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Research Article

IN SILICO PREDICTION OF POTENTIAL INHIBITORS FOR THE M2 PROTEIN OF INFLUENZA A VIRUS USING MOLECULAR DOCKING STUDIES

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ABSTRACT

Objective: In this study, the M2 protein of influenza A virus was selected as a target for various phytochemical compounds and an attempt was made to determine their inhibitory activity against the target protein using computational biology. Thus, seeking novel therapeutic strategies against the influenza A virus.

Methods: With the aid of the computational approach in biology, using *in-silico* techniques, the evaluation of drug-likeness, molecular properties, and bioactivity of the identified eight phytocompounds (Pseudo beta colubrine, Withaferin, Shinjulactone D, 5-Dehydrouzarigenin, Cinchonidine, Corylidin, Amarolide, and Deoxyartemisinin) was carried out using Swiss absorption, distribution, metabolism, and excretion, while Protox-II server was used to identify its toxicity. The *in silico* molecular docking of the phytochemical ligands with the M2 protein motif was carried out using AutoDock (Vina), which evaluated the binding affinity for further selection of the most compatible and pharmacologically significant ligand. All the potent ligands could be considered as lead molecules based on their pharmacokinetic and drug likeness properties.

Results: Results suggested that Shinjulactone D, Cinchonidine, and Deoxyartemisinin ligands with the best binding pose could be selected as promising candidate, showing high potency for drug development.

Conclusion: This study concludes the relevance of selected phytochemical compounds as prospective leads for the treatment of influenza A virus.

Keywords: Molecular docking, M2 ion channel protein, Phytochemicals, Toxicity testing, Bioavailability, Binding energy.

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INTRODUCTION

Influenza infection is considered as a great concern for public health all over the world due to its high number of mortality and morbidity that is caused through epidemics and pandemics [1]. The WHO statistics reveal that the havoc influenza virus has caused to the humankind for ages, it is estimated that in total 1 billion influenza cases are reported around the world each year, including 3–5 million severe cases and 290,000–650,000 fatalities [2].

Influenza viruses belong to the family Orthomyxoviridae, an RNA type virus with a variety of antigenic patterns [3]. These viruses are recognized by possessing segmented and negative-strand RNA genome that displays its dependency on RNA-dependent-RNA polymerase of viral origin for replication purpose [4]. According to the data, the whole family consists of four genera, which include influenza A (genus *Alphainfluenzavirus*) and influenza B (genus *Betainfluenzavirus*) viruses are two types of influenza viruses' influenza C (genus *Gammainfluenzavirus*) and influenza D (genus *Deltainfluenzavirus*). Findings suggest that among the four genera, IAV and IBV genera predominantly circulate in human population resulting in seasonal epidemics with different degree of severity [5].

IAV is a highly contagious respiratory pathogen that is responsible for the most serious medical illness and is considered as the most prevalent cause of seasonal epidemics and pandemics in humans [6]. The virus possesses a broad range of morphological traits, comprising spherical, and filamentous morphologies. These morphologies encase its segmented RNA genome, which encrypts about 10 various sorts of viral proteins. Surface proteins, notably constitute hemagglutinin (HA), neuraminidase (NA), and membrane ion channel (M2) proteins, make up the structural proteins in the fully developed virus particle as well as internal proteins that include viral polymerase that is essential for replication, nucleoproteins in the form ribonucleoparticles (RNP) and the matrix protein (M1), which is regarded as the most abound protein, forming a layer liked to the interior of the viral envelop [7,8]. Influenza A viruses are divided into subcategories based on the antigenic characteristics of their envelope proteins, resulting into 16 distinct HA and nine different NA subtypes found thus far. This might be due to their swift evolution, which causes a lot of variation. This is recognized as a distinguishing feature, which is predominantly found in influenza A viruses [9].

Among the various viral proteins, the M2 ion channel protein holds a significant function in various phases of influenza virus infection. Ion channel (M2) protein is a 97-residue single-pass integral membrane protein that is displayed at the surface of infected cells with three segments: An extracellular N-terminus with residues 1–23, a transmembrane segment with residues 24–46, and an intracellular C-terminus with residues 47–97 oriented toward the virus's periphery [10,11]. It is a pH-regulated homotetrameric proton channel that plays a crucial role in the viral life cycle [12]. The viral lipid membrane has spikes of HA that plays a major role as it binds to the sialic acid found on the surface of host's cell membrane. The viral entry into the host cell is facilitated by receptor-mediated endocytosis as an endosome that takes place when the virus attaches itself to the sialic acid residue of the host cell membrane. As the endosome has low internal environmental pH, which promotes the viral and endosomal membrane to fuse together. Subsequently, this acidic microenvironment not only assist the membrane fusion but also promotes the opening up of the M2 ion channel that act as a proton selective-ion channel. Consequently, opening of the M2 channel protein

acidifies the viral core, which results in the release of the vRNP from M1 protein such that the vRNP freely enters the host cell's cytoplasmic matrix such that the viral genetic material can replicate [13,14].

As the molecular events of viral life cycle are understood up to a good extent and considering the fact that the M2 ion channel protein plays an important role in replication of virus, various antiviral drugs such as amantadine and its methyl derivative rimantadine, a cyclic amine were adopted to suppress the activity of influenza A M2 ion channel within the tetrameric M2 helix bundle and influenza virus replication [15]. However, the long-term use of this drug has become limited due to the spontaneous mutations of viral protein and drug resistance, the easy transmission of drug-resistant viruses, and, in particular, the prediction of central nervous system side effects [16]. This makes it really important to construct a novel and potent chemotherapeutic agents that binds to the M2 transmembrane proton channel to prevents H^+ proton influx, and thus, targeting M2 protein that makes it a feasible approach to building an efficient antiviral drug with natural origin considering their minimal side effects on the subject [14].

The pursuit for a lead compound involved in drug discovery and development is a long and exhausting endeavor, and one is frequently discouraged by the seemingly limitless options. Therefore, the integration of computational and experimental methodologies has simplified the path toward drug discovery. To discover and identify new potential molecules, various *in-silico* approaches have been adopted for instance, ligand-based drug design (pharmacophore), structure-based drug design (drug-target docking), and shape-based screening. Molecular docking is becoming a more efficient *in-silico* methodology in drug development process [17,18]. Docking facilitates the identification of new potential pharmaceutical agents by predicting ligand-target interactions at the molecular level with a reasonable level of certainty [19].

The present research focuses on the use of phytochemicals as a feasible alternative to synthetic medications in the long struggle against IAV-caused influenza. As a consequence, ligands as phytochemicals were used in molecular docking studies against the M2 ion channel protein.

METHODS

Protein retrieval

The M2 protein of Influenza virus was selected for analysis, this protein acts as an ion channel and plays a major role in facilitating viral uncoating. The 3-D structure of the Integrase Protein was obtained from the RCSB PDB data repository in PDB format. The PDB id of the selected structure is 2N70 (https://www.rcsb.org/structure/2N70) [20].

Ligand retrieval

A total of 1600 compounds were selected as ligands, these compounds were derived from Indian Medicinal Plants, Phytochemistry, and Therapeutics (IMPPAT) database [21]. SDF files of these compounds were downloaded and further used for analysis.

Absorption, distribution, metabolism, and excretion (ADME) analysis

Swiss ADME (http://www.swissadme.ch/) a free web-based tool used to evaluate pharmacokinetics, drug likeness, and chemical compatibility of molecules [22]. This tool is used for drug screening based on the Lipinski rule of 5. This analysis helps to prediction the *in vivo* behavior of a ligand thus representing its potential to be a viable drug candidate. To qualify as a ligand, a compound should have a molecular mass of <500 Daltons, an octanol-water partition coefficient (log P) that does not exceed 5, <10 H-bond donors, and no more than 5 hydrogen bond donors (the total number of Nitrogen-Hydrogen and Oxygen-Hydrogen bonds) [23].

Bioavailability radar

The bioavailability radar enables a first glance at the drug-likeness of a molecule and helps to predict the oral bioavailability of the compound. The bioavailability radar of the compounds satisfying the Lipinski's rules was obtained by Swiss-ADME which focuses on the physicochemical indices suitable for oral consumption such as LIPO, Lipophilicity: −0.7 < XLOGP3 < +5; SIZE, Molecular size: 150 g/mol < mol. wt. < 500 g/mol; POLAR, Polarity: 20 Å2 < TPSA <130 Å2; INSOLU, Insolubility: 0 < Log S (ESOL) < 6; INSATU, Instauration: 0.25 < Fraction Csp3 < 1; FLEX, and Flexibility: 0< Number of rotatable bonds <9. The colored zone within the radar is the physicochemical space which indicates oral bioavailability. Any deviation from these parameters on a large scale suggests that the ligand cannot be orally consumed [22].

Protein and ligand preparation

Protein

The 3D structure of M2 protein was prepared for molecular docking analysis using UCSF Chimera 1.15 tool [24] using which the water molecules were removed, Kollman charges and polar hydrogen atoms were added to the protein molecule, and the charged protein molecule was saved in PDB format.

Ligand

Bioactive compounds which satisfied the Lipinski's rule of five were chosen as ligands, and their structures were obtained from PubChem databank in SDF format. PyRx Virtual Screening Tool was used to generate structural variations, to optimize and minimize energy of the ligands [25].

Molecular docking

Molecular docking is a major tool which is used to predict the predominant binding modes of a selected ligand with a protein of known three-dimensional structure [26]. Molecular docking was carried out for the molecules satisfying Lipinski's rule of five. The selected ligand structures were docked with the M2 protein of influenza virus using Auto Dock Vina. The Auto Dock Vina software carries out the prediction of bound confirmation based on free binding energies, which was calculated on the basis of the empirical force field. The docking analysis was performed using the Auto Dock Vina through docking protocol PyRx Virtual Screening Tool [25]. This evaluation helped in narrowing down the potential ligands exhibiting high binding affinity with the protein as probable inhibitors.

Analyzing and output visualization

Biovia Discovery Studio Visualizer was used to analyze the docking pose having the lowest free binding energy to the corresponding protein [27]. The ligands showing an ideal binding energy were selected and analyzed by the two- and three-dimensional protein-ligand complexes, and based on their intermolecular interactions, such as hydrogen bonding, hydrophobic interactions, Van-der Waal forces, alkyl bonds, pi-alkyl bonds, sigma bonds, pi-sigma bonds, pi-cation bonds, pi-anion bonds, and pi-pi T-shaped bonds the best drug candidates were selected.

Toxicity prediction

Selected drugs showing zero violations of the Lipinski's rule were subjected to Toxicity prediction which was performed to evaluate the safety of drugs for human consumption, absorption, distribution, metabolism, excretion, and toxicological characteristics of the compounds employed in this study were calculated (ADMET). ProTox-II, a virtual laboratory for predicting small molecule's toxicity, and ADMET 2.0 were used for the analysis. The drugs were uploaded to the server which gave results representing the toxicity of selected compound. ProTox II tool [28] was used to calculate the toxicity profiles, toxicity class, and LD50 values of the shortlisted phytochemicals. Based on the LD50 value of 3700 dataset compounds, it determines the toxicity of the compound and categorizes the query drug into six broad groups, with Class I being extremely toxic and Class VI being safest. This server also specifies the prescribed mg/kg value of the medication for consumption. ADMET 2.0 [29], an integrated online platform for accurate and comprehensive predictions of ADMET properties, was also used to determine certain toxicity parameters which are required for a

molecule to qualify as an ideal drug. The parameters evaluated through ADMET include Herg Blockers, H-ht, DiliAmes toxicity, Eye irritation, Rat oral acute toxicity, Fdamdd, Skin sensitization, Carcinogenicity, Eye corrosion, Respiratory toxicity, and Environmental toxicity.

RESULTS AND DISCUSSION

Influenza A viruses of *Orthomyxoviridae* family are enveloped, segmented negative single-stranded RNA viruses, and capable of causing severe human respiratory infections [30]. It is a causative agent involved in the outbreak of worldwide epidemics, causing millions of fatalities around the world by respiratory diseases and seasonal illness [31]. Globally, the annual epidemics have accounted for about 3–5 million cases of severe illness and 250,000–500,000 deaths worldwide [32]. Influenza viruses have the ability to undergo rapid and consistent genetic and antigenic evolution due to point mutations in the genome and reassortment of gene segments from intra-species and inter-species influenza viruses (antigenic shift) [33]. Influenza A virus, matrix protein 2 (M2), an ion channel, is crucial for virus infection, and therefore, an important anti-influenza drug target. The M2 protein of influenza virus A is an integral membrane protein expressed on the infected cell surface and incorporated into virions. The M2 protein forms a homotetramer, has H+ ion channel activity that is sensitive to anti-influenza virus drugs and is activated by low pH. When the virus enters cells, the M2 ion channel is activated in endosomes to acidify inside the virion, facilitating viral uncoating [34]. Due to the prevalent resistance to inhibitors that target the influenza A M2 proton channel, it is necessary to develop and continue drug design effort, supported by a study of the mechanism of inhibition and of channel function.

In this study, we aimed to identify new inhibitors of M2 ion protein channel using computational docking approaches. A total of 1600 ligands were selected from the IMPPAT database [21]. The compounds chosen belonged to quinones, carbohydrates, flavonoids, organic compounds, alkaloids, carboxylic acids, steroids, polyphenols, terpenoids, benzene and derivatives, and lipids and fatty acids. These phytochemicals were screened on the basis of Lipinski's rule of five Further, the compounds showing zero violations were docked with the target protein and their binding energies were recorded. The ligands exhibiting least binding energy were assessed for their toxicity levels using ProTox-II and ADMET tools. Binding energies upto −8.2 kcal were recorded and the ligands above −7.9 kcal/mol were shortlisted. The molecular interactions of the complexes of these shortlisted ligands with the target protein were studied using two-dimensional and three-dimensional analysis based on which the number of Hydrogen bonds in each interaction were determined. Depending on this, top 8 compounds were screened on the basis bioavailability, toxicity class, and LD50 value. Further, three highly potential candidates were considered who exhibited better binding affinity in comparison to the standard drugs used in the treatment of influenza A virus infections.

Evaluation of pharmacokinetic and pharmacological properties

The absorption of any drug into the system and its distribution, metabolism, and excretion is based on characteristics such as lipophilicity, molecular weight, and hydrogen donor-acceptor bonds, that not only predicts the absorption of the drug, but also determines the penetration of drug. These characteristics are called Lipinski's rule of 5, and they indicate the compound's drug likeness. When these rules are violated, the interaction between the drug and the membrane is affected. Therefore, the characteristics of the molecule must follow the rule of 5 for increased selectivity and drug-like physicochemical features. Lipinski's oral drug likeness properties were predicted using the swiss ADME web server [35]. This includes (i) Molecular weight (<500 Daltons), (ii) Number of hydrogen bond donors (<5), (iii) Number of hydrogen bond acceptors (<10), and (iv) Log p(<5). In this study, for 1600 bioactive compounds screened using Lipinski's rule of 5 to determine the drug likeness. About 18% of the compounds were organic compounds, 15% of the compounds were terpenoids, 14.25% were flavonoids, and 12.56% of the chemicals were classed as unclassifiable (Others). Alkaloids, polyphenols, carboxylic acids, steroids, quinones, carbohydrates, benzene and derivatives, and lipids and fatty acids made up the remaining components, accounting for 8, 3.31, 4.69, 5.88, 0.31, 5.38, 6.94, and 5.69% of the total. Lipinski's rule of 5 was followed by 44.38% of the compounds with zero violation, whereas 30.06, 19.69, and 5.88% disregarded one, two, and three rules, respectively. Only eight compounds were explored further after compounds that violated one or more of Lipinski's guidelines were removed from the investigation.

Bioavailability radar and toxicity prediction

It is well documented that an inhibitor's antagonistic reaction to an enzyme or a protein receptor does not guarantee its usefulness as a potential medication. Therefore, eight compounds were chosen for further investigation after the ADME analysis, including drug-likeness analysis that is important in the drug discovery which helps to make a rational decision on whether inhibitors can be administered to a biological system or not [36]. Furthermore, inhibitors with poor ADME capabilities and severe toxicity effects on biological systems are often the reason for failure of medicines during clinical trials. The drug-likeness of the molecule was determined using an expository technique called bioavailability radar. Bioavailability radar is based on six physicochemical properties: Size, solubility, lipophilicity, flexibility, polarity, and saturation, to determine drug-likeness. The drug-likeness parameters are related to aqueous solubility and intestinal permeability which determines the first step of oral bioavailability [27]. Oral ingestion is a handy and commonly used form of medication delivery in patients due to its ease of administration, cost-effectiveness, sterility constraints, and dosage design flexibility. As a result, bioequivalent oral medication formulations are more likely to be produced by many drug makers. The bioavailability radar plots of the tested phytochemicals indicated that the phytochemicals were fairly inside the pink area, indicating their drug-likelihood with a better bioavailability profile [37]. Computational methodologies and procedures, which have proven to be more favorable than *in vitro* and *in vivo* research, can be used to avert huge financial losses later in the drug development process. The phytochemicals' *in-silico* toxicity properties were also assessed using ProTox-II, a virtual laboratory for predicting small molecule's toxicity, and ADMET 2.0, in which, toxicity class (Oral toxicity), predicted LD50, hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity were the parameters used to evaluate the substances. The absorption, distribution, metabolism, excretion, and toxicity of a substance in and through the human body are all dealt with by its ADMET characteristics. The pharmacokinetic profile of a therapeutic molecule, which is represented by ADMET, is critical in determining its pharmacodynamic effects. All of the phytochemicals tested had a predicted LD50 (mg/kg) ranging from 10 to 8000, putting them in ProTox-toxicity II's class-2 to class-6. Based on the bioavailability radars (Fig. 1) of the best ligands and the toxicity profile (Table 1), it can be concluded that Shinjulactone D, Cinchonidine, and Deoxyartemisinin are prospective therapeutic candidate that can be utilized to treat influenza A infection.

Molecular docking

Molecular docking is a simulation technique that examines the optimum binding pose for a ligand with a target's active site. This technique involves the selection of 3D-coordinate space of the binding site in the target and calculating the binding affinity of the resultant orientation of the molecule within the binding site which forms the complex [38]. The largest magnitude negative number (highest binding affinity or lowest binding energy) depicts the most favorable conformation of the complex formed when the ligand involved, efficiently binds with the active pockets of the target, and thus determines the significance and sensitivity of binding affinity values. Molecular docking with the influenza A, M2 proton channel was done using bioavailable ligands. The binding energies of the top 8 ligands are shown in Table 2, indicating that they have a high affinity for the target protein, the M2 proton channel. Table 3 displays the binding energy values of medicines used to treat influenza A. Phytochemicals have higher binding energies than medications used to treat influenza A, as shown in Fig. 2. The binding

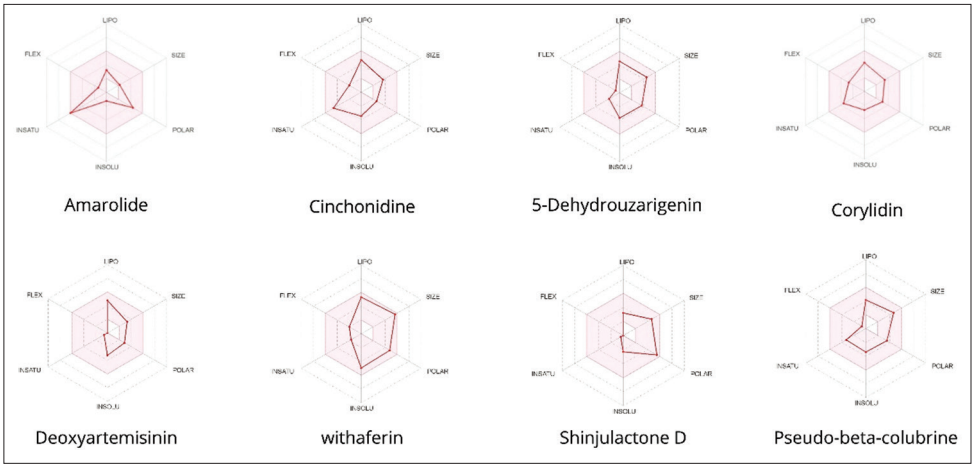

Fig. 1: Bioavailability Radar diagram of the top 8 ligands

Table 1: Toxicity prediction of the top 8 ligands

Ligands	Class	LD 50 (mg/kg)	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
Pseudo beta colubrine	3	150	Inactive	Inactive	Inactive	Inactive	Active
Withaferin	3	300	Inactive	Inactive	Active	Inactive	Active
Shinjulactone D	6	15900	Inactive	Inactive	Active	Inactive	Active
5-Dehydrouzarigenin	2	34	Inactive	Inactive	Active	Inactive	Inactive
Cinchonidine	4	720	Inactive	Inactive	Inactive	Inactive	Inactive
Corylidin	5	2430	Inactive	Active	Active	Active	Inactive
Amarolide	5	3900	Inactive	Inactive	Active	Inactive	Inactive
Deoxyartemisinin	6	8000	Inactive	Inactive	Inactive	Inactive	Inactive

Table 2: Binding energy of Influenza A M2 proton channel with top 8 phytochemicals as ligands

S. No	Ligand	Binding Energy n(ΔG) (kcal/mol)
1	Pseudo beta colubrine	−8.2
2	Withaferin	−8.2
3	Shinjulactone D	−8.1
4	5-Dehydrouzarigenin	−8.1
5	Cinchonidine	−8.1
6	Corylidin	−8.1
7	Amarolide	−8
8	Deoxyartemisinin	−8

Table 3: Binding energy of influenza A M2 proton channel with drugs currently used for treatment

S. No	Ligand	Binding energy n(ΔG) (kcal/mol)
1	Peramivir	−5.9
2	Zanamivir	−5.8
3	Oseltamivir	−5.8
4	Baloxavir	−7.7
5	Ribavirin	−5.5

affinity of complexes was found to be between −8 and −8.2 kcal/mol, indicating their high potency, and in comparison, to the other ligands, Shinjulactone D, Cinchonidine, and Deoxyartemisinin appear to be of superior choice. Lower-binding-energy phytochemicals may include more hydroxyl groups, which form hydrogen bonds with the target protein, indicating a favorable interaction. Alkyl and pi-alkyl linkages also help ligands interact hydrophobically in the receptor's binding pocket and the pi-sigma bond adds stabilizing charges to the medication, allowing it to intercalate into the binding sites of the receptor.

The binding energy of pseudo beta colubrine is −8.2 kcal/mol, and it has two types of interactions. Pi-alkyl bonds were detected in ARG 445 and LEU 446 of chain D, as well as alkyl bonds in ILE 442 and ARG 445 of the same chain. Alkyl bond interaction was also seen in LEU 340, ILE 351, and ILE 351 of chain C. Pseudo beta colubrine did not form hydrogen bonds with the target protein (Fig. 3a and b). According to a study carried out by Vivek-Ananth, *et al*., pseudo alpha colubrine, a monoterpenoid indole alkaloid, exhibited a binding energy of −9.3 kcal/mol with human protease TMPRSS2 protein of SARS CoV-2 virus [39]. Since pseudo alpha and beta colubrine belong to the same class of phytochemicals, it can be concluded that they can be employed as potent antiviral drugs for inhibiting viral proteins. Withaferin also showed a binding energy of −8.2 kcal/mol. It shows a variety of interactions, including the traditional hydrogen bond, the pi-alkyl link, and the alkyl bond. The A chain's LEU 136 established a traditional hydrogen bond. PHE 154 is a chain residue that forms a pi-alkyl bond. LEU 140, ILE 151, ILE139, LEU 140, and LEU 143 are chain residues that form alkyl bonds with the ligand. Between the receptor and Withaferin, one hydrogen bond was formed (Fig. 4a and b**)**. In a study conducted by Jha *et al*., Withaferin showed least binding energy of −9.78 kcal/mol (Ki value = 0.06733 µM) with the target protein that is CHIKV envelope glycoprotein of Chikungunya virus (CHIKV). It also exhibited three different types of interaction with the target protein, comprising of conventional hydrogen bond, pi-alkyl bond, carbon-hydrogen bond, and alkyl bond [40]. Hence, Withaferin can prove to be a good candidate for an antiviral drug against viral infections. In a molecular docking studies conducted by Hasan *et al*., a class of quassinoids including Javanicin derivatives (Javanicin G, I, and K) was found to exhibit binding energies of −8.5 kcal/mol, −8.0 kcal/mol, and −8.3 kcal/mol, respectively, against C3 like protease of SARS CoV virus [41]. Shinjulactone D also belongs to a class of quassinoid and showed a binding energy of 8.1 kcal/mol. Further, it exhibits three types of interactions that is three typical hydrogen bonds are established by ARG 245, ARG 345, and ARG 445, respectively, by the C, B, and A chains. TRP 141, PHE 348 of chains A, and C create pi-alkyl bonds, which are followed by alkyl bonds generated by ARG 345, ARG 145 of chains C, and A. With the target protein, Shinjulactone D forms three hydrogen bonds (Fig. 5a and b).

Amarolide has a binding energy of −8 kcal/mol and two types of interactions. PHE 148 of chain A showed pi-alkyl bonds, while ARG 145 and TRP 241, ARG 245, and ARG 245 of chain A and B, respectively, showed typical hydrogen bonds. The target protein created four hydrogen bonds with amarolide (Fig. 6a and b) Amarolide belongs to the class of macrolides which are employed as antibiotics and hence, it was not previously explored as an antiviral drug. Similarly, there are no studies performed for evaluating the antiviral potential of 5-Dehydrouzarigenin. Therefore, we have evaluated the ability of amarolide and 5-dehydrouzarigenin to inhibit M2 protein of influenza A virus. 5-dehydrouzarigenin has a binding energy of –8.1 kcal/mol. It has two different sorts of interactions: Pi-alkyl bond and alkyl bond. PHE 154, a chain residue, creates a pi-alkyl bond. A and B chain residue LEU 140, LEU 143, ILE1 51, ILE 151, ILE 151, and ILE 242, ILE 242, ILE 151, respectively, interacts with ligand by forming alkyl bonds (Fig. 7a and b). The binding energy of Cinchonidine is –8.1 kcal/mol. There are two types of interactions shown: Alkyl bond and pi-alkyl bond. PHE 448 and ILE 351 of chains D and C are involved in the creation of pi-alkyl bonds. Alkyl bonds are formed by LEU 340, ILE 351, ILE 351, and ARG 445, LEU 446, ILE 442, and LEU 446 of the C and D chains, respectively (Fig. 8a and b) In a study carried out by Rosmalena *et al*., inhibitory activity of cinchonidine salicylate, Cinchonidine, cinchonidine octanoate, and Cinchonidine succinate was evaluated against Pf falcipain-2 protein of *Plasmodium falciparum*. Based on the molecular docking results, it was observed that cinchonidine salicylate, Cinchonidine, Cinchonidine octanoate, and Cinchonidine succinate showed binding energies of −9.1 kcal/mol, −6.8 kcal/mol, −7.6 kcal/mol, and −8.2 kcal/mol, respectively, with the target protein [42]. This highlights the Cinchonidine's ability to be a potent inhibitor of certain parasitic and viral proteins.

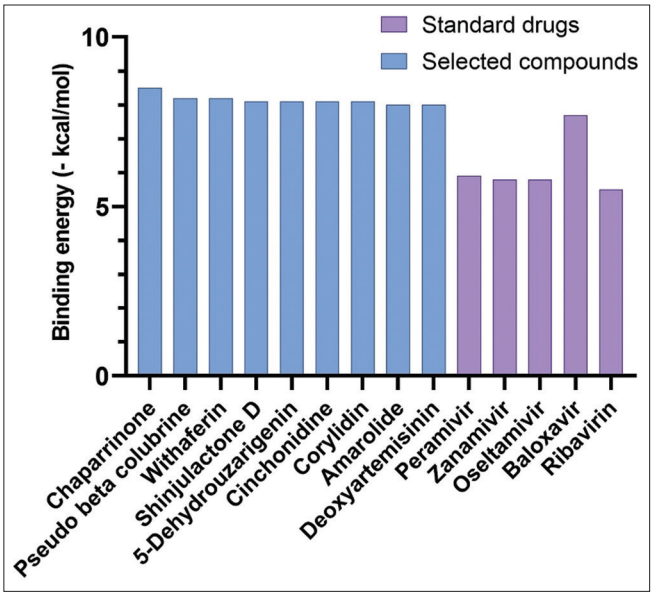

Fig. 2: Bar graph representing comparative binding energy of the best ligands and the drugs currently employed for the treatment of influenza

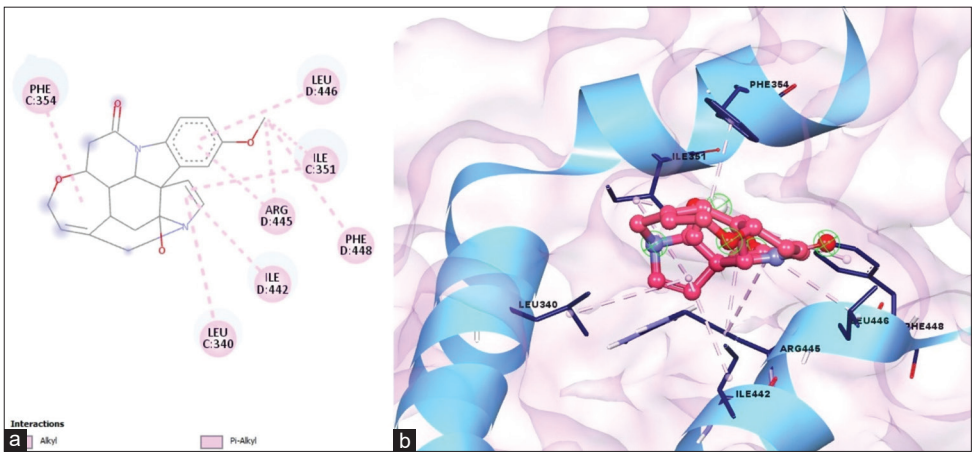

Fig. 3: (a and b) 2D interaction plot of Pseudo beta colubrine docked in the binding pockets of M2 protein. 3D representation showing the position of Pseudo beta colubrine within the binding site of M2 protein

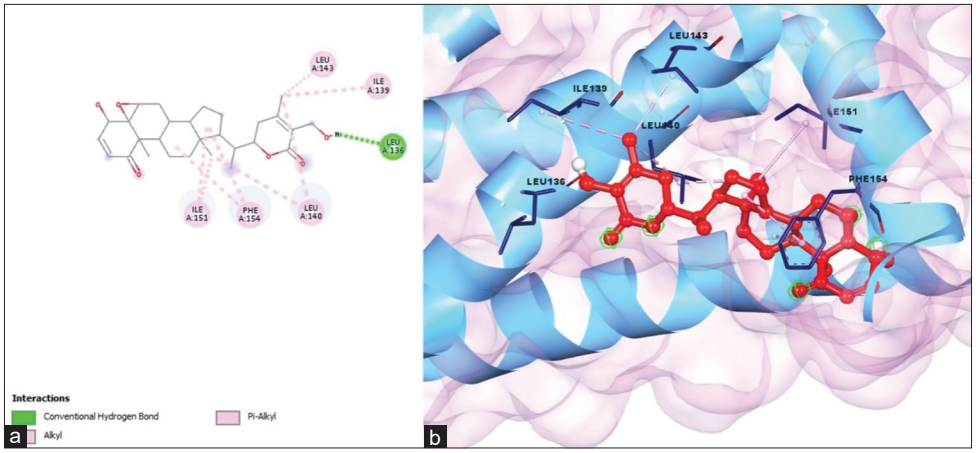

Fig. 4: (a and b) 2D interaction plot of Withaferin docked in the binding pockets of M2 protein. 3D representation showing the position of Withaferin within the binding site of M2 protein

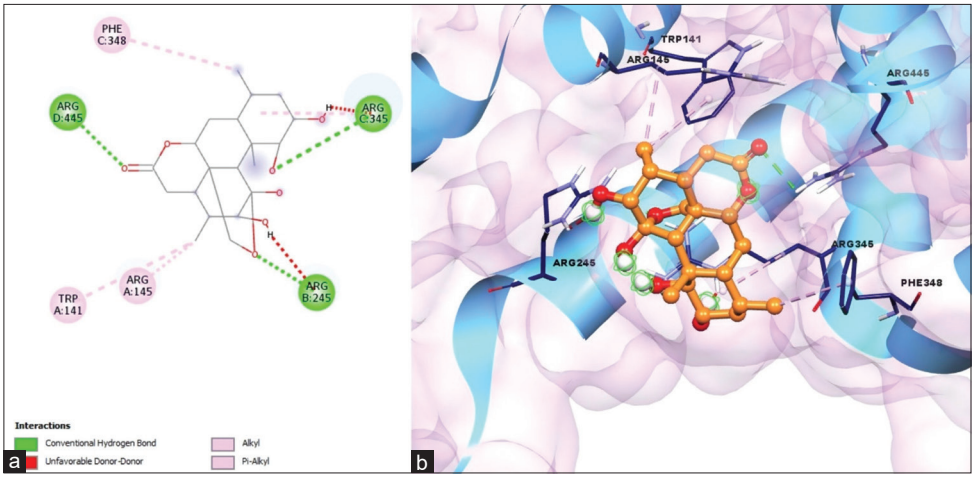

Fig. 5: (a and b) 2D interaction plot of Shinjulactone D docked in the binding pockets of M2 protein. 3D representation showing the position of Shinjulactone D within the binding site of M2 protein

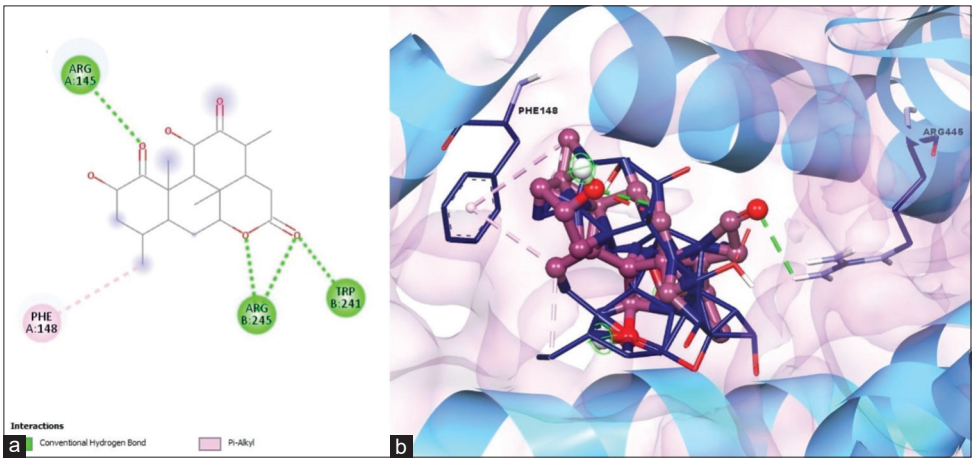

Fig. 6: (a and b) 2D interaction plot of Amarolide docked in the binding pockets of M2 protein. 3D representation showing the position of Amarolide within the binding site of M2 protein

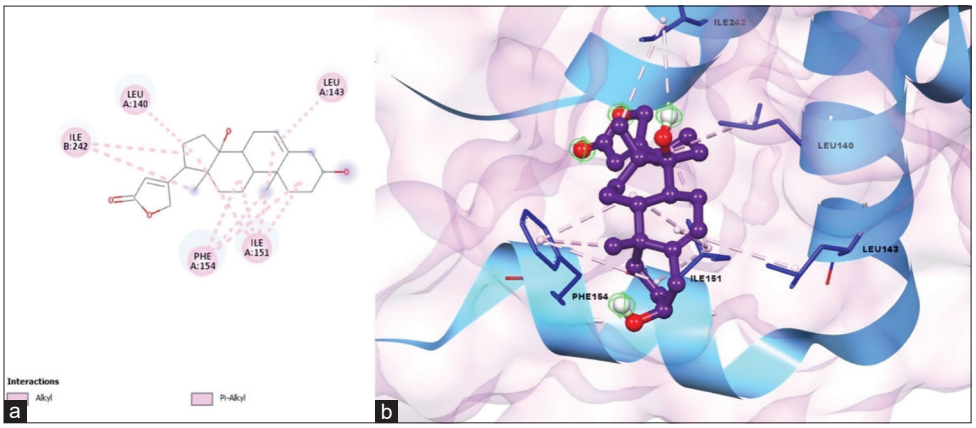

Fig. 7: (a and b) 2D interaction plot of 5-Dehydrouzarigenin docked in the binding pockets of M2 protein. 3D representation showing the position of 5-Dehydrouzarigenin within the binding site of M2 protein

Corylidin is a flavonoid extracted from *Psoralea corylifolia L.* Apart from corylidin, Bavachinin, neobavaisoflavone, isobavachalcone, 40 –O-methylbavachalcone, psoralidin, and corylifol A are the other extracts obtained from *P. corylifolia*. A previous molecular docking study was conducted by Dermawan, *et al*. to screen certain phytochemicals as inhibitors of SARS-CoV-2 main protease. It was seen that Bavachinin, Neobavaisoflavone, Psoralidin, and Corylifol A exhibited binding energies of −7.14 kcal/mol, −7.27 kcal/mol, −7.84 kcal/mol, and −7.79 kcal/mol, respectively, with the target protein. In case of Corylidina, binding energy of −7.6 kcal/mol was seen [43]. The hydrogen bond, the pi-sigma bond, the pi-alkyl bond, the pi-cation bond, and the pi-anion bond are the five types of bonds it has. The hydrogen bond was created by ARG 245 and ARG 245 of chain B. The ligand established pi-cation interactions with ARG 345 and ARG 345 in the C chain. Pi-alkyl bond

produced by ARG 345 of the C chain. Chain B's GLU A-85 makes a pi-anion connection with the target, while chain C's ARG 345 forms a pi-sigma (Fig. 9a and b**).** Deoxyartemisinin has a binding energy of −8 kcal/mol and two types of interactions. PHE 354, PHE 355, and PHE 448 of chains C and D, respectively, were shown to have pi-alkyl linkages. Alkyl bond interaction was also seen in chain D and C's LEU 446, ILE 442, LEU 446, LEU 446, ARG 445, and ILE 351, ILE3 51. Deoxyartemisinin did not create hydrogen bonds with the target protein (Fig. 10a and b). In a study conducted by Fuzimoto AD, antiviral properties of artemisinin were evaluated with respect to its ability to inhibit SARS-CoV-2/host proteins 3CLPRO. Artemisinin exhibited a binding energy of −8.06 kcal/mol in the study which is same as the results obtained after molecular docking of Deoxyartemisinin with the target protein that is M2 ion channel protein of influenza A virus [44]. As we have obtained similar results in case of deoxyartemisinin, it can be considered as a good candidate for antiviral drug for inhibition of influenza A virus.

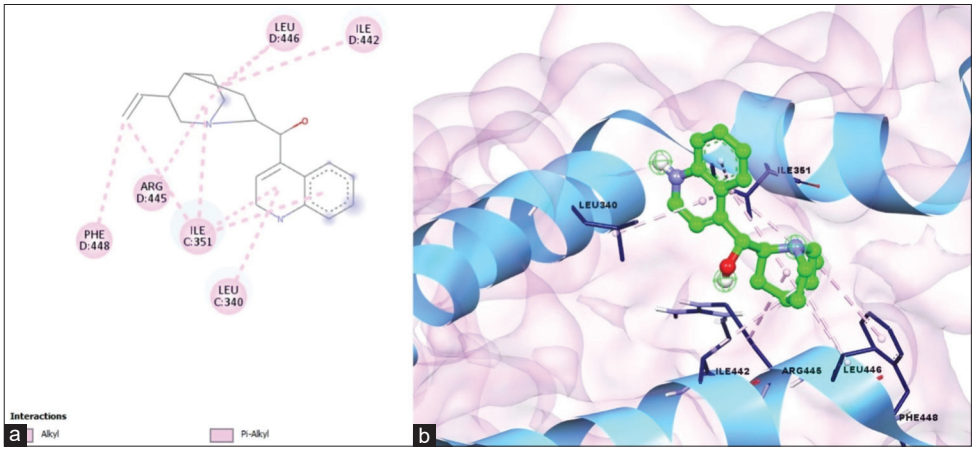

Fig. 8: (a and b) 2D interaction plot of Cinchonidine docked in the binding pockets of M2 protein. 3D representation showing the position of Cinchonidine within the binding site of M2 protein

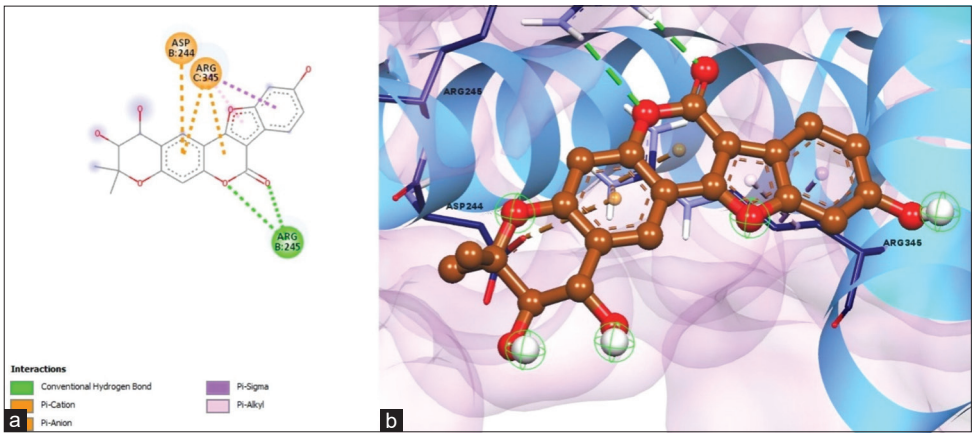

Fig. 9: (a and b) 2D interaction plot of Corylidin docked in the binding pockets of M2 protein. 3D representation showing the position of Corylidin within the binding site of M2 protein

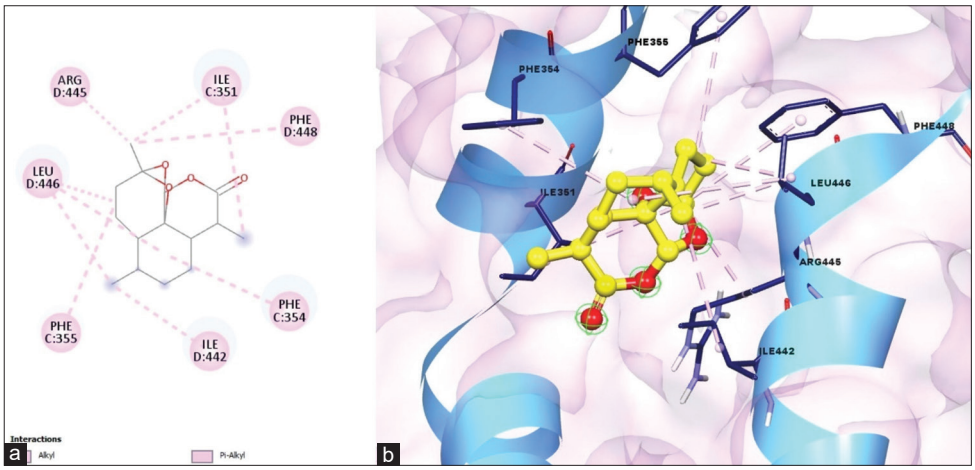

Fig. 10: (a and b) 2D interaction plot of Deoxyartemisinin docked in the binding pockets of M2 protein. 3D representation showing the position of Deoxyartemisinin within the binding site of M2 protein

CONCLUSION

Using a bioinformatic method, this study sets the framework for finding, testing, and creating a novel medication for the influenza A virus. It has been shown a positive view in applying bioinformatics in dealing with emergent pandemic and worldwide epidemics. The interaction of selected drug and target M2 protein can be analyzed through this method; however, more information could be obtained with the aid of the molecular simulation method. The theoretical evaluation of binding affinities (kcal/mol) of some phytochemical ligands was carried out to validate their potency and identify a possible lead molecule for designing a potential drug. The molecular docking studies revealed an excellent docking score ranging from −8 kcal/mol to −8.2 kcal/mol, indicating that the molecules can attach to the active region of the target protein more tightly compared to the standard medications used currently with a docking score ranging from −5.5 kcal/mol to −5.9 kcal/mol. Shinjulactone D, Cinchonidine, and Deoxyartemisinin ligands with the best binding pose could be selected as promising candidate, showing high potency for drug development. In addition, the results of ADME and drug likeness properties indicated good oral bioavailability, less toxicity, and good predicted absorption. The findings of the study support the relevance of these compounds as prospective leads for the treatment of influenza A virus, which could aid medicinal chemists and pharmaceutical professionals in developing and synthesizing more potent therapeutic candidates in the future. It also encourages the *in vitro* and *in vivo* evaluation of the study for proposed designed compounds to validate the computational findings.

ETHICS DECLARATIONS

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Data availability statement
All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Ethical statement
No animals were harmed during this study.

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AUTHOR CONTRIBUTIONS

VJ, TJ, and VD conceptualized this manuscript. VD, NK, MAS, and KT aligned the literature. RP, SD, AB, and SM wrote the manuscript. VD, MAS, KT, and NK prepared the figures. VJ, TJ, and SM did proof reading and corrections. All authors contributed to the revision and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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