

MOLECULAR DOCKING STUDIES ON SCREENING AND ASSESSMENT OF SELECTED BIOFLAVONOIDS AS POTENTIAL INHIBITORS OF COVID-19 MAIN PROTEASESHAILENDRA SANJAY SURYAWANSHI^{1*}, POOJA BHAVAKANA JAYANNACHE², RAJKUMAR SANJAY PATIL², PALLE MS¹, PRIYA SHETTI³¹Department of Pharmaceutical Chemistry, ²Department of Pharmacology, ³Department of Pharmacognosy and phytochemistry. KLE College of Pharmacy, Belagavi, KLE Academy of Higher Education and Research, Nehru Nagar, Belagavi, Karnataka, India. Email: shailendrass80@gmail.com

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ABSTRACT

Objectives: The objective of the study was to screen and assess the selected bioactive bioflavonoids in medicinal plants as potential coronaviruses (CoV) main protease (Mpro) inhibitors using molecular docking studies.

Methods: We have investigated several bioflavonoids which include apigenin, galangin, glycitein, luteolin, morin, naringin, resveratrol, and rutin. Nelfinavir and lopinavir were used as standard antiviral drugs for comparison. Mpro was docked with selected compounds using PyRx 0.8 and docking was analyzed by PyRx 0.8 and Biovia Discovery Studio 2019.

Results: The binding energies obtained from the docking of 6LU7 with native ligand, nelfinavir, lopinavir, apigenin, galangin, glycitein, luteolin, morin, naringin, resveratrol, and rutin were found to be -7.4, -8.3, -8.0, -7.8, -7.3, -7, -7.4, -7.6, -7.8, -6.9, and -9 kcal/mol, respectively.

Conclusion: From the binding energy calculations, we can conclude that nelfinavir and lopinavir may represent potential treatment options and apigenin, galangin, glycitein, luteolin, morin, naringin, resveratrol, and rutin found to possess the best inhibitors of CoV disease-19 main protease.

Keywords: Binding energy, 6LU7, Antiviral, Rutin, Coronavirus, Medicinal plants.

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INTRODUCTION

The 2019-novel coronavirus (nCoV) is a major source of disaster in the 21st century [1]. New CoV strain was identified in Wuhan, China, in the year 2019. CoVs are an infectious agent and found to cause serious diseases of respiratory tract and digestive tract [2]. The Emergency Committee of the World Health Organization declared an outbreak in China on January 30, 2020, which was considered as Public Health Emergencies of International Concern [3]. Officially, the WHO named this disease as CoV disease (COVID)-2019 on February 11, 2020 [4].

At present, no specific therapies for COVID-19 are available and investigations regarding the treatment of COVID-19 are lacking. Potential combinations of protease inhibitor lopinavir/ritonavir, which is commonly used to treat human immunodeficiency virus, for the treatment of COVID-19-infected patients have been investigated and reported. Furthermore, some other reported antiviral drugs such as remdesivir, umifenovir, tenofovir disoproxil, and lamivudine have been reported for COVID-19 [5].

The outbreaks of COVID-19 highlighted their adaptive potential to the changing environmental conditions and they are classified under "emerging viruses." Knowledge about the structure, metabolic pathways of CoV, and pathophysiology of CoV-associated diseases is important to identify possible drug targets [6-9]. Liu *et al.* (2020) have successfully crystallized the main protease (Mpro) from COVID-19, which has been structured and repositioned in the Protein Data Bank (PDB) and is accessible by the public. This protease represents a potential target for the inhibition of CoV replication [10].

Flavonoids are the important class of plant secondary metabolites found to possess wide range of biological activities. These natural products were known for their beneficial effects on health long before

flavonoids were isolated as the effective compounds [11]. Naturally occurring flavonoids with antiviral activity have been recognized since the 1940s and most of the work related with antiviral compounds revolves around inhibition of various enzymes associated with the life cycle of viruses [12]. Antiviral activity of apigenin, galangin, glycitein, luteolin, morin, naringin, resveratrol, and rutin has been reported in different studies [13-20]. Hence, these bioflavonoids were chosen for the present study.

Literature search revealed that selected bioflavonoids have potent antiviral effect against different viruses and may be effective against COVID-19. Hence, there is a need of screening the selected bioflavonoids against molecular targets of COVID-19 using molecular docking techniques. No studies have been reported on molecular docking studies of selected bioflavonoids against selected target of COVID-19. This promoted us to carry out present research work.

METHODS**Standard drugs**

Nelfinavir and lopinavir were used as standard for comparison.

Bioactive bioflavonoids

Apigenin, galangin, glycitein, luteolin, morin, naringin, resveratrol, and rutin used as ligands.

Software

PyRx 0.8, Biovia Discovery Studio 2019, Molsoft, and MarvinSketch.

Determination of drug-likeness properties of selected ligands

In our study, we have selected bioflavonoids as ligands. To find out drug-like properties of each ligand, we have followed the Lipinski's rule of five, which states that molecules with poor permeation and

oral absorption have molecular weights >500, C log p>5, more than 5 hydrogen-bond donors, and more than 10 acceptor groups [16,17]. Data of drug likeness profile of selected bioflavonoids were determined with adherence to Lipinski's rule of five. The canonical simplified molecular-input line-entry system was retrieved from PubChem and used in Molsoft software to obtain drug likeness score [21,22].

Preparation of macromolecules

Structure of COVID-19 3clpro/Mpro (PDB ID: 6LU7) macromolecule was retrieved from PDB (<https://www.rcsb.org/>), website in pdb format. The retrieved protein is associated with water molecules and hetero atoms. All hetero atoms, water molecules and native ligand were removed using Discovery studio 2019 to avoid docking interference and saved in the PDB format. The 6LU7 protein contains two chains, A and C. Chain A contains SARS-CoV-2 main protease enzyme; hence, Chain A was used for macromolecule preparation.

Preparation of ligand

All the three-dimensional structures of the ligand molecules were retrieved from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) in structural data format and converted to PDB format using Discovery studio 2019. In the present study, lopinavir and nelfinavir were used as standard drugs, whereas apigenin, galangin, glycitein, luteolin, morin, naringin, resveratrol, and rutin were used as bioflavonoids.

Determination of active sites

The amino acids in the active site of a protein were determined using the Biovia Discovery Studio 2019. The determination of the amino acids in the active site was used to analyze docking evaluation results [23].

Molecular docking

PyRx 0.8 was used for molecular docking. After the completion of docking, AutoDock preferences were obtained for both ligand and target in PDBQT format. The docking analysis was performed by Biovia Discovery Studio 2019. The pose for minimum binding energy was selected as best interaction.

RESULTS

Drug-likeness properties of selected ligands were calculated. Ligands and drug candidate compounds have been previously selected, based on adherence to Lipinski's rule of five. The drug scanning results were calculated and data are presented in Table 1.

The 6LU7 is the Mpro found in COVID-19, which been structured and repositioned in PDB and can be accessed by the public, as of early February 2020. The PDB ID, structure macromolecules native ligand, and amino acids found in the active site pockets of 6LU7 are presented in Table 2.

Molecular docking analysis of selected bioflavonoids, selected drugs, and its two dimensional interaction with different amino acids on targets is presented in Table 3.

Molecular docking analysis results for several compounds against 6LU7 and its binding energy/Gibbs energy are presented in Table 4. Graph showed molecular docking results between 6LU7 and several drug candidate compounds (the binding energy value ΔG is shown in minus kcal/mol) and presented in Figs. 1 and 2 (a to k) shows the binding between 6LU7 ligands.

DISCUSSION

CoVs are the group of viruses which can infect humans and vertebrate animals. CoV infections affect the respiratory, digestive, liver, and central nervous systems of humans and animals [24]. The present study focused on the main proteases in CoVs PDB ID 6LU7 as potential target proteins for COVID-19 treatment. 6LU7 is the

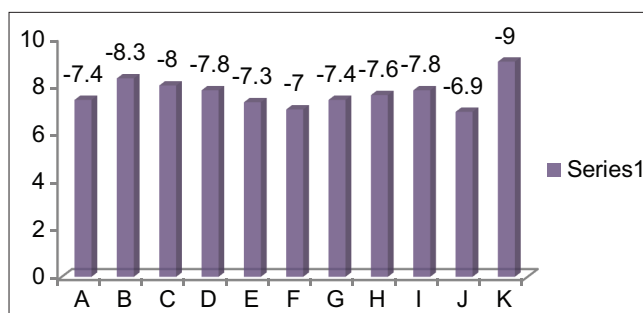
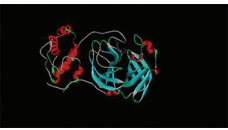
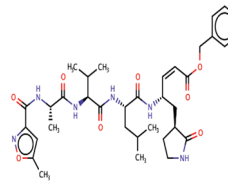


Fig. 1: Graph showing the binding energy value ΔG is shown in minus kcal/mol on Y-axis versus compounds A to K on X-axis. (A - native ligand, B - nelfinavir, C - lopinavir, D - apigenin, E - galangin, F - glycitein, G - luteolin, H - morin, I - naringin, J - resveratrol, K - rutin)

Table 1: Drug-likeness properties of selected drugs and ligands

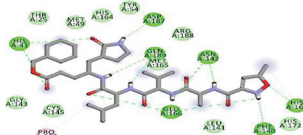
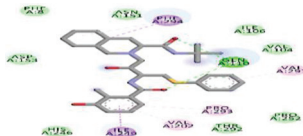
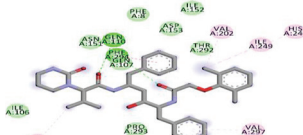
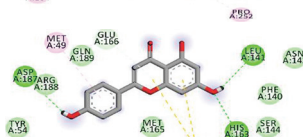
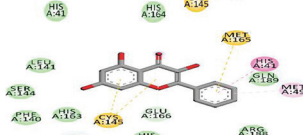
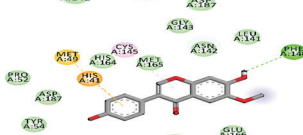
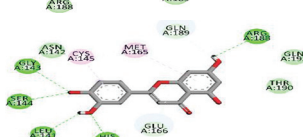
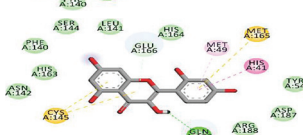
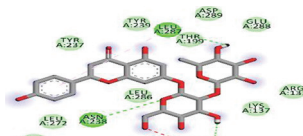
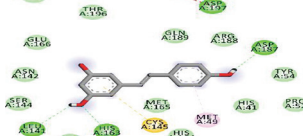
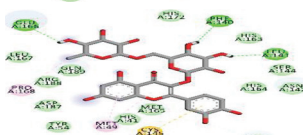
S. No.	Name of the ligands	Mol. formula	Mol. weight	Log p	HBD	HBA	Drug-likeness score
1.	Nelfinavir	C ₃₂ H ₄₅ N ₃ O ₄ S	567.31	5.13	4	6	1.14
2.	Lopinavir	C ₃₇ H ₄₈ N ₄ O ₅	628.36	5.71	4	5	1.10
3.	Apigenin	C ₁₅ H ₁₀ O ₅	270.05	3.22	3	5	0.39
4.	Galangin	C ₁₅ H ₁₅ N ₃ O ₃	257.11	2.68	1	3	0.29
5.	Glycitein	C ₁₆ H ₁₂ O ₅	284.07	2.15	2	5	0.29
6.	Luteolin	C ₁₅ H ₁₀ O ₆	286.05	2.78	4	6	0.38
7.	Morin	C ₁₅ H ₁₀ O ₇	302.04	1.64	5	7	0.46
8.	Naringin	C ₂₇ H ₃₂ O ₁₄	580.18	-0.55	8	14	1.05
9.	Resveratrol	C ₁₄ H ₁₂ O ₃	228.08	2.88	3	3	-1.00
10.	Rutin	C ₂₇ H ₃₀ O ₁₆	610.15	-1.55	10	16	0.91

Table 2: PDB ID, target, native ligand, and active sites

PDB ID	Macromolecule	Native ligand	Active sites
6LU7			THR24, THR26, PHE140, ASN142, GLY143, CYS145, HIS163, HIS164, GLU166, HIS172

PDB: Protein Data Bank

Table 3: Molecular docking analysis data of ligands and target

S. No.	Ligands name	Molecular structure and interaction with 6LU7	Amino acids involved in the interaction
1.	Native ligand		HIS 41, PHE 140, ASN 142, GLU 166, PRO 168, ASP 187, GLN 189
2.	Nelfinavir		GLN 110, VAL 202, ILE 249, PHE 294, VAL 297
3.	Lopinavir		VAL 104, GLN 110, VAL 202, HIS 246, ILE 249, PHE 249, PRO 252, VAL 297
4.	Apigenin		MET 49, LEU 141, CYS 145, HIS 163, ASP 187
5.	Galangin		HIS 41, MET 49, CYS 145, MET 165, GLN 189
6.	Glycitein		HIS 41, MET 49, PHE 140, CYS 145
7.	Luteolin		LEU 141, GLY 143, SER 144, HIS 163, ARG 188
8.	Morin		HIS 41, MET 49, CYS 145, MET 165, GLN 189
9.	Naringin		ASP 197, ASN 238, LEU 287
10.	Resveratrol		LEU 141, CYS 145, HIS 163, ASP 187
11.	Rutin		MET 49, PHE 140, LEU 141, CYS 145, GLU 166, PRO 168

Mpro in COVID-19 that has been structured and repositioned in PDB and has been accessible by the public since early February 2020. The Mpro in CoVs is mainly responsible for proteolytic maturation

of the virus and has been examined as a potential target protein to prevent the spread of infection by inhibiting the cleavage of the viral polyprotein [25].

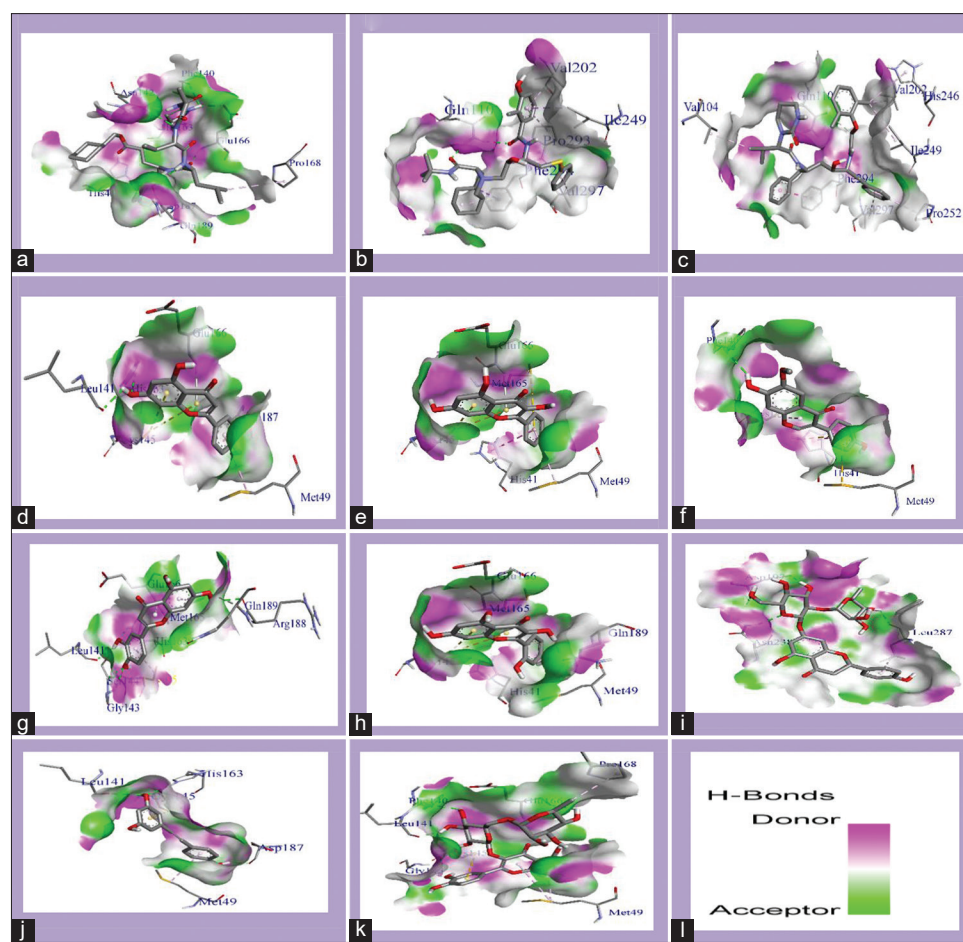


Fig. 2: Docking analysis visualization of 6LU7 binding with (a) native ligand, (b) nelfinavir, (c) lopinavir, (d) apigenin, (e) galangin, (f) glycitein, (g) luteolin, (h) morin, (i) naringin, (j) resveratrol, (k) rutin

Table 4: PubChem ID of ligand and binding energy

Protein	Ligands	PubChem ID	Binding energy (kcal/mol)
6LU7	Native ligand	-	-7.4
	Nelfinavir	92727	-8.3
	Lopinavir	64143	-8.0
	Apigenin	5280443	-7.8
	Galangin	5281616	-7.3
	Glycitein	5317750	-7
	Luteolin	5280445	-7.4
	Morin	5281670	-7.6
	Naringin	442428	-7.8
	Resveratrol	445154	-6.9
	Rutin	5280805	-9

In the present study, we have used nelfinavir and lopinavir as drug standards for comparison. Several bioactive flavonoids, from medicinal plants, have been reported to show antiviral bioactivities [10-12]. We investigated apigenin, galangin, glycitein, luteolin, morin, naringin, resveratrol, and rutin as potential inhibitors of the COVID-19 Mpro. The binding energies obtained from docking 6LU7 with the native ligand, nelfinavir, lopinavir, apigenin, galangin, glycitein, luteolin, morin, naringin, resveratrol, and rutin were -7.4, -8.3, -8.0, -7.8, -7.3, -7, -7.4, -7.6, -7.8, -6.9, and -9 kcal/mol, respectively (Table 4 and Fig. 1).

The docking analysis in the present study showed the inhibition potential of several compounds, ranked by affinity (ΔG); nelfinavir > lopinavir > rutin > apigenin and naringin > morin > luteolin > galangin

> glycitein > resveratrol which were the most recommended flavonoids found in medicinal plants as potential inhibitors of COVID-19 Mpro, which should be explored in future research.

CONCLUSION

Molecular docking studies of apigenin, galangin, glycitein, luteolin, morin, naringin, resveratrol, and rutin were performed using PyRx 0.8 against main protease enzyme of novel CoV. Binding energies obtained by each molecule were compared with standard antiviral drugs nelfinavir and lopinavir. The study concludes that the binding affinity of rutin is higher, whereas resveratrol has lowest affinity among all the compounds. The nelfinavir and lopinavir may represent potential treatment options, and apigenin, galangin, glycitein, luteolin, morin, naringin, resveratrol, and rutin may be recommended as most potential inhibitors of Mpro of CoV. However, further research is necessary to investigate the effect of selected bioflavonoids on other targets of novel CoVs.

AUTHORS' CONTRIBUTIONS

The author declares that all the named authors have contributed equally to this article.

CONFLICTS OF INTEREST

All authors declare that there are no conflicts of interest among the authors.

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