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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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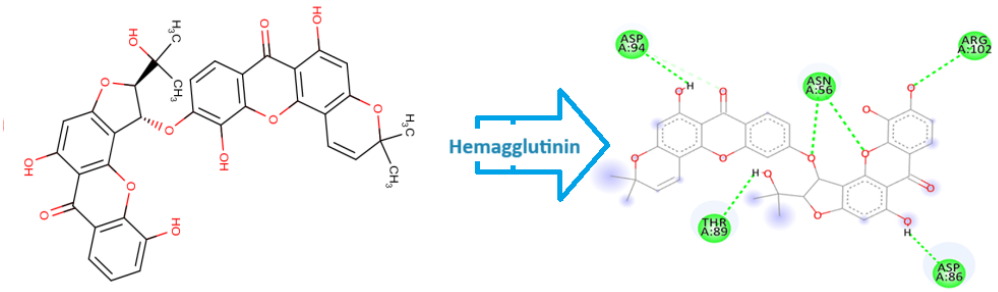
ABSTRACT



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 single-stranded RNA which encodes two glycoproteins named; Hemagglutinin (HA) and Neuraminidase (NA), one Acidic polymerase protein (PA), two Polymerase basic proteins PB1 and 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 through mucus and helps it to reach and infect epithelial cells of the host.[2,3]

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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 α2, 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 *Silybum 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 *Silybum 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]

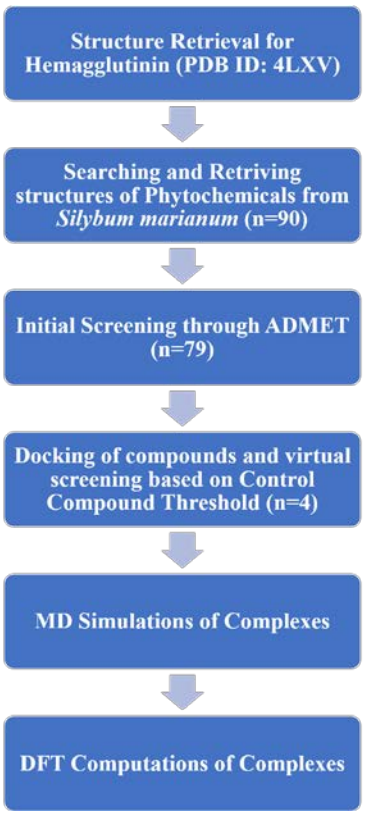


Figure 1. Flowchart of methodology

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 webserver[40] 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 (µM) of these compounds, were calculated. For calculation of K_i, equation 1 was used.

$$Ki = \frac{\Delta G}{e^{R\,x\,T}} \quad (1)$$

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 x 24 x 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 exchange-correlation 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.[52]

RESULTS

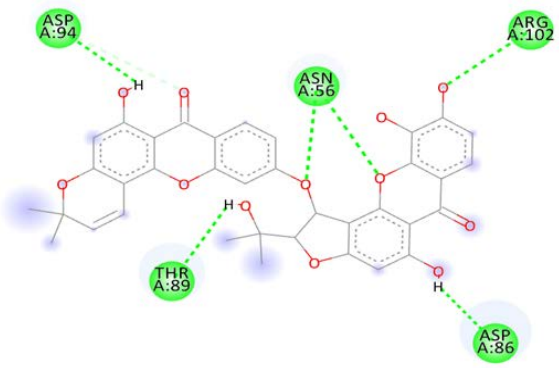
Screening of phytochemicals based upon ADMET analysis

In this study, the phytochemicals library comprised 90 compounds from *Silybum marianum*. Initially, 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 = non-toxic. 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 A, 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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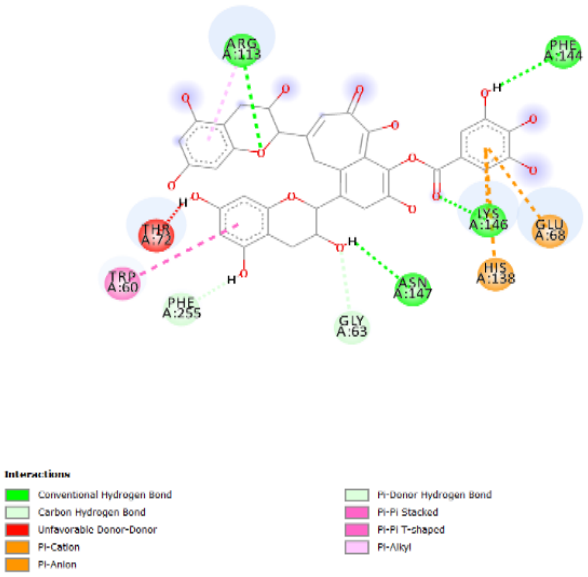

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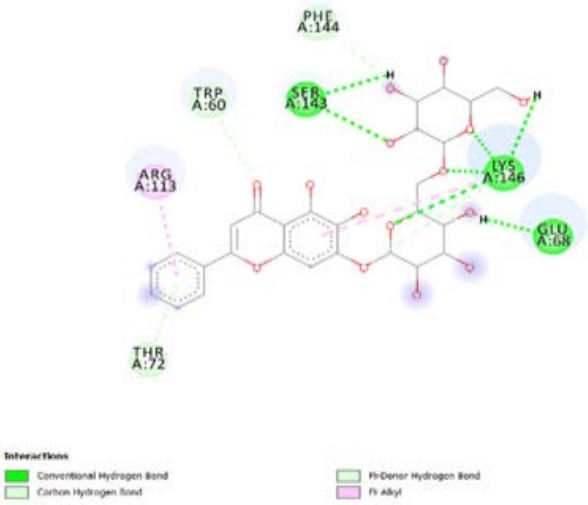

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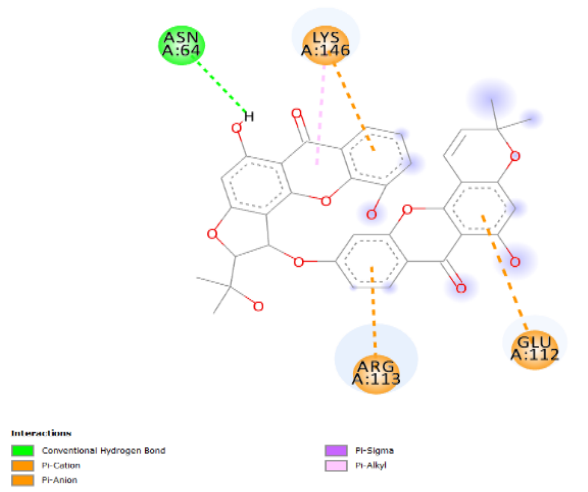

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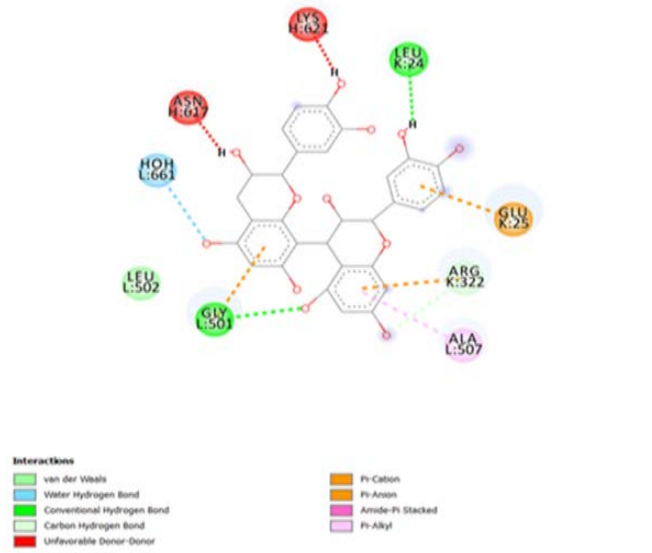


(e)

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 µM, formed a different number of conventional hydrogen bonds with ARG_{102}, ASP_{56}, ASP_{86}, THR_{89}, and ASP_{94} (Fig. 1(A)). Theaflavinegallate showed the binding affinity of -8.8 kcal/mol with the Ki value of 0.349 µM and formed conventional hydrogen bonds with PHE_{14}, LYS_{146} and ASP_{147}. Furthermore, it formed Pi-alkyl bond with ARG_{113}, unfavourable donor-donor interactions with THR_{72}, carbon-hydrogen bonds with GLY_{63} PHE_{255}, GLU_{68}, Pi-cation interactions with HIS_{138}, and Pi-stack interactions with TRP_{60} (Fig. 1(B)).

OroxinB with Ki value of 0.489 µM and binding affinity -8.6kcal/mol, formed conventional hydrogen bonds with SER_{143}, GLU_{68} and LYS_{146}. Furthermore, carbon-hydrogen bond and Pi-donor hydrogen bond with TRP_{60}, THR_{72}, PHE_{144}, and Pi alkyl interaction with ARG_{113} and LYS_{146} were also observed (Fig. 1(C)). JacarelhyperolA with Ki value of 0.489 µM and binding affinity of -8.6kcal/mol, formed a conventional hydrogen bond with ASP_{64}, and Pi-cations and anions with GLU_{112}, ARG_{113} and LYS_{146}. LYS_{146} also made Pi-alkyl interactions with ligand and formation of Pi-sigma was also observed (Fig. 1(D)).

Compactness of complexes through MD simulation

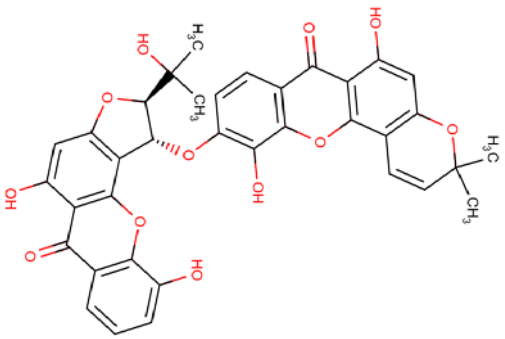
Compactness and stability of phytochemical-ligand complexes were analyzed by monitoring the radius of gyration (Rg) and RMSD scores were computed to observe changes between the initial state of complex and after MD simulation of the complex through MD simulation. Each complex was subjected to 20ns MD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

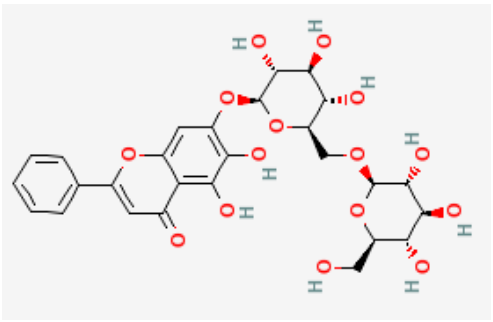
even better than the Rg of control i.e. < 2.94 Å. RMSD values reported in Table 1, depicted high stability as these values ranged between 0.55Å- 2.87 Å.

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

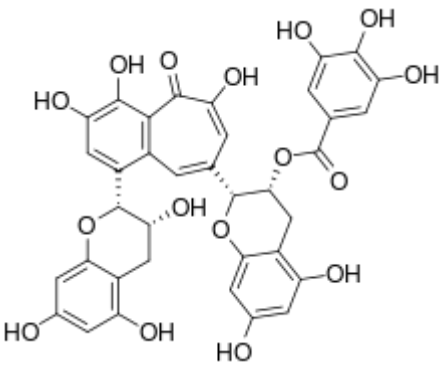
COMPLEX OF HEMAGGLUTININ AND PHYTOCHEMICALS	AVERAGE RMSD AT VARIOUS TEMPERATURES (Å)			
	300K	325K	340K	350K
JacarelhyperolB	0.55	0.77	1.25	1.36
Theaflavinegallate	0.59	1.04	1.29	1.61
OroxinB	1.20	1.70	1.89	1.96
JacarelhyperolA	1.65	1.88	2.62	2.87
ProanthocyanidinB2-di-gallate (Control)	1.70	1.93	2.72	2.94



Jacarelhyperol B



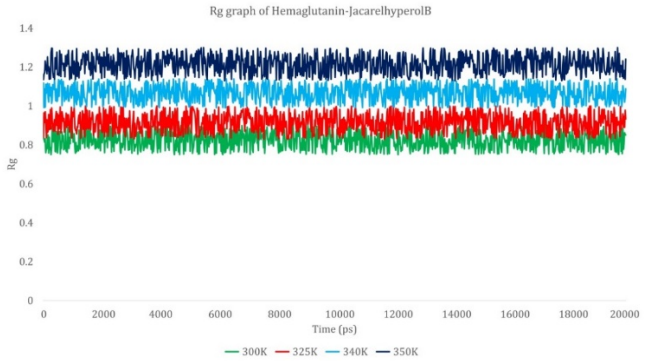
Oroxin B



Theaflavin-3-gallate

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.



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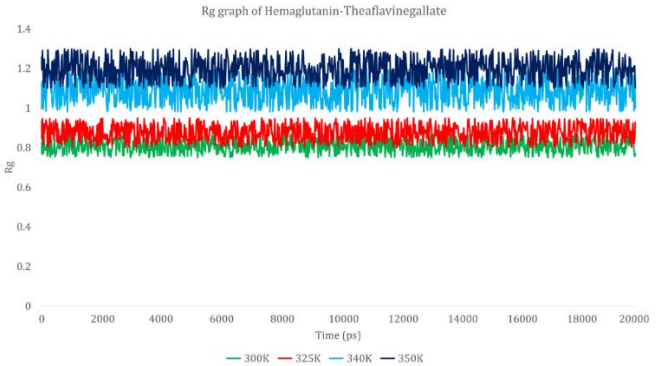

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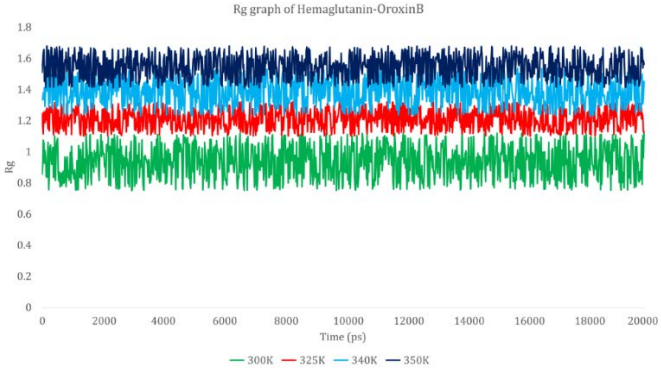

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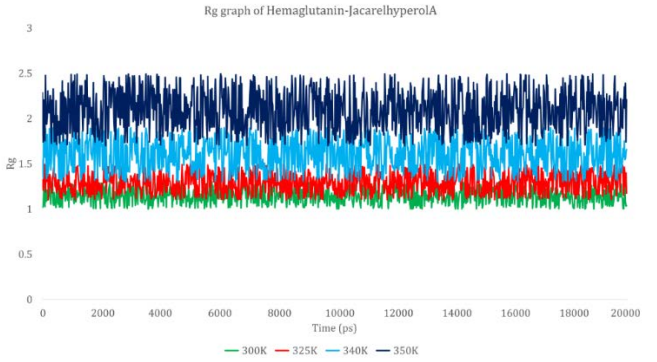

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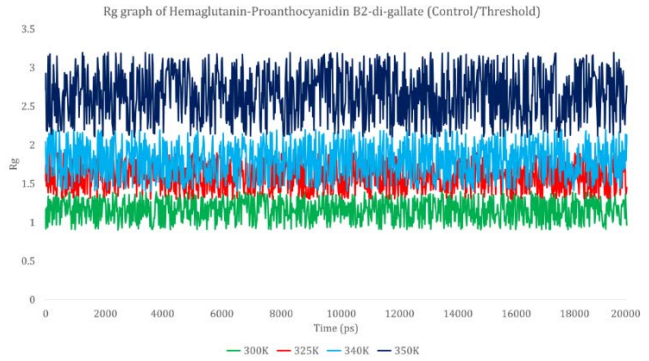

(e)

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/MOL)	E_{LUMO} (KCAL/MOL)	E_{HOMO} (KCAL/MOL)	BAND ENERGY GAP (ΔE) (KCAL/MOL)
JacarelhyperolB	-8.9	-0.274	-0.389	0.114
Theaflavinegallate	-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
ProanthocyanidinB2-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 anti-bacterial 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 receptor-based 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.

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