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ORIGINAL ARTICLE

In silico Molecular docking study of Antiviral Phytochemical compounds and synthetic drugs as Potential binding compounds with SARS-CoV-2 Proteins

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ABSTRACT
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing a respiratory disease called COVID-19 spread rapidly around the globe. In the present study, in silico molecular docking analysis was performed to identify natural or synthetic drug which directly inhibits the SARS-CoV-2. Initially, we conduct a depth literature search for compounds that had biologically confirmed antivirus activity. We performed a molecular docking study of 11 natural and 5 synthetic drugs against the SARS-CoV-2 spike protein, RNA-binding domain of nucleocapsid phosphoprotein and nsp9 RNA binding protein as the probable target proteins using iGemdock v. 2. The selected antiviral compounds were cross-checked for listing in the pharmacology database and were subjected to absorption, distribution, metabolism and excretion (ADME) evaluation for its effective oral administration. Based on the docking results, nafamostat mesylate exhibited a significantly strong interaction (-115.1kcal/mol) compared to the other compounds with the spike protein of coronavirus. However, it could not clinically apply due to its carcinogenic nature. However, delphinidin also efficiently bind (-94.4 kcal/mol) with spike protein. Additionally, plant-based compound cimicifugin has shown good binding efficacy with binding energy (-129.1 kcal/mol) to the RNA-binding domain of nucleocapsid phosphoprotein of SARS-CoV-2. Among the others, daclatasvir and amentoflavone were found to interact with the nsp9 RNA binding protein of coronavirus (Binding energy: -123.5 Kcal/mol and -118 Kcal/mol respectively) besides violated 2 Lipinski's rule. Moreover, silymarin a naturally-occurring bioflavonoid expressed a better binding affinity (-108.1 Kcal/mol) toward the nsp9 RNA binding protein. It has been concluded that delphinidin could bind efficiently with the spike protein and prevent the entry of coronavirus in the host cells. In addition, cimicifugin, and silymarin have potential in inhibiting the targeted replicative polyprotein required for the life cycle of coronavirus. These drugs could also be used in combination for antiviral treatment to fight particularly against SARS-CoV-2. However, further in vitro and in vivo research are necessary to explore its preventive therapeutic use for COVID-19.
Keywords: *SARS-CoV-2, Molecular Docking, COVID-19, coronavirus*

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INTRODUCTION

Genetic similarity between SARS-CoV-2 and bat coronaviruses indicates that SARS-CoV-2 is considered as a bat-borne virus. Many genetically different coronaviruses cause Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus (SARS-CoV) in humans. Recently in December 2019, a novel coronavirus named SARS-CoV-2 was recognized in a cluster of patients in Wuhan, China. The SARS-CoV-2 virus can cause a respiratory illness named coronavirus disease 2019 (COVID-19) [1]**.** Since COVID-19 is considered as an airborne disease and vigorously

spreading worldwide. World Health Organization (WHO) has confirmed and declared it as pandemic disease. The transmissions of COVID-19 from humans to humans mostly take place via respiratory droplets produced during patient coughs or sneezes [2, 3]**.** As of March 18, 2020, around 313266 fatalities have been reported due to COVID-19 worldwide (https://www.worldometers.info/coronavirus/). Several studies have reported that the coexisting disorder like hypertension, cardiovascular diseases, diabetes mellitus in patients increase the severity towards the COVID-19 [4,5]. Recently, the elevated pro-inflammatory cytokines and chemokines are associated with the severity of COVID19 disease [6].

Many structural proteins of SARS, MERS, and other related coronaviruses have been explored and also studied its biological interaction with compounds having antiviral properties [7]. The molecular effect of virus protein on human protein plays a critical role in designing pharmacological strategies. Coronavirus belong to RNA viruses and contains a single-stranded RNA (ssRNA) as genetic material. The genome of the coronavirus encodes a variety of structural and non-structural proteins, some of which are essential for viral entry and replication [8]. The structure of spike glycoprotein S contains two subunits S1 and S2 from which S1 involved in regulating tropism and S2 make the possible virus and cell membrane fusion. The spike glycoprotein S1 of coronavirus binds to the Angiotensin-Converting Enzyme 2 (ACE 2) receptor of the host cell and allowed to enter the human respiratory cells [9, 10]. In a previous study, it was well documented that the S protein of virus activates the host immune response which could be attractive targets for drug development [11]. The nucleocapsid protein (N protein) of SARS CoV-2 is RNA-binding protein essential for the viral RNA transcription and replication. N protein bound to the leader RNA plays an essential role in packaging the RNA genome during replication and transcription in the virus-infected cell [12]. In addition to this, highly conserved N protein is more immunogenic in nature and expressed abundantly during infection which leads to the development of immune response against the SARS-CoV-2 [13]. The non-structural proteins (nsp), especially non-structural protein9 (nsp9) bind to the RNA have an important role in the replication and transcription of CoV-2 [14]. These envelope glycoproteins (S), nucleocapsid proteins (N), and new genomic RNA integrate together to produce new virus particles [15, 16]. Understanding the structural aspect of SARS-CoV-2 proteins could help to discover of antiviral agents that predominantly block the replication, transcription, and viral assembly of coronaviruses.

At the present time, there are not any precise and effective antiviral drugs targeting the novel coronavirus. There are a numbers of research institutes, and the pharmaceutical companies have an established pipeline to bring specific vaccines against this novel coronavirus to late-stage human clinical trials but still, the road ahead remains uncertain. There is an urgent need to search for possible medications. Based on the traditional principle, scientists were not able to carry out new drug development in such an unexpected epidemic. A study reported the effectiveness of Remdesivir and Chloroquine combination in reducing the *in vitro* replication of SARS-CoV-2 [5]. Another study proved that the administration of Lopinavir/Ritonavir could significantly reduce SAR-CoV-2 viral loads in COVID-19 patients [17]. Administration of chloroquine phosphate, an anti-malarial drug proved to limited curative effect on the COVID19 in many clinical investigations [18, 19]. Currently, various FDA approved antiviral drugs including ribavirin, Kaletra, Remdesivir and favivir have been assessed for the treatment of SARS CoV-2 [20]. Recent studies suggested that naturally occurring substances like phytochemicals have essential anti-viral activities. It is well reported that the gene sequence of SARS-CoV-2 had high similarity with the genome of SARS-Cov and MERS-Cov. Moreover, the phytochemical as naturally occurring compounds exhibit a broad safety profile and less pharmacological side effects [21]. Phytochemical compounds isolated from Chinese herbs had been reported effective antiviral agents having a high binding affinity towards SARS-CoV-2 proteins [22]. The drug repurposing strategies in which drugs approved for one disease may exert biological activity for others too. Molecular docking studies are used to predict the conformation and orientation of the drug candidate with target protein as well as explain its biochemical interaction.

In view of the above information, we employed a computational drug docking approach for identifying potential and specific phytochemicals and synthetic inhibitors of selected protein of the coronaviruses. We selected spike protein (PDB: 6LXT) as a target to block entry of SARS CoV-2 and RNA-binding domain of nucleocapsid phosphoprotein (PDB: 6WKP), Nsp9 RNA binding protein (PDB: 6W4B) as a molecular target to inhibit the virus replication. In the present research, we have performed a drug docking study of some of the potential therapeutic drug candidates that are being used against various viral diseases worldwide by using computational methods. The study was conceived with a strategy of exploring the compound which may impede SARS-CoV-2 infection by blocking the viral entry into the host cell or by inhibiting the viral polyprotein processing and virus cycle in the cell.

MATERIAL AND METHODS

Literature search and compound selection

Literature survey leads to the identification of 11 natural plant compounds including luteolin, [23] p-coumaric acid, [24] ladanein, [25] delphinidin, [26] cimicifugin, [27] cinanserin, [28] amentoflavone, [29] silymarin, [30] scutellarein, [31] resveratrol [32] and myricetin [33] having an antiviral effects. In the present study, 5 synthetic chemical drugs viz. Azodicarbonamide, [34] daclatasvir, [35] levofloxacin, [36] reproterol [37] and nafamostat mesylate [38] approved by the Food and Drug Administration (FDA) having an antiviral activity or effective against mild-to-moderate respiratory diseases are also selected. The molecular structure, formula and Canonical SMILES of 11 natural plant compounds shows evidence of antiviral effect presented in Table 1. The molecular structure, formula and Canonical SMILES of five FDA approved synthetic drug used for the treatment of respiratory disorder are depicted in Table 2.

Drug Likness Activity and Prediction of ADME by computational analysis

The drug-likeness analysis was performed to determine the lipophilicity (logP value), H-bond donor and acceptor by DruLiTo an open-source virtual screening tool [39]. ADME is useful at an early phase of the drug development process to remove molecules with poor ADME properties. On the basis of canonical SMILES of the selected ligands obtained from PubChem, Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADME) profiling of all potential compound at pH 7 was determined using online Swiss ADME program [40]. Insilco analysis of ADME studies was carried out to screen for compounds that may be bioactive via oral administration. The Lipinski's rules of five and solubility of drug [41] were deliberate and calculated values are presented in **Table 3.**

Preparation of Ligands and Receptors

For the current study, 11 natural plant compounds and 5 synthetic chemical drugs were considered based on known potent antiviral drugs. The 3-dimensional structure along with Canonical SMILES file of ligands (Azodicarbonamide, Chebulagic Acid, Daclatasvir, Levofloxacin, Myricetin, Reproterol, Resveratrol, Saikosaponin, Scutellarein, Silymarin, Amentoflavone, Cinanserin, Cimicifugin) for targeting RNA-binding domain of nucleocapsid phosphoprotein (PDB id: 6WKP) and Nsp9 RNA binding protein (PDB id: 6W4B) of SARS-CoV-2 whereas lafamostat mesylate, luteolin, p-coumaric acid, ladanein, delphinidin for targeting spike protein (PDB id: 6LXT) were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). The Canonical SMILES file of all ligands were converted into Molecular file format (MOL file) by using http://www.cheminfo.org/Chemistry/Generate_molfiles. The three-dimensional crystal structure of spike protein (PDB id: 6LXT), the RNA-binding domain of nucleocapsid phosphoprotein (PDB id: 6WKP), Nsp9 RNA binding protein (PDB id: 6W4B) of SARS-CoV-2S protein (Fig. 1) were downloaded from the RCSB protein data bank (https://www.rcsb.org/). Protein target was prepared for the molecular docking process by removing water molecules and other ligand attached. The structure of the proteins was saved in PDB format for further analysis.

Protein-molecular docking:

Molecular docking was performed with iGemDock v.2 a prevalent molecular screening tool for identifying binding energy between the 3D structure of each ligand and target protein [42]. For the docking experiment, whole spike protein consists of chains A, B, C was used. Docking was performed with iGEMDOCK v.2 an established molecular screening tool for identifying binding energy between the 3D structures of each ligand and target proteins. All the 11 phytochemicals and the 5 chemical compounds are subjected to the drug docking process against SARS-CoV-2 protein using iGEMDOCK v.2. The docking accuracy parameter such as Population Size = 300, Generations = 70, number of solutions =3 were set with the default parameters. Blind docking was performed to know the probable binding site. Docking scores are reported in kcal/mol, the more negative the number, the better binding. The interactions analysis indicating Hydrogen Bonding and other non-bonded terms between the docked Phytochemicals or chemical drug and the protein target are seen using post screening analysis of iGEMDOCK v.2.

RESULT AND DISCUSSION

The results obtained from these docking studies revealed that all selected ligand exhibited significant binding affinity towards RNA-binding domain of nucleocapsid phosphoprotein, Nsp9 RNA binding protein, and spike protein of SARS-CoV-2. SARS-CoV-2 is a novel human coronavirus that leads to a severe respiratory illness known as COVID-19 declared it a pandemic disease [43]. As per the report of WHO, there are no established anti-COVID-19 drugs or vaccines on hand. The number of infected cases increasing gradually and medical scientists desperately looking for efficient natural or synthetic antiviral compounds used treatment of COVID-19. The most important step to begin drug design is to identify and select the most suitable drug target. In the present study, we identify RNA-binding domain of nucleocapsid phosphoprotein (PDB: 6WKP), Nsp9 RNA binding protein (PDB: 6W4B) and spike protein

(PDB: 6LXT) could be an ideal target proteins for inhibiting COVID-19. The nucleocapsid phosphoprotein is an important protein that interacts with the viral genomic RNA and participates in RNA package along with virus particle release [44]. SARS-CoV-2 nsp9 or replicase is a single-stranded RNA-binding protein involved with viral RNA synthesis and the replicative cycle of the coronaviruses [45]. Viral spike (S) protein combines with Angiotensin-Converting Enzyme 2 (ACE2) receptor present on the human's lower respiratory tract is important for SARS-CoV-2 entry and infection of cells [46].

Molecular docking is extensively employed to indentify types of binding interaction of the potential drugs with active sites on targeted molecules. Drug binding affinity towards the target molecules has been widely explained by the number and pattern of hydrogen bonding [47]. The binding energy data obtained in Kcal/mol allow us to predict the binding affinity of a different ligand with the corresponding target protein. The least binding energy utilized during binding mode is considered as the most excellent mode of binding as it is largely stable for the ligand.

In the present study, nafamostat mesylate, luteolin, p-coumaric acid, ladanein, delphinidin were docked with spike protein of SARS-CoV-2. Whereas azodicarbonamide, daclatasvir, levofloxacin, myricetin, reproterol, resveratrol, scutellarein, silymarin, amentoflavone, cinanserin and cimicifugin were docked with RNA-binding domain of nucleocapsid phosphoprotein and Nsp9 RNA binding protein of coronavirus. Lipinski's rules of five are usually employed to assess potential interaction between drug and other target molecules. This method could act as a filter to screen probable therapeutic agents and also reduced the labour and cost exercises during clinical drug development. The entire compounds used in this study were found in compliance with all the five criteria mentioned in Lipinski's rule except daclatasvir and amentoflavone. The results of Lipinski properties are reported in Table 3. *In silico* ADMET predictions revealed that all studied compounds except azodicarbonamide and nafamostat mesylate had non-toxic effects and had good absorption as well as solubility characteristics. Table 4 illustrates the various ADMET parameters obtained in the present study. Thus we also suggest that these entire compounds selected in the present study have the potential ability to work efficiently as drug. The docking study revealed that all selected compounds exhibited a good binding affinity to targeted proteins. The range of the binding affinities of all the compounds lies 67.0 kcal/mol to 129 kcal/mol (Table 5-7). These docking studies also revealed the specific amino acids which are involved in interact with the functional group of the drug. We select ligand with the lowest binding energy which may have a higher affinity as a potential drug against SARS-CoV-2. Ligand presented in the stick model whereas targeted protein is shown as a ribbon structure. The total number of hydrogen bonds and various amino acids contribute to ligand-protein interaction with different bonds is shown in Table 5-7. The protein-drug interactions are illustrated in Figures 2-4.

The molecular docking analysis and visualisation of spike S2 subunit with nafamostat mesylate (A), ladanein (B), delphinidin (C), luteolin (D) and p-coumaric acid (E) is shown in Fig. 2. Nafamostat mesylate is a synthetic serine protease inhibitor docked with spike protein that showed significant binding with lowest energy (-115.1kcal/mol) compared to other compounds. The interaction of nafamostat mesylate with spike protein chain showed hydrogen bonding with GLN-935, ASP-1184, ASN-1194, SER-939, SER-940 and LYS-947. The van der Waals interaction of nafamostat mesylate with ASN-1178, GLN-1180, ASP-1184, LYS-1181, GLU-1182, and ARG-1185 amino acid residues have been observed (Table 5). But ADME study suggested the carcinogenic nature of this compound needs to take consideration for further use. Instead of carcinogenic nafamostat mesylate, delphinidin a natural anthocyanidin could be effective to bind with spike protein due to lower binding energy (-94.4 Kcal/mol). Delphinidin ligand could strongly interact with spike protein with 6.0 H-bond with GLU-1195, LEU-1197, ASP-1199, GLN-926, ILE-1198, LEU-1200 amino acid residue (Fig. 2C). From this observation, it can be predicted that delphinidin may prevent the initial attachment of coronavirus with ACE2 receptor on the host cell specifically at the respiratory tract. Previously, nafamostat mesylate drug is used to treat acute pancreatitis. Recent work demonstrates that nafamostat mesylate, a serine protease inhibitor reduce SARS-CoV-2 infection *invitro* in lung-derived human cell line [38, 48]. It is assumed that nafamostat can inhibit the fusion of envelope spike protein of the coronavirus with host cell surface receptor ACE2. Therefore, it is expected that nafamostat will prevent SARS-CoV-2 from entering human cells [5, 49]. Additionally, nafamostat could diminish the Middle East respiratory syndrome (MERS) coronavirus infections and the ebola virus disease in *in-vitro* studies [50]. Despite this, nafamostat mesylate exhibited a number of side effects like causing cardiac arrest and cancer [51]. Delphinidin (Del) is well explored and having broad-spectrum antiviral activity against the West Nile virus, Zika virus, and Dengue virus at early steps of the viral infection [26].

The molecular interaction between protein RNA-binding domain of nucleocapsid phosphoprotein with ligands cimicifugin (a), reproterol (b), amentoflavone (c), myricetin (d), silymarin (e), scutellarein (f),

levofloxacin (g), resveratrol (h), daclatasvir (i), cinanserin (j) and azodicarbonamide (k) are presented in Fig. 3. The result of a molecular docking study revealed that ARG 107, THR 54 and TRP-52 amino acid play a crucial role in the active pocket of the RNA-binding domain of nucleocapsid phosphoprotein. It was clearly evident that ARG 107 is essential for binding interaction for activity [52]. The lowest binding energy observed with cimicifugin with the RNA-binding domain of nucleocapsid phosphoprotein could be attributed to the presence of six H-bond with PRO-67, ARG-68, GLY-69, GLN-70, TYR-123, and ALA-134 amino acid residues (Table 6). Due to higher affinity, cimicifugin is a potential candidate to inhibit the activity of the RNA-binding domain of nucleocapsid phosphoprotein required for virus replication and life cycle. Cimicifugin is a major phenolic constituent of obtained from *Cimicifuga foetida* possessed inhibitory activity against the human respiratory syncytial virus (RSV) that causes infection of the lower respiratory tract [27]. The signs and symptoms of respiratory syncytial virus infection are almost similar to COVID-19 like fever, runny nose, difficulty in breathing, and lung inflammation [53].

The ligands and their docking interactions with nsp9 RNA binding protein are shown in Fig. 4a-k. In comparison with selected 11 compounds, daclatasvir demonstrated good binding affinity with binding energy (-123.5 Kcal/mol) forming two H-bond with GLY-38 and ARG-40 residue of nsp9 RNA binding protein as a target in the coronavirus (Table 7). Daclatasvir is a clinically approved antiviral therapeutic agent against the hepatitis C virus (HCV). Both Hepatitis C (HCV) and SARS-CoV-2 are positive-sense RNA viruses and share many similar features. Sacramento et al., [54] demonstrated the inhibitory effect of daclatasvir on the SARS-CoV-2 replication cycle and also prevent the production of IL-6 and TNF-α, inflammatory mediators during infection. Daclatasvir could be inhibiting SARS-CoV-2 RNA replication by specific inhibition of the viral nps9 protein similar like to viral NS5A protein in hepatitis C virus [55]. But this compound has violated 2 Lipinski's rule during ADME studies. The number of H bonds involved in interaction suggested that ligand would be established stable confirmation. So instead daclatasvir, silymarin could be a potential natural compound that shows lower binding energy (-108.1 Kcal/mol) with good affinity to attach with nsp9 RNA binding protein. Silymarin shows binding to nsp9 RNA binding protein with SER-14, ASP-27, ASN-28, SER-47, ASP-48 and LYS-87 amino acid residues participating in the interaction with the H-bonds (Fig. 4c).

We performed a molecular docking study of natural and synthetic antiviral compounds selected based on review and literature against SARS-CoV-2 proteins. The results obtained in the present study demonstrate the strong interaction of the potential drug nafamostat mesylate and delphinidin with spike protein inhibiting the entry of coronaviruses. Our study also suggested that cimicifugin display higher affinity to bind with RNA-binding domain of nucleocapsid phosphoprotein and daclatasvir as well as silymarin with nsp9 RNA binding protein which may inhibit the further replication cycle of coronavirus.

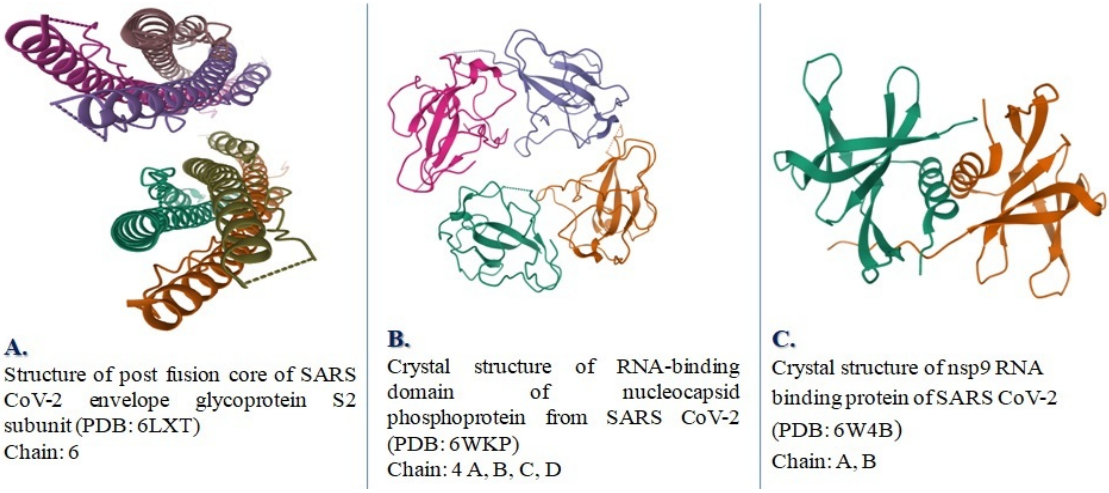


Figure 1: Crystal 3-D structure of (A) S2 subunit of envelope spike glycoprotein (B) RNA-binding domain of nucleocapsid phosphoprotein (C) nsp9 RNA binding protein of SARS- CoV-2.

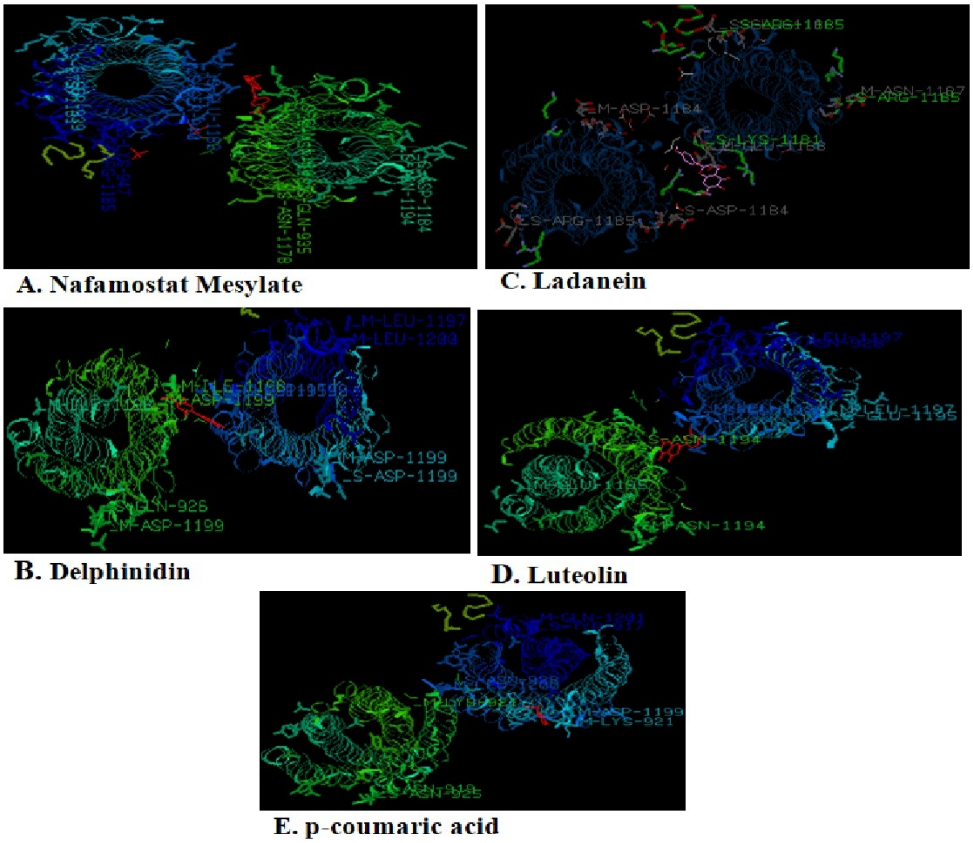


Figure 2: Docking analysis and visualization of S2 subunit of spike protein with nafamostat mesylate (A), ladanein (B), delphinidin (C), luteolin (D) and p-coumaric acid (E)

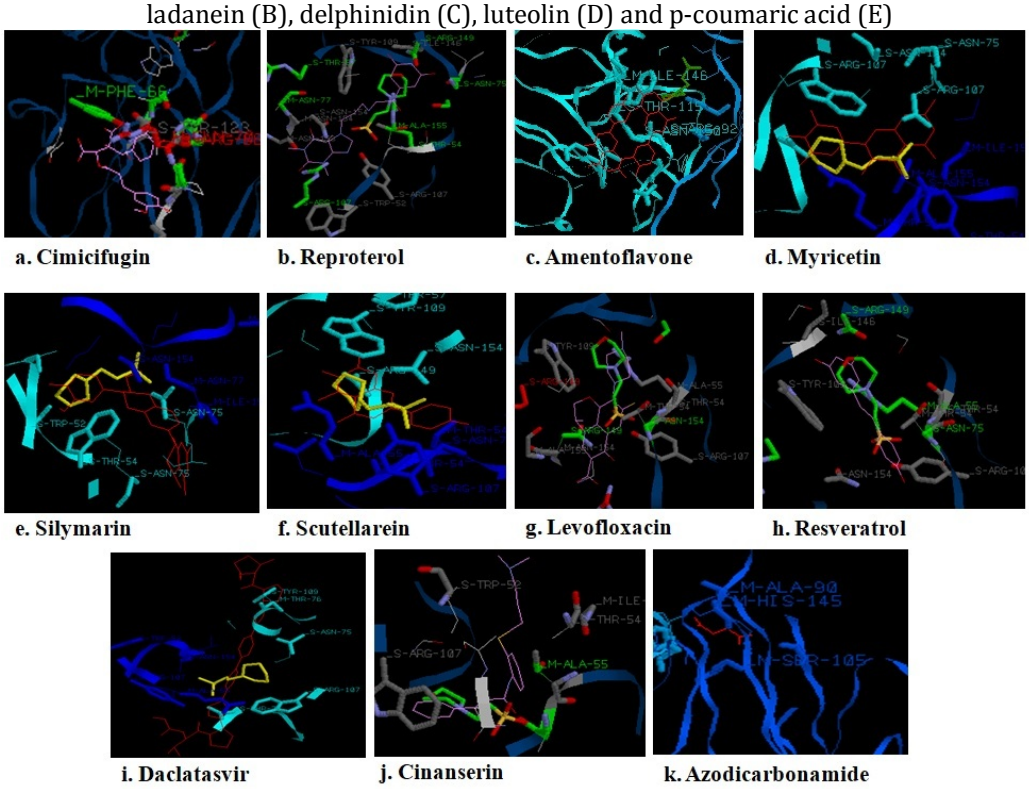


Figure 3: Molecular docking analysis and visualization of RNA-binding domain of nucleocapsid phosphoprotein with ligands cimicifugin (a), reproterol (b), amentoflavone (c), myricetin (d), silymarin (e), scutellarein (f), levofloxacin (g), resveratrol (h), daclatasvir (i), cinanserin (j) and azodicarbonamide (k)

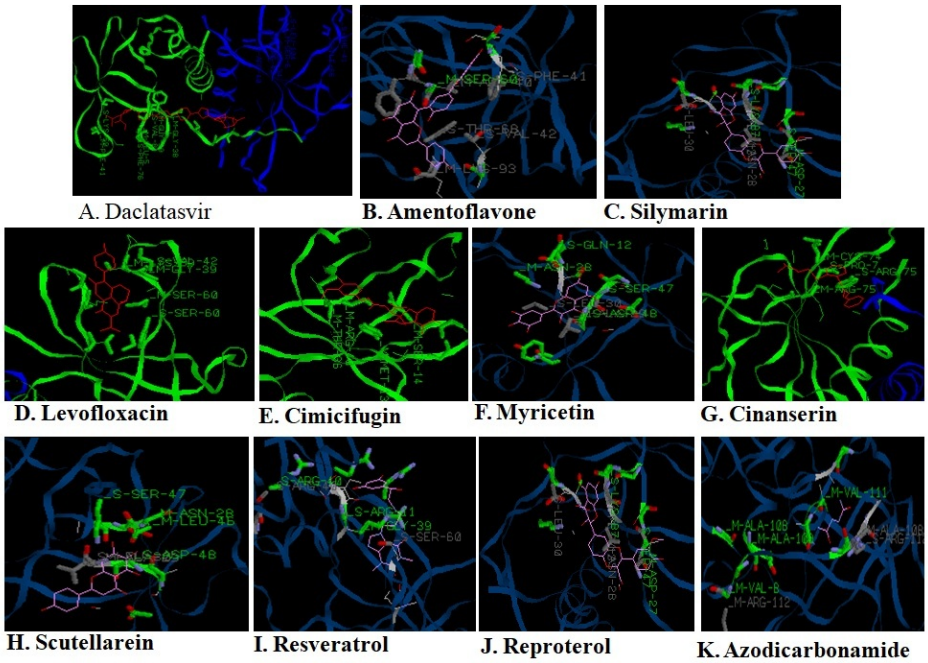
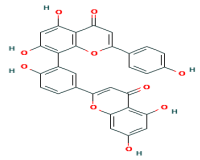
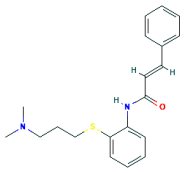
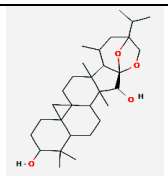
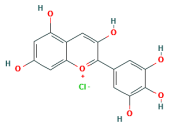
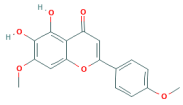
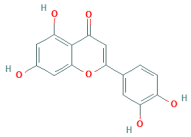


Figure 4: Molecular docking analysis and visualization of nsp9 RNA binding protein with ligands daclatasvir (a), amentoflavone (b), silymarin (c), levofloxacin (d), cimicifugin (e), myricetin (f), cinanserin (g), scutellarein (h), resveratrol (i), reproterol (j) and azodicarbonamide (k)

Table 1: Molecular structure, Formula, Canonical SMILES format and medicinal properties of 11 natural plant compounds having antiviral activity

No.	Compound	Molecular Formula	Molecular Structure	Medicinal Properties	References
1.	Amentoflavone	$C_{30}H_{18}O_{10}$		Identify natural HCV inhibitors	Lee *et al*., 2018
2.	Cinanserin	$C_{20}H_{24}N_2OS$		Strongly Reduces Corona Virus Replication	Chen *et al*., 2005
3.	Cimicifugin	$C_{30}H_{48}O_4$		Antiviral Effect against Human Respiratory Syncytial Virus	Wang *et al.*, 2012
4.	Delphinidin	$C_{15}H_{11}ClO_7$		Exhibit virucidal effect on broad spectrum of virus	Vázquez-Calvo *et al*., 2017
5.	Ladanein	$C_{17}H_{14}O_6$		Inhibits entry of all HCV	Haid *et al*., 2012
6.	Luteolin	$C_{15}H_{10}O_6$		Antiviral effects on both HIV-1 and Epstein-Barr virus (EBV)	Fan *et al*., 2016

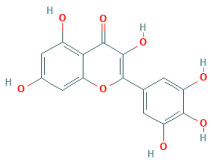
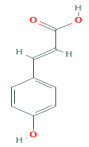
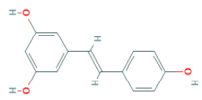
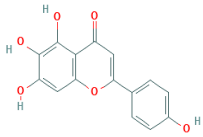
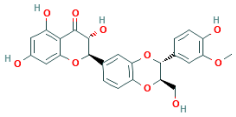
7.	Myricetin	$C_{15}H_{10}O_8$		Novel chemical inhibitors of the SARS coronavirus helicase	Yu *et al.*, 2012
8.	p-coumaric acid	$C_9H_8O_3$		Inhibits HCV entry via its direct effect on viral particles	Shirasago *et al.*, 2019
9.	Resveratrol	$C_{14}H_{12}O_3$		antiviral activities against Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV)	Lin *et al.*, 2017
10.	Scutellarein	$C_{15}H_{10}O_6$		Exhibited broad spectrum antiviral activities.	Liu *et al.*, 2020
11.	Silymarin	$C_{25}H_{22}O_{10}$		Antiviral activity against CHIKV, reducing both replication and production of viral proteins.	Wagoner *et al.*, 2010.

Table 2: Molecular structure, Formula, Canonical SMILES format and medicinal properties of 5 FDA approved synthetic drug having antiviral activity and use for the treatment of respiratory disorder.

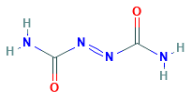
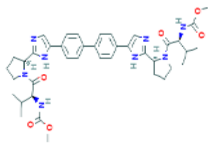
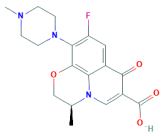
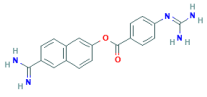
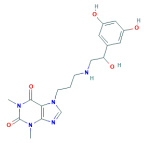
No.	Compound	Molecular Formula	Molecular Structure	Medicinal Properties	References
1.	Azodicarbo-namide	$C_2H_4N_4O_2$		Agents used to treat AIDS and/or stop the spread of the HIV infection.	Clercq, 2002
2.	Daclatasvir	$C_{40}H_{50}N_8O_6$		Oral antiviral agents to treat chronic hepatitis C.	McCormack, 2015
3.	Levofloxacin	$C_{18}H_{20}FN_3O_4$		Used in the treatment of mild-to-moderate respiratory	Torres & Liapikou, 2012
4.	Nafamostat mesylate	$C_{21}H_{25}N_5O_8S_2$		Used to treat acute pancreatitis,	Yamamoto et al., 2016
5.	Reproterol	$C_{18}H_{23}N_5O_5$		Used in the treatment of asthma	Tien et al., 2020

Table 3 Lipinski properties of 11 natural plant compound and 5 synthetic chemical drugs analyzed using DruLiTo an open source virtual screening tool.

No.	Name of Ligand	Molecular weight (<500 Da)	Log P (<5)	H-bond donor (5)	H-bond acceptor (<10)	No. of violations
1.	Amentoflavone	538.09	2.03	6	10	2
2.	Cinanserin	340.16	3.205	1	3	0
3.	Cimicifugin	472.36	7.119	2	4	1
4.	Delphinidin	303.05	2.315	6	6	1
5.	Ladanein	314.08	2.128	2	6	0
6.	Luteolin	286.05	1.486	4	6	0
7.	Myricetin	318.04	2.182	5	8	0
8.	p-coumaric acid	164.05	0.751	2	3	0
9.	Resveratrol	228.08	2.048	3	3	0
10.	Scutellarein	286.05	1.915	4	6	0
11.	Silymarin	482.12	0.855	5	10	0
12.	Azodicarbo-namide	116.03	-2.564	2	6	0
13.	Daclatasvir	738.39	-2.883	4	14	2
14.	Levofloxacin	361.14	1.995	1	7	0
15.	Nafamostat mesylate	347.14	0.512	4	7	0
16.	Reproterol	389.17	-0.832	4	10	0

Table 4: ADME properties of selected ligands

No.	Compound	HIA	BBB	AMES toxicity	Carcinogenicity	LD50 in rat (mol/kg)
1.	Amentoflavone	0.6602	0.9739	Non AMES toxic	Non-carcinogens	3.1298
2.	Cinanserin	0.9834	0.9326	Non AMES toxic	Non-carcinogens	2.4316
3.	Cimicifugin	0.6824	0.9227	Non AMES toxic	Non-carcinogens	2.5171
4.	Delphinidin	0.9177	0.9029	Non AMES toxic	Non-carcinogens	3.0873
5.	Ladanein	0.7881	0.9678	Non AMES toxic	Non-carcinogens	2.9037
6.	Luteolin	0.5711	0.9650	Non AMES toxic	Non-carcinogens	3.0200
7.	Myricetin	0.5711	0.9650	Non AMES toxic	Non-carcinogens	3.0200
8.	p-coumaric acid	0.5237	0.9938	Non AMES toxic	Non-carcinogens	1.3698
9.	Resveratrol	0.9952	0.5900	Non AMES toxic	Non-carcinogens	1.6791
10.	Scutellarein	0.5711	0.9650	Non AMES toxic	Non-carcinogens	3.0200
11.	Silymarin	0.7675	0.9698	Non AMES toxic	Non-carcinogens	2.2206
12.	Azodicarbo-namide	0.9941	0.9731	AMES toxic	Carcinogens	1.2897
13.	Daclatasvir	0.7766	0.9956	Non AMES toxic	Non-carcinogens	2.5746
14.	Levofloxacin	0.9659	0.9545	AMES toxic	Non-carcinogens	2.3639
15.	Nafamostat mesylate	0.6432	0.6752	Non AMES toxic	Carcinogens	2.4215
16.	Reproterol	0.9684	0.9712	Non AMES toxic	Non-carcinogens	2.1221

Table 5: Binding energy and interaction residues obtained in molecular docking with post fusion core of SARS CoV-2 S2 subunit of spike protein (PDB: 6LXT)

No.	Name of Compound	Binding Affinity (Kcal/mol)	No. of Hydrogen Bond	H Bond Interaction Residue	Other Interaction Residue
1.	Nafamostat Mesylate	-115.1	6	GLN-935, ASP-1184, ASN-1194, SER-939, SER-940, LYS-947	ASN-1178, GLN-1180, ASP-1184, LYS-1181, GLU-1182, ARG-1185
2.	Delphinidin	-94.4	6	GLU-1195, LEU-1197, ASP-1199, GLN-926, ILE-1198, LEU-1200	ILE-1198, ASP-1199, LEU-1197
3.	Ladanein	-90.8	3	ARG-1185, LYS-1181, ARG-1188	ASP-1184, ARG-1185, GLU-1188
4.	Luteolin	-85.5	4	LEU-1197, GLN-926, ASN-928, ASN-1194	ASN-1194, GLU-1195, LEU-1197
5.	p-coumaric acid	-71.6	3	ASN-919, LYS-921, ASN-928	TYR-917, LYS-921, ASN-925, ASP-1199, LUE-1200, GLN-1201

Table 6: Binding energy and interaction residues obtained in molecular docking with RNA-binding domain of nucleocapsid phosphoprotein from SARS CoV-2 (PDB: 6WKP)

No.	Name of Compound	Binding Affinity (Kcal/mol)	No. of Hydrogen Bond	H Bond Interaction Residue	Other Interaction Residue
1.	Cimicifugin	-129.1	6	PRO-67, ARG-68, GLY-69, GLN-70, TYR-123, ALA-134 (ARG-68 E-S)	LYS-65, PHE-66, PRO-67, ARG-68, TYR-123, VAL-133
2.	Reproterol	-125.1	6	THR-57, ARG-107, ARG-149, ASN-75, ASN-77, ALA-155	THR-54, ARG-107, TYR-109, TRP-52, ILE-146, ASN-153, ASN-154, ALA-155
3.	Amentoflavone	-118.3	4	ARG-92, ARG-107, GLY-116, ASN-150,	ARG-92, TRP-52, THR-115, GLY-116, ASP-144, ILE-146, GLY-147
4.	Myricetin	-115.2	4	ARG-107, ASN-75, ASN-154, ALA-155	THR-54, ARG-107, TYR-109, TRP-52, ASN-154, ILE-157
5.	Silymarin	-110.5	4	ASN-75, ASN-77, ASN-154, ILE-157	THR-54, ALA-156, TRP-52, ASN-75, ASN-154, ILE-157
6.	Scutellarein	-109.2	4	THR-57, ARG-107, ARG-149, ASN-75	THR-54, ALA-55, ARG-107, TYR-109, TRP-52, ASN-75, ASN-154
7.	Levofloxacin	-108.0	3	THR-57, ARG-149, ASN-154 (ARG-149 –E-S)	THR-54, ALA-55, ARG-107, TYR-109, TRP-52, ASN-154, ALA-155
8.	Resveratrol	-94.2	3	ARG-107, ARG-149, ASN-75	THR-54, ALA-55, ARG-107, TYR-109, TRP-52 ILE-146, ASN-154
9.	Daclatasvir	-90.3	4	ARG-107, TYR-109, THR-76, ASN-154	ALA-55, TRP-52, ARG-107, ASN-75, THR-76
10.	Cinanserin	-78.4	1	ALA-55	THR-54, ARG-107, ALA-156, TRP-52, ILE-157
11.	Azodicarbonamide	-74.8	4	ALA-90, SER-105, ARG-107, HIS-145	LEU-104, SER-105, ARG-107

Table 7: Binding energy and interaction residues obtained in molecular docking with Nsp9 RNA binding protein of SARS CoV-2 (PDB: 6W4B)

No.	Name of Compound	Binding Affinity (Kcal/mol)	No. of Hydrogen Bond	H Bond Interaction Residue	Other Interaction Residue
1.	Daclatasvir	-123.5	2	GLY-38, ARG-40	PHE-76, LEU-104, LUE-5, SER-6, VAL-8, ASN-34, GLY-39, ARG-40, PHE-41, LYS-59, ILE-66
2.	Amentoflavone	-118.2	3	ARG-40, SER-60, GLU-69	ARG-40, PHE-41, VAL-42, PHE-57, THR-68, ILE-92, LYS-93
3.	Silymarin	-108.1	6	SER-14, ASP-27, ASN-28, SER-47, ASP-48, LYS-87	GLN-12, MET-13, SER-14, ASN-28, ALA-29, LEU-30, ASP-48
4.	Levofloxacin	-99.9	3	VAL-42, SER-60, THR-68	GLY-39, ARG-40, VAL-42, PHE-57, LYS-59, SER-60, ILE-66
5.	Cimicifugin	-97.9	5	SER-14, TYR-33, THR-36, GLY-39, ARG-40	MET-13, TYR-33, ARG-40
6.	Myricetin	-90.3	7	GLN-12, ASP-27, ASN-28, TYR-32, SER-47, ASP-48, LYS-87	ALA-29, LEU-30, LEU-46, LYS-87
7.	Cinanserin	-85.4	2	ARG-40, THR-68	GLY-39, ARG-40, VAL-42, PHE-57, LYS-59, SER-60, ILE-66
8.	Scutellarein	-83.3	7	ASP-27, ASN-28, ALA-29, LEU-46, SER-47, ASP-48, LYS-87	ALA-29, LEU-30
9.	Resveratrol	-80.1	2	ARG-40, PRO-58	ARG-40, PHE41, VAL-42, PHE-57, LYS-59, ILE-66
10.	Reproterol	-75.32	2	ARG-11, ARG-40	ARG-40, SER-60, GLY-39
11.	Azodicarbonamide	-67.86	4	ALA-108, ALA-109, VAL-111, VAL-8	ALA-108. ARG-112

CONCLUSION

The result strongly suggests that nafamostat mesylate and daclatasvir from synthetic compounds while cimicifugin, delphinidin and silymarin from natural compounds could be potential induced conformation change in targeted protein complexes. The combination of this compound should be explored further as a preventive therapeutic for COVID-19. We also recommended the *in vitro* and *in vivo* study of these compounds will give a clear path for the development of drugs for COVID-19 therapy.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ABBREVIATIONS

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2, ADME: absorption, distribution, metabolism and excretion, MERS-CoV: Middle East respiratory syndrome coronavirus.

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