Natural compounds as potential inhibitors of novel coronavirus (COVID-19) main protease: An *in silico* study

Amaresh Mishra
Gautam Buddha University, Greater Noida, India
Yamini Pathak
Gautam Buddha University, Greater Noida, India
Vishwas Tripathi (drvishwastripathi@gmail.com)
Gautam Buddha University, Greater Noida, India

Research Article

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Abstract

COVID-19 pandemic, a novel coronavirus disease is caused by severe acute respiratory syndrome corona virus, SARS-CoV-2. It was first reported in Wuhan, China and has now expanded to more than 190 countries across the world. Till date, there is no specific medication available to prevent or target SARS CoV-2 infection. Very recently, the crystal structure of COVID- 19 main protease (M^{pro}) was revealed by Liu et al. (2020). SARS-CoV-2 main protease (M^{pro}) is a key enzyme that plays a crucial role in viral replication and transcription. Thus, M^{pro} could be a promising target to inhibit SARS-CoV-2 infection. Natural compounds due to their structural diversity and safety are considered as an excellent source of antiviral drugs. In this study, we selected Herbacetin, Rhoifolin, Pectolinarin, Apigenin, Luteolin, Amentoflavone, Daidzein, Puerarin, Epigallocatechin, Gallocatechin gallate, Resveratrol, Maslinic acid, Piperine and Ganomycin B to target the SARS-CoV-2 main protease (M^{pro}) using in silico tools. These compounds were examined based on ADME, drug likeness, docking studies, MD simulations using CABS-flex 2.0, and prediction of major toxicity parameters (hepatotoxicity & cytotoxicity) to check the safety aspects of the selected compounds. We also investigated the similarity of these compounds, if any, with FDA approved drugs using Swiss similarity. The docking results were found in the order of Amentoflavone (-9.13 kcal/mol), Ritonavir (-8.52 kcal/mol), Lopinavir (-8.5 kcal/mol), Puerarin (-7.97 kcal/mol), Maslinic acid (-7.97 kcal/mol), Piperine (-7.65 kcal/mol), Gallocatechin gallate (-7.59 kcal/mol), Luteolin (-7.58 kcal/mol), Apigenin (-7.42 kcal/mol), Resveratrol (-7.41 kcal/mol), Herbacetin (-7.4 kcal/mol), Daidzein (-7.32 kcal/mol), Rhoifolin (-6.71 kcal/mol), Ganomycin B (-6.46 kcal/mol), Epigallocatechin (-6.13 kcal/mol), and Pectolinarin (-5.88 kcal/mol). Among these selected natural compounds, Amentoflavone and Puerarin were the two top leads which showed the lowest binding energies. Interestingly, Amentoflavone showed highest binding affinity among all the selected compounds. Our promising findings based on in-silico studies warrants further clinical trial in order to use these compounds as potential inhibitors of SARS-CoV-2 protease.

Introduction

Coronavirus disease (COVID–19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV–2) which primarily affects the lungs and shows pneumonia-like symptoms [1]. SARS-CoV–2 is a novel strain of coronavirus, first noticed in December 2019, during an outbreak in Wuhan, China and subsequently expanded to all over the world in a very short period of time [2]. The World Health Organization (WHO) declared it as a pandemic on 11 March 2020 [3]. As on April 6, 2020, 12,89,380 COVID–19 cases have been confirmed globally with 70,590 deaths

[4]. Till date, we don't have any specific treatment for this ongoing pandemic. Some preliminary study results investigated potential drug combination of Lopinavir and Ritonavir to treat COVID–19 infected patients, which were earlier used in human immunodeficiency virus (HIV) and SARS CoV or Middle East respiratory syndrome (MERS) coronavirus patients [5, 6].

The drugs which can specifically target the virus replication cycle and subsequent infection are urgently required so as to develop the effective antiviral therapies as early as possible. Natural compounds due to the presence of enormous structural and chemical diversity, availability of more chiral centers and relative biosafety are considered as an excellent source of drugs in several diseases including the viral infections. This is further strengthened by the fact that around 45% of today's bestselling drugs have either originated from natural products or their derivatives [7].

Natural compounds having anti-viral property could become a valuable resource in this regard. Liu et al. (2020) crystallized the COVID–19 main protease (M^{pro}), which has been structured and repositioned, in the Protein Data Bank (PDB) and is accessible to the public [8]. SARS-CoV–2 main protease (M^{pro}) is reported to play an inevitable role in virus replication and transcription, suggesting it to be a promising target for inhibition of SARS-CoV–2 cycle [7,9]. Keeping this in mind, in this study we have selected several natural compounds based on extensive literature. [10–12]. Herein, we screened and explored the potential of these selected natural compounds in inhibition of the main protease of novel coronavirus using ADME, drug likeness, specific binding to the main protease active sites using docking studies, MD simulations using CABS-flex 2.0, a coarse-grained simulations study of protein motion, prediction of main toxicity endpoints (hepatotoxicity & cytotoxicity) in order to predict specificity & safety of the selected compounds so as to propose them as potential drug candidate against the novel SARS- CoV–2.

Methods

Proteins/Macromolecules: The crystal structure of COVID–19 main protease Mpro (PDB ID: 6LU7) was obtained from Protein Data Bank (PDB; http://www.rcsb.org/pdb/), in.pdb format. PDB is a database for the crystal structures of biological macromolecules, worldwide [13]. The 6LU7 protein contains two chains, A and B, which form a homodimer. The PDB files to be used under further computational analysis were optimized by Chain A for macromolecule preparation.

Literature search and compound selection: Extensive literature search has been done from the open-source databases like PubMed concerning drugs against noble coronavirus were selected using different queries "coronavirus, their inhibitor, SARS-Cov, anti-viral natural compounds etc." Further, with prior to our interest, top 15 natural compounds obtained from public libraries (Drugbank and PubChem).

3-dimensional (3D) structures of selected natural compounds obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/), in.sdf format. PubChem is a database of chemical molecules consisting of three databases, including substance, compound, and bioassay databases [14]. The compounds used in the present study were ritonavir (CID_392622), lopinavir (CID_92727), herbacetin (CID_5280544), rhoifolin

(CID_5282150), pectolinarin (CID_168849), apigenin (CID_5280443), luteolin (CID_5280445), amentoflavone (CID_5281600), daidzein (CID_5281708), puerarin (CID_5281807), epigallocatechin

(CID_72277), gallocatechin gallate (CID_199472), Resveratrol (CID_445154), Maslinic acid (CID_73659), Piperine (CID_638024), and Ganomycin B (CID_10246918).

ADME compound screening: An in-silico tool for analysis of absorption, distribution, metabolism, and excretion (ADME) was used to screen the above mentioned compounds which could be bioactive via oral administration. Drug-like properties were calculated using Lipinski's rule of five using SWISSADME prediction (http://www.swissadme.ch/). [15, 16].

Determination of Active Sites: Structural analysis of the binding pocket was developed by using CASTp 3.0 software using the http://sts.bioe.uic.edu/castp/ web server [17]. The ligand-binding pocket located in the catalytic site was obtained manually and then verified by a priori docking approach. The determination of the amino acids in the active site was used to analyze the Grid box and docking evaluation results.

Molecular Docking: Molecular docking study was done in order to get insight about the most preferred binding mode of the selected natural compound into the binding active site of enzyme. The ligand binding affinity with the active site of enzyme was confirmed by energy score (S, Kcal/mol), lower energy score indicates better affinity. Molecular docking was performed using the crystal structure of SARS-CoV-2/COVID–19 main protease using Autodock 4.2. The results obtained from the docking simulation were visualized with the Pymol version 1.7.4.5 Edu.

Molecular dynamics (MD) simulation: Structural conformations of proteins were improved by Molecular dynamic (MD) simulation [18]. The structural flexibility of the selected natural compounds was analyzed by CABS-flex 2.0 server [19]. This web server gives RMS fluctuation by 10 ns simulation of all amino acid residues present in a specific protein and considered similar to NMR data [20]. A structural PDB file provided to the server with default parameters for the MD simulation output. This server gives protein structural model on the basis of protein flexibility, contact map and all residue fluctuation between the residues.

Toxicity analysis: Toxicity analysis of selected natural compounds was done by the ProTox-II web server [21]. ProTox-II is a kind of virtual lab which integrates several parameters like molecular similarity, fragment propensities and most frequent features. It predicts various toxicity endpoints and incorporates a total of 33 models for the prediction of various toxicity aspects of small molecules.

Similar FDA approved drug compound search with SWISS similarity: The compounds which were giving the best binding energy among the selected natural compounds were checked for similarity, if any, with FDA approved drugs using SWISS similarity tool (http://www.swisssimilarity.ch). [22].

Results

Determination of Active Sites: Table 1 shows the structure and amino acids found in the active site pockets of 6LU7. 6LU7 is the main protease (Mpro) found in COVID–19, which has been structured and

repositioned in PDB and can be accessed by the public, as of early February 2020.

ADME: Absorption, distribution, metabolism, and excretion (ADME) properties were found by obtaining the canonical smiles from PubChem. These smiles were used to identify ADME properties using SWISS ADME. Then compounds were analyzed on various parameters like lipophilicity, molecular weight, hydrogen-bond donors, hydrogen-bond acceptors, Clog P-value, Ghosh violations, Lipinski violations, etc. Ligands/natural compounds have been selected based on adherence to soft or classical Lipinski's rule of five. The selected ligands that did not incur more than 2 violations of Lipinski's rule were further used in molecular docking experiments with the target protein. The drug scanning results (Table 2) show that most of the tested compounds in this study was accepted by Lipinski's rule of five. These compounds were selected for docking to find their binding affinity with COVID19 main protease M^{pro}. List of compounds with suitable ADME properties given in Table 2.

Molecular docking: Molecular docking is an extensively used in-silico way to predict protein-ligand interaction. To perform the docking analysis, the structures and amino acids found in the active site pockets of 6LU7. 6LU7 is the main protease (Mpro) found in COVID–19, which has been structured and repositioned in PDB databank. Thereafter, Ligand-protein docking was performed and the interactions were determined based on the binding affinity of our compounds. Each individual analysis gave positive results, suggesting that the selected natural compounds may directly inhibit COVID–19 main protease M^{pro}. The 14 selected natural compounds were docked with COVID–19 main protease M^{pro} along with the standard ritonavir and lopinavir to compare the results. Further, like previous other findings, our results also indicated a good binding affinity of ritonavir and lopinavir to the COVID–19 main protease M^{pro}.

The results obtained are in Table 3:

Molecular dynamics (MD) simulation: CABS-flex 2.0 server was used to calculate route mean square fluctuation value (RMSF) of the protein residues of 6LU7 with the selected natural compounds. Residues with the highest degree of flexibility were predicted with the help of this web server. Here, high RMSF value indicates high residue flexibility. The results of this web server for MD simulation study have been proved to be in concordance with NMR measurements. The Comparative analysis of the protein model Vis a Vis fluctuation plot suggests that the residues with higher fluctuation did not form reliable secondary structure. Fluctuating residues represent the coil structure of the 6LU7 protein. The appropriate secondary structure residues with α- helix & β-sheet show a minimal fluctuation. The results of docking analysis suggest that the compounds selected based on detailed literature survey, all of them are showing good binding energy in terms of ligand-protein binding. However, Amentoflavone and Puerarin were the two top leads which showed the lowest binding energies. We perform MD simulation for both compounds based on the RMSF value given in Figure 3.

Toxicity analysis: In-silico toxicities of selected natural compounds were predicted by using ProTox-II. As shown in Table 4, ProTox-II toxicity prediction was done to check the safety of the compounds based on two major toxicity end points, hepatotoxicity & cytotoxicity. According to the toxicity analysis, none of the

selected natural compounds showed potential hepatotoxicity or cytotoxicity except Pectolinarin which showed potential cytotoxicity.

Similarity checks with FDA approved drugs using SWISS similarity: We further checked the similarity of our two top hit natural compounds (Amentoflavone and Puerarin), if any, with the FDA approved drugs using SWISS similarity check. Swiss Similarity web tool is used for rapid ligand-based virtual screening. We did not find any reported similar FDA approved drug in Swiss Similarity database indicating that these compounds could be very important and unique with pharmaceutical perspectives and need to be explored at in vitro and subsequent pre-clinical and clinical trials.

Discussion

The ongoing Covid–19 pandemic caused by SARS-CoV–2 has shattered the whole world and created a situation of public health emergency [2]. Keeping in the mind the immediate urgency, we are in dire need of some effective drug against the novel coronavirus Covid–19. Since the virus is new to the Human population therefore the information regarding the ins and outs of this virus is very limited. Considering the scenario, we can get the possible lead from the old SARS virus (SARS-CoV–1) emerged in 2003. Fortunately, in a recent study Liu et al. (2020) has revealed the crystal structure of SARS-CoV–2/COVID–19 main protease (Mpro/3CL protease PDB-ID–6LU7 [8]. This protease is considered an attractive target as it is essential for virus functionality, replication, and entry competence. The main protease Mpro has been investigated as a potential target to inhibit previous coronavirus infections also like SARS and MERS [12]. Thus, in this study we selected natural compounds based on extensive literature survey taking a lead from old coronavirus infections. We observed their pharmacokinetic properties absorption, distribution, metabolism and excretion (ADME) and drug likeness to target the main protease of SARS-CoV–2. Some of these compounds were earlier found to have antiviral effect against other viruses also. The compounds which satisfied the ADME parameters were further selected for molecular docking. Lopinavir and ritonavir are well known protease inhibitor of HIV [37]. Both drugs were also recommended as repurposed drug in the treatment of SARS and Middle East respiratory syndrome (MERS) [6]. Therefore in this study we have taken these drugs as standard reference drugs to compare the efficacy of the binding of our selected compounds. After identification of the active sites of the SARS-CoV–2 protease (PDB–6LU7), we further performed docking study of our selected compounds as potential inhibitors of the COVID–19 main protease Mpro. The binding energies obtained from docking 6LU7 with selected natural compounds Herbacetin, Rhoifolin, Pectolinarin, Apigenin, Luteolin, Amentoflavone, Daidzein, Puerarin, Epigallocatechin, Gallocatechin gallate, Resveratrol, Maslinic acid, Piperine, and Ganomycin B were—7.4, −6.71, −5.88, −7.42, −7.58, −9.13, −7.32, −7.97, −6.13, −7.59, −7.41, −7.97, −7.65, and −6.46 kcal/mol respectively. The docking analysis in the present study showed the order of inhibition potential of these natural compounds, ranked by affinity (ΔG); in the order of Amentoflavone > Puerarin > Maslinic acid > Piperine> Gallocatechin > gallate > Luteolin > Apigenin > Resveratrol > Herbacetin > Daidzein > Rhoifolin > Ganomycin B > Pectolinarin.

Further, in order to investigate the safety of these natural compounds, we also observed the prediction of two major toxicity endpoints hepatotoxicity & cytotoxicity using ProTox- II, a virtual lab for the prediction of toxicities of small molecules. Our toxicity prediction experiment suggests that none of the selected natural compound were having potential hepatotoxicity and cytotoxicity except Pectolinarin which showed potential cytotoxicity. In order to further validate the findings of our docking results, we used molecular dynamic simulation studies of Amentoflavone and Puerarin based on the RMSF value. Considering the promising results of specific binding of our compounds Amentoflavone, Puerarin, Maslinic acid, Piperine, Gallocatechin gallate, Luteolin, Apigenin, Resveratrol, Herbacetin, and Daidzein targeting the key protease of novel coronavirus SARS-CoV–2, supported by the prediction of pharmacokinetic and toxicity results, these compounds could be proposed as a potential drug candidate against novel coronavirus. Our study warrants further preclinical and clinical trials in order to further validate these potential compounds as an inhibitor of SARS-CoV–2 protease. To sum up, our findings highlight a promising pharmaceutical perspective for targeting main protease of novel coronavirus SARS-CoV–2.

Conclusion

COVID–19 has appeared as a potential threat to human health worldwide. However, till date, no approved drug exists to consider for the treatment of COVID–19. This study aimed to screen the natural compounds based on their pharmacokinetic properties, drug likeness and ability to specifically bind to the active sites of SARS-CoV–2 main protease so that these leads can be proposed as potential inhibitor to check the virus replication cycle. In our in silico prediction experiment, none of the selected compound showed hepatotoxicity and cytotoxicity except Pectolinarin which showed potential cytotoxicity. The compounds which were found to potentially inhibit the viral protease based on the binding energy were Herbacetin, Rhoifolin, Pectolinarin, Apigenin, Luteolin, Amentoflavone, Daidzein, Puerarin, Epigallocatechin, Gallocatechin gallate, Resveratrol, Maslinic acid, Piperine, and Ganomycin B Among these Amentoflavone and Puerarin were the top two leads showing lowest binding energy and satisfying our studied parameters. Therefore, we propose that these natural compounds may further be validated as potential inhibitors of COVID–19 main protease Mpro. Our promising findings based on preliminary in silico analysis could become a basis for further studies at in vitro and in vivo levels in order to use these compounds as potential inhibitors of SARS-CoV–2 protease.

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Tables

Due to technical limitations, Tables 1-4 are provided in the Supplementary Files section.

Figures

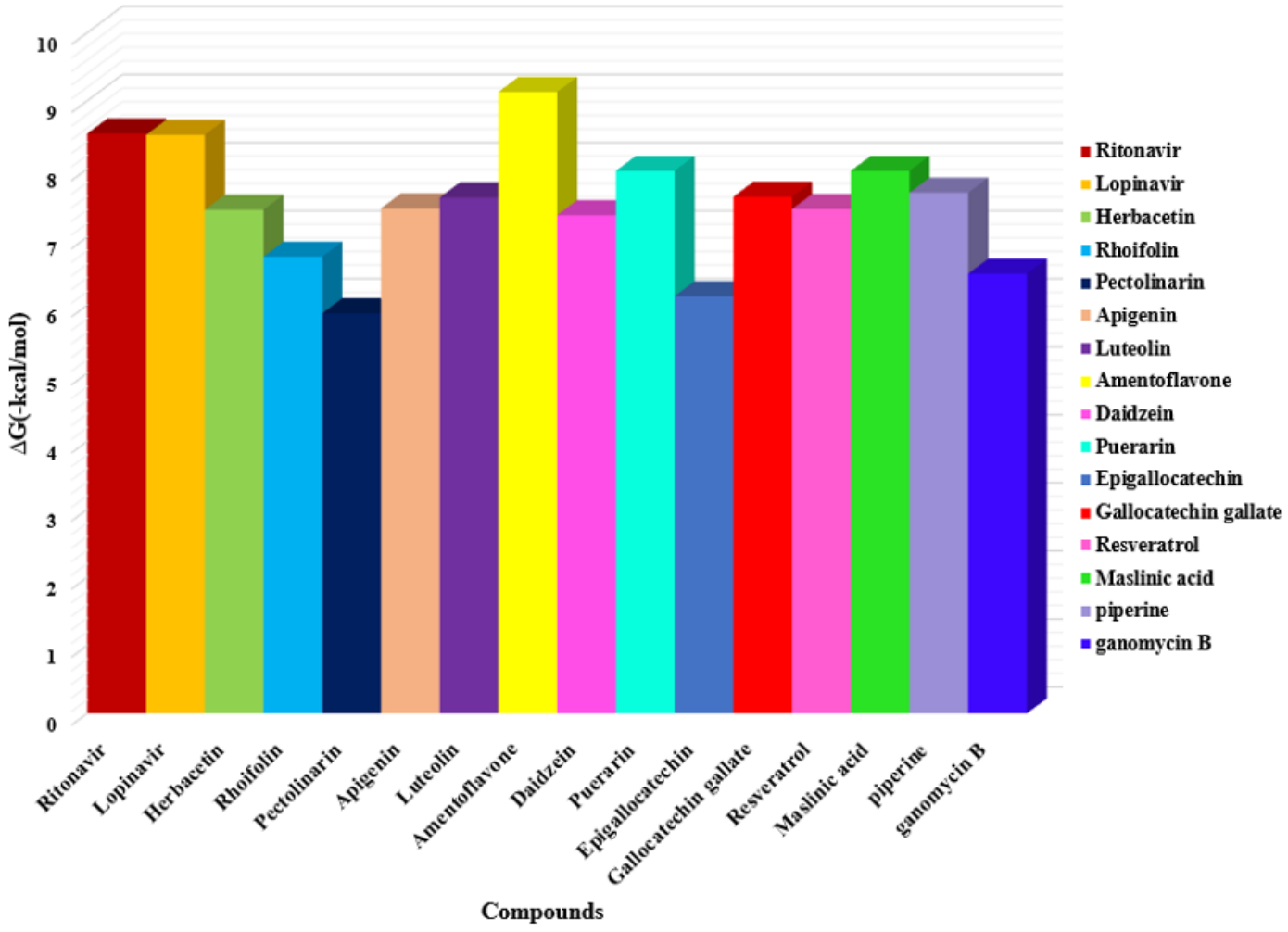


Figure 1

Histogram showing molecular docking results between COVID-19 main protease Mpro (PDB-6LU7) and selected natural compounds (the binding energy value ΔG is shown in minus kcal/mol).

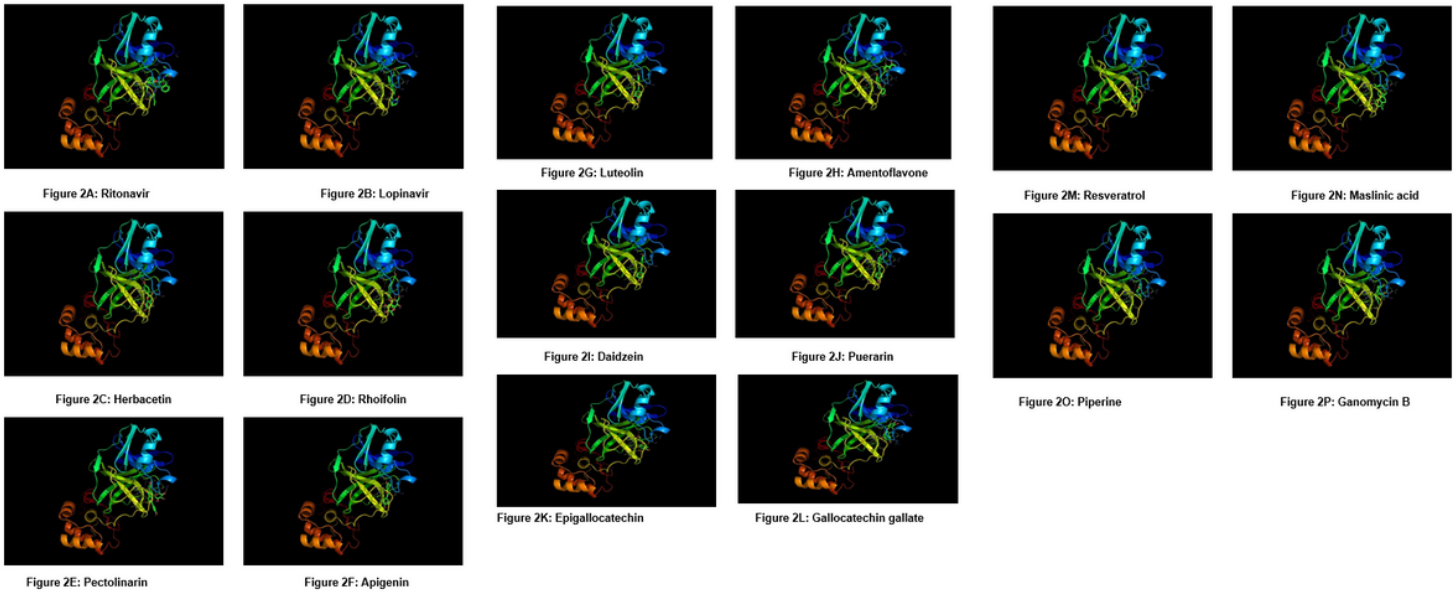
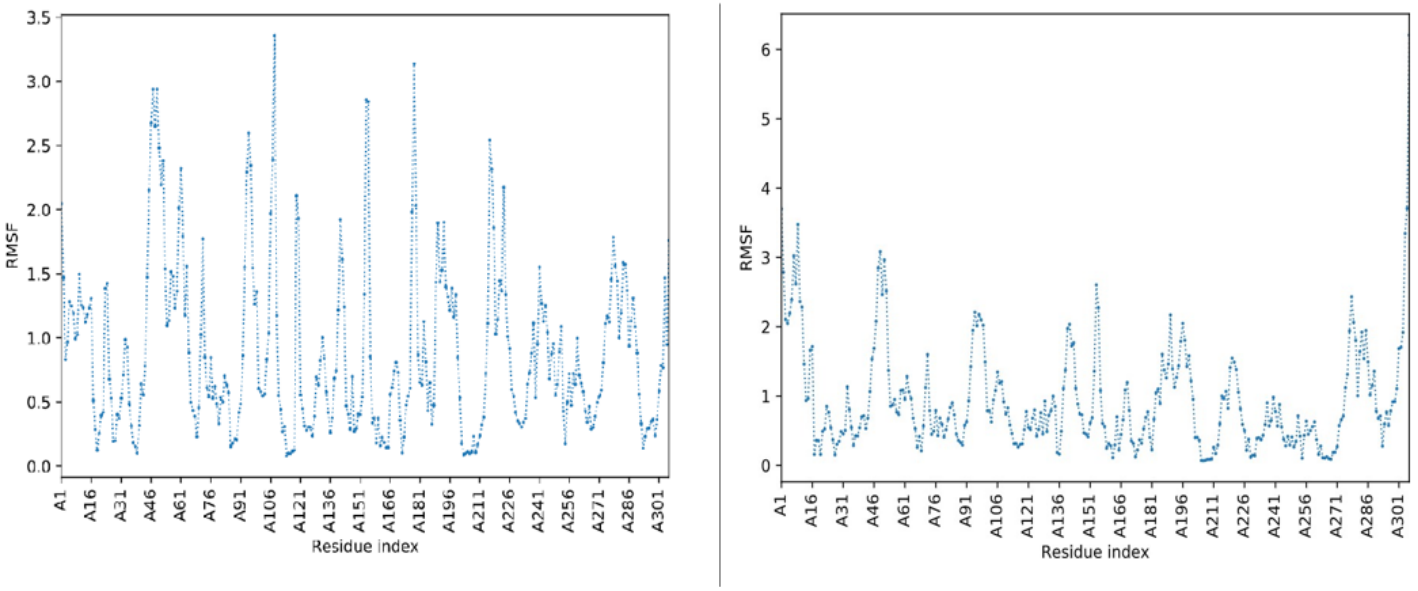


Figure 2

2A to 2P: Docking analysis visualisation of COVID-19 main protease Mpro (PDB- 6LU7) binding with Ritonavir, Lopinavir, Herbacetin, Rhoifolin, Pectolinarin, Apigenin, Luteolin, Amentoflavone, Daidzein, Puerarin, Epigallocatechin, Gallocatechin gallate, Resveratrol, Maslinic acid, Piperine, Ganomycin B and Baicaleinas. The 3D structures of protein-ligand interactions were visualised by pymol software. The binding residues and their chains were identified from the protein-ligand complex as shown in the above images.



(A). Amentoflavone **(B).** Puerarin

Figure 3

CABS-flex predicted molecular dynamics simulation results of selected natural compounds against 6LU7 based on RMSF value.

Supplementary Files

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