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Insilico Screening of Bioactive Phytochemicals against Spike Protein of COVID-19

Muhammad Hamza Tariq[1], Mahjabeen Akram[2] and Yasir Sharif[3]*

[1]*Atta-ur-Rehman School of Applied Biosciences, National University of Science and Technology, Islamabad, Pakistan*
[2]*Paediatrics Medicine Unit 1, Services Hospital, Lahore 54000, Pakistan*
[3]*Institute of Biochemistry and Biotechnology, University of the Punjab, Lahore-54590, Pakistan*

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ABSTRACT

Coronavirus disease 2019 (COVID-19) is a pandemic and this disease has infected millions of people globally now. COVID-19 is caused by a novel beta coronavirus strain known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Once SARS-CoV-2 manages to enter the body, it identifies and binds to the angiotensin converting enzyme 2 (ACE2) receptors through the binding receptor of Spike Protein (S-protein). The present study aimed to investigate the phytochemicals as potential inhibitors of the binding domain of S protein so that the binding of COVID-19 with ACE2 receptors could be restrained. For this purpose, the library of 2113 phytochemicals was docked against the binding domain of the S-protein. Top ten compounds with maximum binding affinity to the active sites of target protein were further screened for ADMET properties by adopting SwissADME and ADMETsar online servers. The compounds namely Morin, Curcumin, Apigenin, Cedronolactone A and Matairesinol showed acceptable drug-like properties therefore these compounds can be proposed as effective inhibitors, disrupting the S-protein- ACE2 interaction. This study might help in the development of a natural and cost-effective drug against COVID-19. Further, *in vivo* and *in vitro* examinations are required to validate our results.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel strain of coronavirus that is reported to cause infectious Coronavirus disease 2019 (COVID-19) (Nadeem *et al*., 2020). SARS-CoV-2 is characterized by positive stranded non-segmented RNA genome with spike glycoproteins (S-proteins) on its envelope (Walls *et al*., 2020). These S-proteins gives it crown like appearance under electron microscope. Soon after its incidence it has become a pandemic now, it has affected millions of people globally and the cases are still increasing day by day due to higher degree of virus contagiosity. Pathogenesis of SARS-CoV-2 includes invasion of virus through the respiratory tract by disrupting the intercellular epithelial tight junctions (which are present in human respiratory tract and serve as a first line of defense against foreign invaders). After incursion, SARS-CoV-2 recognizes angiotensin converting enzyme 2 (ACE2) receptors through spike proteins, followed by viral entry into cell, its replication and ultimately severe COVID-19 infection (Zhang *et al*., 2020). Once binding is accomplished, series of conformational changes start to occur that leads to conversion of S-protein from pre-fusion to a post-fusion state. S2 subunit is the other subunit of S-protein that causes the virus to get fused with the cell membrane, allowing it to enter the cell. It is now clear that the entry of SARS-CoV-2 requires ACE2 receptors and these receptors are found in different body cells of all age group human being, however, their level is much more elevated in children, making them more susceptible towards severe form of COVID-19 (Cristiani *et al*., 2020). Currently there is no therapeutic agent is available to treat this infectious disease while number of infected people is increasing every day.

Active biological agents that are extracted straightforwardly from the plants are called as phytochemicals, these show remarkable sustenance and pharmaceutically effectiveness because of their prominent natural qualities. Phytochemicals have been reported to have very good anti-viral activities with minimum side-effects (Kapoor *et al*., 2017; Shahzad *et al*., 2019), therefore, the current study is aimed to identify some bioactive phytochemicals that might be able to act as a potential inhibitor of S protein so that the attachment of SARS-CoV-2 with ACE2 receptors could be restricted.

* Corresponding author: s.yashel36@hotmail.com
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MATERIALS AND METHODS

Structure retrieval and optimization

SARS-CoV-2 receptor binding domain was retrieved from Protein databank (Berman *et al*., 2000) as PDB ID 6W41. This selected protein contains the Crystal structure of SARS-CoV-2 receptor binding domain in complex with human antibody CR3022. This structure was downloaded as PDB file and opened in Molecular Operating Enviornment (MOE) software. This target protein was first optimized by removing Human receptor and antibody. Human receptor and antibody was removed while receptor binding domain of Spike Protein of SARS-CoV-2 was taken for further study. This target protein was first optimized by; 3D protonation and energy minimization using MOE software and taken as receptor for docking study.

Ligand library preparation

Three dimensional structure of 2113 bioactive phytochemicals was downloaded from PubChem database (Kim *et al*., 2015), out of which 227 were alkaloids, 81 were aromatic, 11 were carbohydrates, 999 were flavonoids, 88 were lignans, 63 were polycylclicaromatic, 51 were saponins, 77 were steroids, 6 were tanins and 510 were terpenoids. All these phytochemicals were also optimized by 3D protonation and energy minimization. A library containing all these optimized phytochemicals was established for docking study.

Molecular docking

Docking analysis was performed by using Molecular Operating Environment software (Vilar *et al*., 2008). For this purpose, docking was done between ligand library and active residues of target protein, these active residues were determined by site finder tool of MOE software. Once the docking was completed, phytochemicals with best conformations were identified according to Root Mean Square Deviation (RMSD) value and S-score. RMSD represents the mean distance amongst the backbone atoms of superimposed proteins and S-score is a mathematical value that demonstrates the binding affinity of ligands with their receptors with all potential binding geometries. LigX tool of MOE was adopted to examine the clear vision of 2D and 3D plots of receptor ligand interactions.

In silico analysis of drug likeness and ADMET properties

Top ten Phytochemicals with minimum docking score were further appraised for Lipinski rule of five to evaluate their drug-like attributes by engaging Molinspiration online tool (URL: http://www.molinspiration.com//cgi-bin/properties). Any compound violating more than two parameters of Lipinski rule was not considered as a good drug against TBX19. All those compounds that followed Lipinski rule of five were further considered for ADMET (Adsorption, Distribution, Metabolism, Excretion and Toxicity) like properties by practicing admetSAR (URL: http://lmmd.ecust.edu.cn/admetsar1) (Cheng *et al*., 2012) and SwissADME software (URL: http://www.swissadme.ch/) (Daina *et al*., 2017).

RESULTS AND DISCUSSION

Drug designing has been radically revolutionized due to in silico analysis and bioinformatics. It has decreased both cost and time required for drug discovery. Due to advancements in Chemoinformatics, libraries of 3D structures of compounds are accessible that could be tested for their potential therapeutic effects via different computational tools. Molecular Docking involves the Insilco estimation of best suitable three-dimensional conformation of ligand- target complex and also predict the free energy of resulting complex (Bortolato *et al*., 2013), therefore, it is considered as a common protocol in drug-design (de Ruyck *et al*., 2016). The current study employed a library of 2113 bioactive phytochemicals to evaluate their ability to inhibit the receptor binding domain of S-protein. For this purpose, docking analysis was performed between phytochemical library and receptor binding domain of S-protein.

Docking analysis

Library of energy minimized 2113 phytochemicals was docked with receptor binding domain of COVID-19 spike protein. Once the docking was completed, phytochemicals were ranked on the basis of four different parameters i.e. minimum Gibbs free energy, maximum occupancy of the binding pocket and Power of different binding forces. Out of 2113 phytochemicals, top ten were selected on the basis of lower RMSD value, minimum S-score and maximum binding site occupancy (Table I). All of these ten compounds had an S-score within the range of -14.49 kcal/mol to -11.64 kcal/mol. These ten compounds include; Tannic acid, Oenin, Morin, Chebulinic Acid, Uncarinic Acid C, Cedronolactone A, Matairesinol, Apigenin, Curcumin, D-Chicoric acid. Amino acids; Phe 342, Ala 344, Asp 363 and Ser 373 were found to be most active amino acid residues as they showed interactions with three out of ten phytochemicals.

Evaluation of drug-like properties

These ten compounds were further assessed for drug-like properties by considering them for Lipinski rule of five according to which a compound shows drug like properties must have a molecular weight of <500 Da, it should

contain < 5 Hydrogen bond Donor (HBD), <10 Hydrogen Bond Acceptor (HBA) and lipophilicity of ClogP< 05 (Lipinski *et al*., 1997). Out of ten, only five compounds followed this rule namely; Morin, Matairesinol, Apigenin, Cedronolactone A and Curcumin (Table II) with binding energy of -12.6072, -12.0830, -11.9076, -11.8840 and -11.8678 respectively. Curcumin is a flavonoid that is derived from *Curcuma longa* plant. It has been reported as a therapeutic substance against different viruses including; Chikungunya, dengue and Zika viruses (Mounce *et al*., 2017). Apigenin is also a flavonoid, produced by *Ocimum basilicum*. It is also known for its anti-viral activity against african swine fever virus, hepatitis B virus and adenoviruses (Chiang *et al*., 2005). Morin is also a flavonoid that is isolated from *Maclura pomifera* and reported for its activity against Equid herpesvirus 1 (Gravina *et al*., 2011). Matairesinol is a plant lignin whereas Cedronolactone A is terpenoid in nature, both of have no reported anti-viral activity however their bioactivity is well known (Shoeb *et al*., 2004; Guo *et al*., 2005).

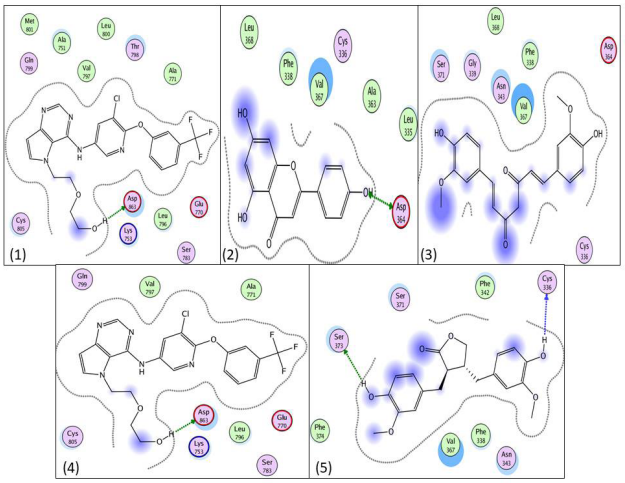

Fig. 1. 2D interaction images of top five phytochemicals, having potential drug-like properties, against COVID-19 spike protein where (1) shows interactions of Morin in which side chain atom, Asp 863 is involved in hydrogen bond interaction, (2) shows interactions of Apigenin in which side chain atom Asp 364 is involved in hydrogen bond formation, (3) shows interactions of Curcumin in which Asp 364 is involved in hydrogen bond formation, (4) shows interactions of Cedronolactone A in which Asp 863 is involved in hydrogen bond interaction and (5) shows interactions of Matairesinol in which Ser 373 and Cys 336 are involved in hydrogen bond interaction.

These five compounds were then assessed for ADMET attributes so that their potential drug-like properties could be evaluated, results of which are shown in Table II. Among ADMET properties; Blood Brain Barrier (BBB) represents the capability of a drug to cross BBB, Gastro-intestinal (GI) tract displays that how well the drug is absorbed in GI tract whilst Caco-2 permeability depicts the ability of a drug to get absorbed in intestine. All of the five compounds were non-carcinogenic in nature and non-permeant towards BBB. All of them were found to be highly absorbent in GI tract. Apigenin, Matairesinol and Cedronolactone A were Caco-2 Permeable whereas Morin and Curcumin were non-permeable for Caco-2.

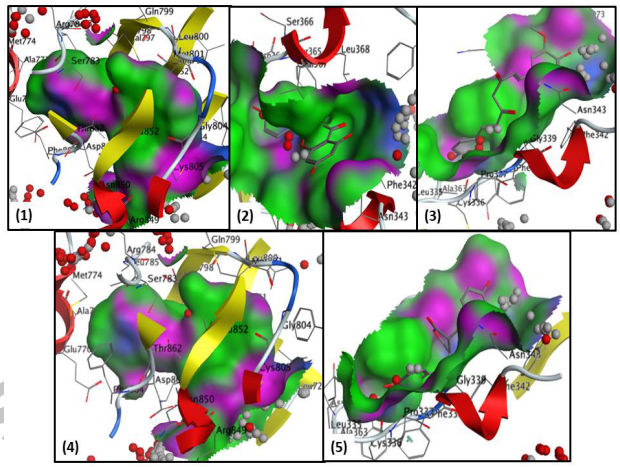

Fig. 2. 3D interaction images of top five phytochemicals, having potential drug-like properties, against COVID-19 spike protein where (1) shows interactions of Morin (2) shows interactions of Apigenin (3) shows interactions of Curcumin (4) shows interactions of Cedronolactone A and (5) shows interactions of Matairesinol.

Molecular Docking analysis has been used previously to predict potential therapeutic agent of COVID-19. Hall and Ji (2020) performed the docking analysis between library of different compounds and two target proteins of COVID-19 i.e. spike glycoprotein, and the 3CL protease. Thuy *et al*. (2020) also performed docking between phytochemicals of garlic (Allium sativum) essential oil and ACE2 receptors of human and main protease enzyme of COVID-19.

CONCLUSION

The COVID-19 is an infectious disease that has infected millions of people globally and it is still spreading all over the world. There is no well-known therapeutic agent currently available to treat this disease. The current study proposed that morin, curcumin, apigenin, cedronolactone A and matairesinol are the phytochemicals that have a potential to constrain the attachment of SARS-

Table I. Top ten phytochemicals with minimum S-score and maximum binding capabilities for spike protein of COVID-19, generated with MOE software.

Sr. no	Pubchem ID	Phytochemical name	S-score	Rmsd value	Interacting residues
1	16129778	Tannic Acid	-14.4967	3.9527	Ala 344, Phe 342, Arg 509, Cys 336
2	72284	Chebulinic Acid	-13.9819	2.2131	Ala 344, Asn 343, Ser 371
3	443652	Oenin	-13.2615	2.0824	Ser 373, Asn 343, Phe 342
4	44583694	Uncarinic Acid C	-12.9015	1.8111	Asp 863
5	5281670	Morin	-12.6072	1.3346	Asp 863
6	119205	Matairesinol	-12.0830	1.3719	Ser 373
7	5280443	Apigenin	-11.9076	0.7896	Asp 364
8	11754814	Cedronolactone A	-11.8840	1.3047	Asp 863
9	969516	Curcumin	-11.8678	0.5795	Asp 364
10	5470299	D-Chicoric Acid	-11.6446	2.4099	Ala 344, Phe 342, Arg 509, Ser 373

Table II. Results of top 10 phytochemicals, examined for Lipinski rule of five by using Molinspiration.

Phytochemical	Molecular weight (g/mol)	Number of hydrogen bond acceptor	Number of hydrogen bond donor	MLogP
Lipinski rule of five	<500	<10	<05	<05
Tannic acid	1701.21	46	25	7.06
Chebulinic acid	956.11	27	13	-0.36
Oenin	493.44	12	7	0.48
Uncarinic acid C	632.88	6	3	7.57
Morin	302.04	7	5	1.64
Matairesinol	358.14	6	2	2.03
Apigenin	270.05	5	3	3.22
Cedronolactone A	478.22	9	3	0.48
Curcumin	368.13	6	2	2.83
D-Chicoric acid	474.08	12	6	1.12

Table III. ADMET profiling enlisting absorption, metabolism and toxicity related drug like parameters of candidate compounds.

Phytochemical	Morin	Apigenin	Curcumin	Matairesinol	Cedronolactone A
A. Absorption					
Blood-brain barrier permeant	No	No	No	No	No
Gastro- intestinal absorption	High	High	High	High	High
Caco-2 permeability	No	Yes	No	Yes	No
P-glycoprotein substrate	No	No	No	No	Yes
B. Metabolism					
CYP450 1A2 inhibitor	Yes	Yes	No	No	No
CYP450 2C19 inhibitor	No	No	No	No	No
CYP450 2C9 inhibitor	No	No	Yes	No	No
CYP450 2D6 inhibitor	Yes	Yes	No	Yes	No
CYP450 3A4 inhibitor	Yes	Yes	Yes	Yes	No
C. Toxicity					
Human either-a-go-go inhibition	No	No	No	Yes	No
Carcinogens	No	No	No	No	No
AMES mutagenesis	Yes	No	No	Yes	No

CoV-2 with human body cells, thereby preventing the body from being infected by it. Additional in vitro and in vivo experiments are highly advised to assess the efficacy of above mentioned phytochemicals.

Statement of conflict of interest

The authors have declared no conflict of interest.

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