energy descriptors. The result showed that these screened phytochemicals exhibited high reactivity ranging from 0.114 to 0.127 (Table 2). All of these phytochemicals showed reactivity higher than the reactivity of *Proanthocyanidin B2-di-gallate*. However, among these screened phytochemicals, again the *JacarelhyperolB* after the comparison of its band energy gap with the band energy gap of *Proanthocyanidin B2-di-gallate* and screened phytochemicals was proved to have the highest reactivity i.e. 0.114 kcal/mol.

 Table 2: DFT results for compounds passing threshold

 (Proanthocyanidin B2-di-gallate, binding affinity -8.6kcal/mol)

Compound	Binding affinity (kcal/m ol)	e _{lumo} (kcal/m ol)	e _{homo} (kcal/m ol)	Band energy gap (δe) (kcal/m ol)
JacarelhyperolB	- 8.9	-0.274	-0.389	0.114
Theaflavinegallat e	- 8.8	-0.288	-0.407	0.118
OroxinB	- 8.6	-0.293	-0.418	0.125
JacarelhyperolA	- 8.6	-0.273	-0.401	0.127
Proanthocyanidin B2-di-gallate (Control)	- 8.6	-0.304	-0.451	0.147

DISCUSSION

Almost 250 000 to 500 000 deaths are caused by Influenza Virus annually worldwide.¹¹ Although in 1976, the establishment of the expanded program on immunization (EPI) in Pakistan included vaccination for six major health threats such as measles, diphtheria, tetanus, pertussis, poliomyelitis and tuberculosis has been done. Yet Pakistan and various other developing countries have no official influenza vaccine policy. Almost 1000 deaths each day in less than 5 years old children is estimated to occur in Pakistan and the reason is the unavailability of an effective standard. However, the treatment of extended target groups such as older children, adolescents and adults would eliminate the chances of cross-infection, leading to the establishment of an effective and dependable standard.¹⁰

In a previous study, Oximacro®; a compound drug having many components along with Proanthocyanidin A, A2 and Proanthocyanidin B2-di-gallate, PAC-B2 was investigated to find its ability to interact with the crystal structure of Hemagluttinin of Human Influenza A and B virions.¹² PAC-B2 showed -8.6 kcal/mol by forming un-favourable donor forces with ASN (H:617) and LYS(H:621) Van der Waals forces with LEU(L:502). It also forms a conventional hydrogen bond with GLY(L:501) and LEU(K:24). It forms Pi-ions with ARG(K:322), GLU(K:25), along with the carbon-hydrogen bond. It forms Pi-alkyl with ALA(L:507) and GLY(L:501) (Fig 2(E)).

Upon incubation, the direct involvement of Oximacro® to Hemagglutinin has been indicated by the altered electrophoretic mobility of HA ectodomain, moreover, the presence of PAC-A2 in the extract also mediates such interactions. The direct comparison between PAC-A2 and PAC-B2 was done by Luganini et al, and results of this in silico docking showed much lower binding affinity to HA of the latter.¹² However, these two findings supported the view regarding the propensity and the potential binding affinity of different plant-derived polyphenols to the envelop proteins of influenza virus; hemagglutinin and neuraminidase. Hence, the overall described action of Oximacro® against influenza virus supports its use for the prevention of influenza infection. Therefore, the local application of Oximacro®, either in the form of tablets of chewable gums or through inhaling devices can allow the active PAC-A to inactivate the infecting virus in the upper respiratory tract, leading to the prevention of overall infection.¹²

Due to the limited availability of antiviral resources, the development of anti-influenza approaches can prove to be effective. One of the various strategies is the use of biologically active compounds with broad-spectrum antiviral activities. *Vaccinium macrocarpon Aiton*, also known as chinaberry has mines of potential compounds with anti-adhesive and antibacterial activities. It is also thought to provide certain compounds with abilities to resist the viral attachment to the targeted cells.

Nature has provided plants with a thriving disparity of entirely effective phytochemicals for most human diseases. This feature of the plant has boosted up the pharmaceutical industry (~25%-50% dependence) to explore and pull out the extracts from them and use their beneficial products.¹³ Flavonoids have been suggested to possess certain antitumor and anti-inflammatory activities that can serve to treat upper respiratory tract disorders. *OroxinB* is also a flavonoid, it has been found to have antitumor action and was chiefly attributed to induce apoptosis in a cell, which leads to the inhibition of tumor angiogenesis, regulation of cell signal transduction, adjustment of body's immune function, etc. It can be obtained from a traditional and herbal Chinese medicine *Oroxylum Indicum* (L.) Vent.¹⁴

JacarelhyperolA and *JacarelhyperolB* are members of pyranoxanthones class of phytochemicals; isolated from *Hypericum japonicum* and are famous for inhibitory activities against platelet-activating factor (PAF)-induced hypertension and for treating diseases like hepatitis and tumors due to its apoptotic ability.¹⁵

Theaflavinegallate, being a component of black tea and traditional Chinese medicines, has been reported to have antioxidant, antipathogenic substance, and cancer-suppressing properties. It has become the centre of interest for researchers. Moreover, Theaflavins have also been evaluated for advantageous effects to obviate coronary heart disease and hypertension and to prevent diabetes.¹⁶

Various computational approaches such as hit identification and lead optimization are used for the docking and scoring of small molecules to check their potential for the binding sites present in the structure of various macromolecules.¹⁷ Proteins can be the targets of drug designing as they have significant roles in the progression of the disease through various interactions which may lead to signalling involved in the disease proliferation. In the receptor-based *in silico* screening, X-ray crystallography and NMR are used for the determination of 3D structures as well as the active sites of the target proteins. This is important for the initial steps of drug discovery as well as for the generation of testable hypotheses.¹⁷

In order to develop drugs through an in-vivo and in-vitro method, long time span and high expenditure is required.^{18,19} Moreover, to introduce drugs through pharmaceutical industry, multistep and time-consuming processes are required for the biological validation and investigation of active drug candidates for certain properties such as their metabolism, potential toxicity as well as their pharmacokinetic properties (ADME).²⁰

In silico drug designing in this regard, put forward a useful, time saving and cost-effective way to not only predict the active molecules but also, it gives an idea about their certain properties and drug discovery process. Today, with the advent of genomics, proteomics, bioinformatics and effective technologies including virtual screening, de novo design, high throughput screening (HTS), ADMET analysis, Molecular docking Analysis, DFT analysis and MD simulations, it is now possible to observe the efficiency of certain molecules by providing information about their pharmacological properties, affinities to block certain targets by production complexes, compactness of resulting complexes as well as certain properties describing the energy associated with these complexes.²¹⁻²⁸

Being an abbreviation for the absorption, distribution, metabolism, excretion, and toxicity; ADMET analysis is imposed to predict these properties of drugs, as a tool to implement certain groups of experimental screens (assays). Low drug-likeness and poor ADMET properties of compound exclude it before doing any advance preclinical research, no matter if it has the highest bioactivity.²⁹

Being defined as the process of the determination of binding affinities between two molecules, molecular docking is an important computational tool that uses either ligand or receptorbased procedure to arbitrate the potential expectant for the achievement of particular goals such as promising drug designing efficiently and cost-effectively.^{30,31} It is the method through which one can foretell the chosen alignment and exposure of one molecule to another molecule during the formation of a stable complex structure. It indicates the binding of the certain ligand with a target that can be a protein molecule. The procedure of molecular docking is used chiefly to predict the drug interactions through the inspection and modelling of certain drug molecular interactions between ligand and target receptor. Several molecular docking tools such as AutoDock, FRED, eHITS and FTDOck etc. are used to develop and select ligand conformations and orientations.³² Molecular docking analysis provides deep information about the interaction types of a ligand with targeted protein. Several highly computationally intensive algorithms allow the understanding of interaction in the presence or absence of water molecules in a microenvironment. It doesn't only provide the ability to analyze the cellular level complexities of small molecules but also provides regular practice of protein DNA, protein-protein and protein-RNA analysis.^{33,34}

One of the effective procedures that characterize alternative methods for the sample configuration space is Molecular dynamics simulations. It depends upon the molecular motion simulation and solves Newton's equation of motion for individual atom by increasing the speed and position of each atom through the increase in time duration. Based on these rules, MD simulation overcomes the small barriers and local area around the sample points. Inherent dynamics of a system are used for the sampling of conformational spaces within a largely confined system. It also searches the deformation modes of low energy ²⁷. It aids the understanding of the functionality of a large molecule, its structure and reactions in a sequential manner. Various types of simulations such as free energy-based calculation, targeted MD simulations, steered molecular dynamics simulation, coarse gain simulations and RMSD of the molecule with each step, can be done.35

Another *in silico* technique known as density functional theory analysis is based upon quantum mechanics and is used to analyze the electronic structures. Being one of the best methods to calculate the orbital energy values, DFT provides insight into the stability of the structure through HOMO and LUMO energies. Energy gap, also known as the energy difference between HOMO and LUMO energies, indicates the reactions occurring due to charge transfer within a molecule.²⁴⁻²⁶

In our study, above mentioned computational tools were used to screen and analyze the potential phytochemicals against Hemagglutinin protein of H1N1 (pdm09) virus. Overall, this study provided with exceptional and dependable results.

CONCLUSION

The present study against Human Influenza A virus, directing Hemagglutinin protein suggests that phytochemicals *OroxinB*, *JacarelhyperolA*, *Theaflavinegallate* and *JacarelhyperolB* can successfully inhibit Hemagglutinin protein of H1N1 (pdm09) virus because their binding affinity values are equal to the threshold value. Moreover, after MD simulation and DFT analysis, *JacarelhyperolB* came forward as the best inhibitor against Hemagglutinin protein. Altogether, these potential phytochemicals can be assessed in vitro and in vivo for determining their efficacy and safety and can be used as drugs in future due to their marvellous inhibitory effects against the genetic re-assortment and higher mutation rates of influenza viruses. The development of phytochemicals for Human Influenza A (pdm09) treatment, would be medicinally and economically practical.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. M.J. Farrukh, L.C. Ming, S.T. Zaidi, T.M. Khan. Barriers and strategies to improve influenza vaccination in Pakistan. *Journal of Infection and Public Health* **2017**, 10, 881-83.

2. Y. Shtyrya, L. Mochalova, N. Bovin. Influenza virus neuraminidase: structure and function. *Acta Naturae (англоязычная версия)* **2009**, 1.

3. X. Yang, L. Steukers, K. Forier, R. Xiong, K. Braeckmans, K. Van Reeth, H. Nauwynck. A beneficiary role for neuraminidase in influenza virus penetration through the respiratory mucus. *PloS one* **2014**, 9, e110026.

4. L. Vaccaro, K.J. Cross, J. Kleinjung, S.K. Straus, D.J. Thomas, S.A. Wharton, J.J. Skehel, F. Fraternali. Plasticity of influenza haemagglutinin fusion peptides and their interaction with lipid bilayers. *Biophysical journal* **2005**, 88, 25-36.

5. N. Sriwilaijaroen, Y. Suzuki. Molecular basis of the structure and function of H1 hemagglutinin of influenza virus. *Proceedings of the Japan Academy, Series B* **2012**, 88, 226-49.

6. Y. Wu, M. Cho, D. Shore, M. Song, J. Choi, T. Jiang, Y.-Q. Deng, M. Bourgeoiset. al. A potent broad-spectrum protective human monoclonal antibody crosslinking two haemagglutinin monomers of influenza A virus. *Nature communications* **2015**, 6, 1-11.

7. M. Mukhtar, M. Arshad, M. Ahmad, R.J. Pomerantz, B. Wigdahl, Z. Parveen. Antiviral potentials of medicinal plants. *Virus research* **2008**, 131, 111-20.

8. A.N. Singh, M.M. Baruah, N. Sharma. Structure Based docking studies towards exploring potential anti-androgen activity of selected phytochemicals against Prostate Cancer. *Scientific reports* **2017**, 7, 1-8.

9. A. Kerviel, S. Dash, O. Moncorgé, B. Panthu, J. Prchal, D. Decimo, T. Ohlmann, B. Lina*et. al.* Involvement of an arginine triplet in M1 matrix protein interaction with membranes and in M1 recruitment into virus-like particles of the influenza A (H1N1) pdm09 virus. *PloS one* **2016**, 11, e0165421.

10. A. Loregian, B. Mercorelli, G. Nannetti, C. Compagnin, G. Palù. Antiviral strategies against influenza virus: towards new therapeutic approaches. *Cellular and molecular life sciences* **2014**, 71, 3659-83.

11. R. Hollingsworth, C. El Guerche-S éblain, T. Tsai, Y. Vasiliev, S. Lee, H. Bright, P. Barbosa. Assessment of the benefits of seasonal influenza vaccination: Elements of a framework to interpret estimates of vaccine effectiveness and support robust decision-making and communication. *Influenza and Other Respiratory Viruses* **2021**, 15, 164-74.

12. A. Luganini, M.E. Terlizzi, G. Catucci, G. Gilardi, M.E. Maffei, G. Gribaudo. The cranberry extract Oximacro® exerts in vitro virucidal activity against Influenza virus by interfering with hemagglutinin. *Frontiers in microbiology* **2018**, 9, 1826.

13. S. Suganya, B. Nandagopal, A. Anbarasu. Natural Inhibitors of HMG-CoA Reductase—An Insilico Approach Through Molecular Docking and Simulation Studies. *Journal of cellular biochemistry* **2017**, 118, 52-57.

14. N.-N. Li, X.-S. Meng, Y.-R. Bao, S. Wang, T.-J. Li. Evidence for the involvement of COX-2/VEGF and PTEN/PI3K/AKT pathway the mechanism of oroxin B treated liver cancer. *Pharmacognosy magazine* **2018**, 14, 207.

15. S. Zhang, J. Yin, X. Li, J. Zhang, R. Yue, Y. Diao, H. Li, H. Wanget. *al.* Jacarelhyperol A induced apoptosis in leukaemia cancer cell through inhibition the activity of Bcl-2 proteins. *BMC cancer* **2014**, 14, 689.

16. M.A. Vermeer, T.P. Mulder, H.O. Molhuizen. Theaflavins from black tea, especially theaflavin-3-gallate, reduce the incorporation of cholesterol into mixed micelles. *J. Agricultural Food Chem.* **2008**, 56, 12031-36.

17. D. Kitchen, H. Decornez, J. Furr, J. Bajorath. Structure-based virtual screening and lead optimization: methods and applications. *Nature Rev Drug Discov* **2004**, 3, 935-49.

18. J.A. DiMasi. Trends in drug development costs, times, and risks. *Drug Information Journal* **1995**, 29, 375-84.

19. J.A. DiMasi, R.W. Hansen, H.G. Grabowski. The price of innovation: new estimates of drug development costs. *Journal of health economics* **2003**, 22, 151-85.

20. B. Hileman. Accounting for R&D-Many doubt the \$800 million pharmaceutical price tag. *Chemical & Engineering News* **2006**, 84, 50-50.

21. N. Arif, A. Subhani, W. Hussain, N. Rasool. In silico inhibition of BACE-1 by selective phytochemicals as novel potential inhibitors: molecular docking and DFT studies. *Current drug discovery technologies* **2020**, 17, 397-411.

22. S.N. Chaudry, W. Hussain, N. Rasool. Inhibitory role of selective phytochemicals against HIV-2 protease: a study of molecular docking,

ADMET and DFT computations. *International Journal of Computational Biology and Drug Design* **2020**, 13, 390-414.

23. W. Hussain, A. Majeed, A. Akhtar, N. Rasool. Computational Studies of 3D-QSAR on a Highly Active Series of Naturally Occurring Nonnucleoside Inhibitors of HIV-1 RT (NNRTI). *Journal of Computational Biophysics and Chemistry* **2020**, 2050036.

24. K. Kousar, A. Majeed, F. Yasmin, W. Hussain, N. Rasool. Phytochemicals from Selective Plants Have Promising Potential against SARS-CoV-2: Investigation and Corroboration through Molecular Docking, MD Simulations, and Quantum Computations. *BioMed research international* **2020**, 2020.

25. N. Rasool, A. Akhtar, W. Hussain. Insights into the inhibitory potential of selective phytochemicals against Mpro of 2019-nCoV: a computer-aided study. *Structural Chemistry* **2020**.

26. N. Rasool, A. Bakht, W. Hussain. Analysis of Inhibitor binding combined with reactivity studies to discover the potentially inhibiting phytochemicals targeting Chikungunya viral replication. *Current drug discovery technologies* **2020**.

27. N. Rasool, W. Hussain. Three Major Phosphoacceptor Sites in HIV-1 Capsid Protein Enhances its Structural Stability and Resistance Against the Inhibitor: Explication Through Molecular Dynamics Simulation, Molecular Docking and DFT Analysis. *Combinatorial Chemistry & High Throughput Screening* **2020**, 23, 41-54.

28. N. Rasool, A. Majeed, F. Riaz, W. Hussain. Identification of novel inhibitory candidates against two major Flavivirus pathogens via CADD protocols: in silico analysis of phytochemical binding, reactivity, and pharmacokinetics against NS5 from ZIKV and DENV. *Structural Chemistry* **2020**, 31, 2189-204.

29. H. Yang, L. Sun, W. Li, G. Liu, Y. Tang. In silico prediction of chemical toxicity for drug design using machine learning methods and structural alerts. *Frontiers in chemistry* **2018**, 6, 30.

30. M.-E. Suh, S.-Y. Park, H.-J. Lee. Comparison of QSAR methods (CoMFA, CoMSIA, HQSAR) of anticancer 1-N-substituted imidazoquinoline-4, 9-dione derivatives. *Bulletin of the Korean Chemical Society* **2002**, 23, 417-22.

31. S. Wold, A. Ruhe, H. Wold, I. Dunn, WJ. The collinearity problem in linear regression. The partial least squares (PLS) approach to generalized inverses. *SIAM Journal on Scientific and Statistical Computing* **1984**, 5, 735-43.

32. R.N. dos Santos, L.G. Ferreira, A.D. Andricopulo In Computational Drug Discovery and Design; Springer: **2018**, pp 31-50.

33. J.J. Gray, S. Moughon, C. Wang, O. Schueler-Furman, B. Kuhlman, C.A. Rohl, D. Baker. Protein–protein docking with simultaneous optimization of rigid-body displacement and side-chain conformations. *Journal of molecular biology* **2003**, 331, 281-99.

34. M. Van Dijk, A.M. Bonvin. A protein–DNA docking benchmark. *Nucleic acids research* **2008**, 36, e88-e88.

35. P. Pattnaik, S. Raghunathan, T. Kalluri, P. Bhimalapuram, C.V. Jawahar, U.D. Priyakumar. Machine learning for accurate force calculations in molecular dynamics simulations. *The Journal of Physical Chemistry A* **2020**, 124, 6954-67.

36. U.A. Ashfaq, A. Mumtaz, T. ul Qamar, T.J.B. Fatima. MAPS Database: Medicinal plant activities, phytochemical and structural database. **2013**, 9, 993.

37. A. Mumtaz, U.A. Ashfaq, M.T. ul Qamar, F. Anwar, F. Gulzar, M.A. Ali, N. Saari, M.T.J.N.p.r. Pervez. MPD3: a useful medicinal plants database for drug designing. **2017**, 31, 1228-36.

38. N. Rasool, A. Jalal, A. Amjad, W. Hussain. Probing the Pharmacological Parameters, Molecular Docking and Quantum Computations of Plant Derived Compounds Exhibiting Strong Inhibitory Potential Against NS5 from Zika Virus. *Brazilian Archives of Biology and Technology* **2018**, 61.

39. A. Occhipinti, A. Germano, M.E. Maffei. Prevention of urinary tract infection with Oximacro®, a cranberry extract with a high content of a-type proanthocyanidins: a pre-clinical double-blind controlled study. *Urology journal* **2016**, 13, 2640-49.

40. A. Daina, O. Michielin, V. Zoete. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific reports* **2017**, 7, 42717.

41. J.V. Cruz, R.B. Serafim, G.M. da Silva, S. Giuliatti, J.M. Rosa, M.F.A. Neto, F.H. Leite, C.A. Taft*et. al.* Computational design of new protein kinase 2 inhibitors for the treatment of inflammatory diseases using QSAR, pharmacophore-structure-based virtual screening, and molecular dynamics. *Journal of molecular modeling* **2018**, 24, 225.

42. A. Akhtar, A. Amir, W. Hussain, A. Ghaffar, N. Rasool. In Silico Computations Of Selective Phytochemicals As Potential Inhibitors Against Major Biological Targets Of Diabetes Mellitus. *Curr Comput Aided Drug Des* **2019**.

43. W. Hussain, M. Ali, M. Sohail Afzalv, N. Rasool. Penta-1,4-Diene-3-One Oxime Derivatives Strongly Inhibit the Replicase Domain of Tobacco Mosaic Virus: Elucidation Through Molecular Docking and Density Functional Theory Mechanistic Computations. *Journal of Antivirals & Antiretrovirals* **2018**, 10.

44. W. Hussain, I. Qaddir, S. Mahmood, N.J.V. Rasool. In silico targeting of non-structural 4B protein from dengue virus 4 with spiropyrazolopyridone: study of molecular dynamics simulation, ADMET and virtual screening. **2018**, 29, 1-10.

45. I. Qaddir, N. Rasool, W. Hussain, S.J.J.o.v.b.d. Mahmood. Computeraided analysis of phytochemicals as potential dengue virus inhibitors based on molecular docking, ADMET and DFT studies. **2017**, 54, 255. 46. N. Rasool, A. Ashraf, M. Waseem, W. Hussain, S.J.T.J.o.B. Mahmood. Computational exploration of antiviral activity of phytochemicals against NS2B/NS3 proteases from dengue virus. **2019**, 44.

47. O. Trott, A.J. Olson. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of computational chemistry* **2010**, 31, 455-61.

48. M.R. Koebel, G. Schmadeke, R.G. Posner, S. Sirimulla. AutoDock VinaXB: implementation of XBSF, new empirical halogen bond scoring function, into AutoDock Vina. *Journal of Cheminformatics* **2016**, 8, 27.

49. G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell, A.J. Olson. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of computational chemistry* **2009**, 30, 2785-91.

50. H. Yang, J.C. Chang, Z. Guo, P.J. Carney, D.A. Shore, R.O. Donis, N.J. Cox, J.M. Villanueva*et. al.* Structural stability of influenza A (H1N1) pdm09 virus hemagglutinins. *Journal of virology* **2014**, 88, 4828-38.

51. M.J. Abraham, T. Murtola, R. Schulz, S. Páll, J.C. Smith, B. Hess, E. Lindahl. GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX* **2015**, 1, 19-25.

52. N. Rasool, S. Iftikhar, A. Amir, W. Hussain. Structural and quantum mechanical computations to elucidate the altered binding mechanism of metal and drug with pyrazinamidase from Mycobacterium tuberculosis due to mutagenicity. *Journal of Molecular Graphics and Modelling* **2018**, 80, 126-31.