Journal of Ayurveda & Holistic Medicine

www.jahm.co.in

eISSN-2321-1563

ORIGINAL RESEARCH ARTICLE – EXPERIMENTAL OPEN ACCESS

IDENTIFICATION OF POTENT COVID-19 MAIN PROTEASE (MPRO) INHIBITORS FROM FLAVONOIDS*:* AN IN-SILICO APPROACH

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ABSTRACT:

Background: The scientific researches on COVID-19 pandemic topics are headed to an explosion of scientific literature. Despite these global efforts, the efficient treatment of patients is an in-progress challenge. Based on a meta-study of published evidence about the compounds and their plant sources in the last six decades [1,2,3,],we have identified two potential flavonoids with high binding capacity and possible drug candidates against MPRO. Methods: AUTODOCK tools 1.5.6. LigPlot + and Protein-ligand Interaction Profiler was used to visualize the binding interactions. ADMET and drug-likeness study of ligands was done using OSIRIS Property Explorer and admetSAR. MD simulation was performed to check the stability of the ligand-enzyme complexes using UNRES web server. Results: 14 Flavonoids having antiviral properties and 4 existing drugs (Fig. 1) were used as ligands to bind to the COVID-19 main protease. Out of these ligands, flavonoids procyanidin b2 and mangiferin showed high binding affinity with the Mpro having Docking affinity of -9.4 and -8.5kcal/mol respectively. Conclusion: Our results propose that flavonoids such as Procyanidin b2 and mangiferin have a better binding affinity to Mpro of COVID-19 than hydroxyhloroquine, favipiravir and remdesivir. These compounds with good binding potency support the potential as starting points for therapeutics against COVID-19.

Keywords: COVID-19, main protease, Molecular docking, Flavonoids, MD simulations.

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1. INTRODUCTION

COVID-19 presented an unprecedented epidemic in human history that commenced in Wuhan [4,5]..Many awry treatment regimes have been adopted so far whether it was FDA's emergency drug Remdesivir, monoclonal antibody bamlanivimab or molnupiravir, a nucleotide analog[6]. These drugs have iMPROved the outlook of COVID-19 patients but we still have limited data on lingering symptoms of post COVID-19 recovery.

Since then the SARS-CoV-2 pandemic has proceeded in multiple waves and is driven by the emergence of successive variants such as Alpha, in late 2020, and Delta in the early portion of 2021. Until now, Omicron displaced Delta as the most widely transmitted strain since late 2021, which results in millions of infected persons per day [7]. In general, eliminating the source of infections by wearing a mask and disinfecting the virus are the major ways to control the propagation of COVID-19. Additionally, several vaccines and approved drugs have proved to be efficient to protect and treat the disease [8].

In silico based screening has proven to be a very useful tool to meet the challenges of antiviral drug discovery[9]. Natural compounds turned out to be cheaper and safer drug candidates against several diseases [10]. Besides "old" drugs [11,12], flavonoids are of new interest. It has been reported that natural products could offer effective antiviral activity against SARS-CoV-2[13]. Ccomputational methods offer novel reasonable hypotheses for potential drugs against COVID-19, and a virtual screening technique was well developed to estimate the active binding site on the target protease for many natural compounds through molecular docking[14,15].

Flavonoids are naturally occurring polyphenolic biomolecules widely found in plants and perform a wide variety of biological [16]. Many of these are known to be effective antivirals that can act at different stages of viral infection, particularly at the molecular level to inhibit viral growth. In fact, flavonoids lack systemic toxicity, their ability to synergize with conventional drugs has been largely demonstrated and, finally, they are "pleiotropic" compounds, meaning that their functional groups can interact with different cellular targets and intercept multiple pathways[17]. These features make flavonoids potential candidates to interfere with the coronavirus life cycle.

Studies using flavonoids against a wide range of DNA and RNA viruses have been extensively done. In general, flavonoids work by several mechanisms. They can block attachment and entry of viruses into cells, interfere with various stages of viral replication processes or translation and polyprotein processing to prevent the release of the viruses to infect other cells [18].

Covid 19 being multisystemic disease have strengthened the idea of using flavonoids as therapeutic as well as prophylactic options[19]. Post Covid-19 symptoms include damaged lung, brain, kidneys, inflammation, impaired immune system and hemorrhoid disease [20]. Flavonoids have proven efficacy in dealing with these post Covid symptoms [21,22].

Our study is based on emerging concept by focusing on flavonoids which have shown considerable antiviral activities against numerous viral diseases including HIV (Human Immunodeficiency Syndrome), Adenoviruses (ADV), Herpes simplex virus (HSV-1 and HSV-2), Hepatitis C virus (HCV) and poliovirus type 2.

In the current study, we have docked 14 antiviral flavonoids against MPRO using AutoDock Vina and out of these ligands, three potential candidates have been identified. We also docked four drugs; azithromycin, remdesivir, favipiravir and hydroxychloroquine with MPRO which have shown potential against COVID-19 [23]. The importance of MPRO in the life cycle of COVID-19 and its stability inside the coronavirus identifies the MPRO as an attractive target for antiviral drug design. MPRO is a key enzyme of coronaviruses and has a pivotal role in mediating viral replication and transcription. The interruption of its catalytic activity could represent a relevant strategy for the development of anti-coronavirus drugs.

2. METHODS

2.1 Ligand and Receptor molecule Preparation

The crystal structure of MPRO having PDB ID 6lu7 essential for virus replication was retrieved from the protein data bank web site (http://www.rcsb.org/pdb.). Structures of small molecules investigated for docking studies were obtained from https://pubchem.ncbi.nlm.nih.gov/ as SDF form and in the 3D Conformer.

2.2 Molecular docking

To understand the binding interaction of these molecules with COVID-19 virus MPRO, AutoDock Vina[24]was used to predict their binding poses. Docking studies were attempted to explore the binding mode of the suggested protease inhibitors onto the 3D structure of MPRO of COVID-19 using AUTODOCK tools 1.5.6. Before docking, polar-H atoms were added to the MPRO followed by the addition of Kollman charges. The macromolecule file was then saved in pdbqt format and ready to be used for docking. Ligands were also saved in pdbqt format after detecting the torsion angles. Default settings were used for all other parameters. PyMOL4.3.0 (The PyMOL Molecular Graphics System, version2.0Schrondinger, LIC.), LigPlot+ and Protein-ligand Interaction Profiler[25]was used to visualize the binding interactions between these ligands and main protease of COVID-19.

2.3 Visualization of docking results

To analyze the docking results PyMOL 4.3.0and Ligplot + were used. Binding site residues were identified -Protein-ligand Interaction Profiler was used to analyze the binding site residues of azithromycin as ligPlot+ could not plot and show the interacting amino acid residues due to the complex structure of azithromycin.

2.4 Drug-Likeness Prediction

The OSIRIS Property Explorer uses chemical structures and calculates various drug-relevant properties whenever a structure is valid (https://www.organic-chemistry.org/prog/peo/). Prediction results are valued and color coded. Properties analyzed are TPSA, log calculation, log calculation, molecular weight, fragment based drug-likeness, and drug score.

2.5 ADMET Prediction

In this study, we have used the admetSAR prediction tool to study ADMET properties (http://lmmd.ecust.edu.cn:8000/). ADMET properties of a compound include its absorption, distribution, metabolism, excretion, and toxicity in and through the human body. ADMET, which constitutes the pharmacokinetic profile of a drug molecule, is very essential in evaluating its pharmacodynamic activities.

2.6 Molecular Dynamic Simulations

MD simulation was performed for 20 ns to check the stability of the ligand-enzyme complexes using UNRES web server, which offers ~1000-4000-fold speed up in molecular dynamics simulations compared to all-atom approaches [26]. . The UNRES force field has been developed on a solid statistical-mechanical basis, by expanding the potential of mean force of a system containing polypeptide chain(s) in water into cluster-cumulant series and parameterization of the terms of the series (factors) based on simple model system.

3. RESULTS

3.1 Selection of flavonoids for the docking analysis

Through a literature search, it was found that all the selected 14 phytocompounds (Fig. 1) have depicted significant inhibitory potential against several viral diseases, including HIV and Hepatitis, via several ways such as DNA polymerase inhibition, inhibition of reverse-transcriptase and protease inhibition, etc. We have selected 4 standard drugs; favipiravir, remdesivir hydroxychloroquine and azithromycin a semi-synthetic antibiotic for our study as they currently being used for the treatment of COVID-19.

3.2 Molecular Docking

14 Flavonoids and 4 existing drugs were used as ligands to bind to the COVID-19 main protease. Their structures and docking scores are shown in Fig.1 and Table 1, respectively. Out of these ligands, flavonoids procyanidin b2 and mangiferin showed high binding affinity with the MPRO having docking affinity values of -9.4 and -8.5 kcal/mol respectively. Azithromycin showed the affinity -13.4 kcal/mol which took it to the top of all the docked ligands. Other previously proposed inhibitors of COVID -19 like favipiravir, remdesivir and hydroxychloroquine were predicted to show binding with MPRO with docking scores of -7.2, -8.1 and -6.2, respectively.

Table 1. Docking scores of all the ligands with the target protein.

Flavonoid/Ligand	Binding affinity (kcal/mol)
3-Glucosylquercetin	-8.1
Anthraquinone	-7.0
Azithromycin	-13.4
Beta-Amyrinacetate	-7.6
Beta-sitosterol	-7.0

Betulin	-7.0
Catechin	-7.2
Curcumin	-7.0
Dibutylphthalate	-5.3
Epicatechin	-7.1
Epigallactocatechin	-7.2
Mangiferin	-8.5
Procyanidin b2	-9.4
Quercetin	-7.4
Squalene	-5.6
Hydroxychloroquine	-6.2
Favipiravir	-7.2
Remdesivir	-8.1

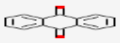
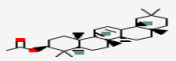
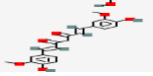
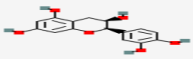
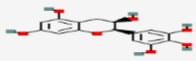
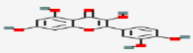
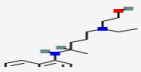
Flavonoid/Ligand	Structure	Target Protein
3-Glucosylquercetin		Covid-19 main protease
Anthraquinone		Covid-19 main protease
Azithromycin		Covid-19 main protease
Beta-Amyrinacetate		Covid-19 main protease
Beta-sitosterol		Covid-19 main protease
Betulin		Covid-19 main protease
Catechin		Covid-19 main protease
Curcumin		Covid-19 main protease
Dibutylphthalate		Covid-19 main protease
Epicatechin		Covid-19 main protease
Epigallactocatechin		Covid-19 main protease
Mangiferin		Covid-19 main protease
Procyanidin b2		Covid-19 main protease
Quercetin		Covid-19 main protease
Squalene		Covid-19 main protease
Hydroxychloroquine		Covid-19 main protease
Flavipiravir		Covid-19 main protease
Remdesivir		Covid-19 main protease

Fig. 1. Various flavonoids and existing drugs used for docking with Covid-19 MPRO.

3.3 Analysis and visualization of best docked ligands

The docking poses of all the ligands were visualized using Pymol, LigPlot+ and Protein-ligand Interaction Profiler. Three ligands with the highest binding affinity to MPRO; azithromycin, procyanidin b2 and Mangiferin were visualized and the binding residues of MPRO in the binding pocket were analyzed. Table 2 shows three lowest binding energy ligands and their binding residues.

Table 2. Target (protein) and the ligands with their best docking score (lowest binding energy) and binding residues.

Flavonoid/Ligand	Binding affinity (kcal/mol)	Binding residues
Azithromycin	-13.4	Tyr54, Asp187, Gly143, Glu166
Procyanidin b2	-9.4	Gln189, His41, Glu166, Gly143, Asn142, Cys145
Mangiferin	-8.5	His41, Gln192, Asn 142, Ser144, Cys145, Gly143, Leu141, Thr190, Arg188

Azithromycin was interacting with Tyr54, Asp187, Gly143, Glu166 residues in the binding pocket of MPRO (Fig.2). The residues Gln189, His41, Glu166, Gly143, Asn142 of binding pocket were responsible for the binding of procyanidin b2 in the binding pocket (Fig.3) and mangiferin showed interaction with His41, Gln192, Asn 142, Ser144, Cys145, Gly143, Leu141, Thr190, Arg188 residues of MPRO (Fig.4).

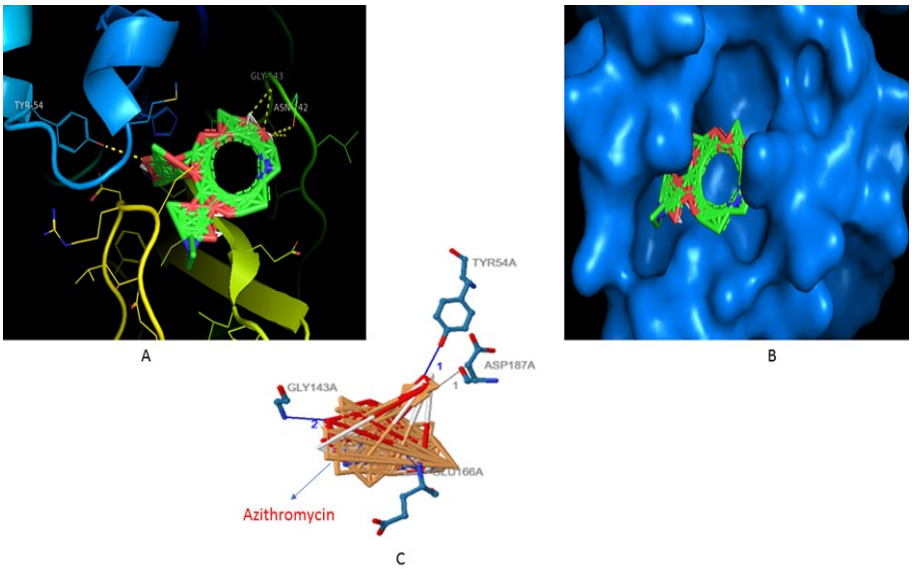


Fig.2. Docking of Azithromycin with the main protease of COVID-19.

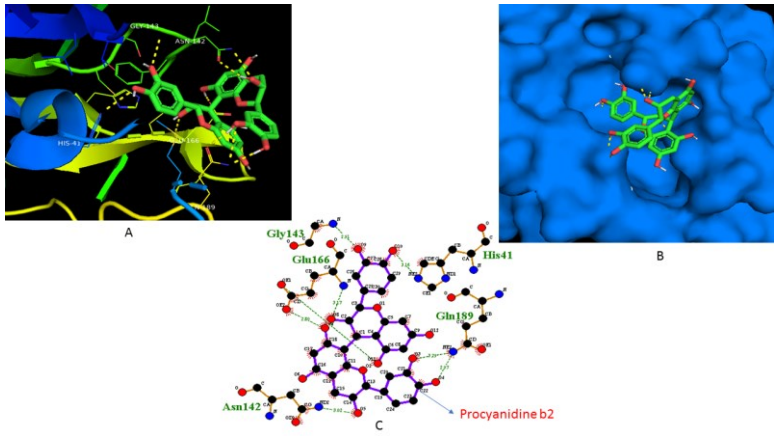


Fig.3. Docking of Procyanidin b2 with the main protease of COVID-19.

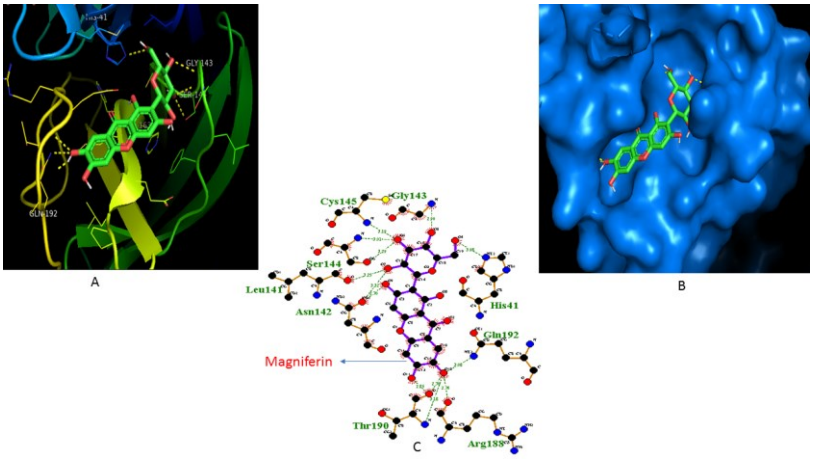


Fig.4. Docking of Mangiferin with the main protease of COVID-19.

Details of H-Bonding between residues in the binding pocket of MPRO with mangiferin, procyanidin b2 and azithromycin are shown in Table 3, Table 4 and Table 5, respectively.

Table 3. Details of H-Bonding between residues in the binding pocket of MPRO with Mangiferin.

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Sidechain	Donor Atom	Acceptor Atom
1	41A	HIS	3.06	3.41	119.02	✖	✔	50 [O3]	419 [N2]
2	46A	SER	2.14	2.70	115.46	✖	✔	91 [O3]	455 [O3]
3	54A	TYR	3.38	3.85	113.21	✔	✔	523 [O3]	49 [O3]
4	142A	ASN	3.68	4.08	127.22	✖	✔	51 [O3]	1213 [O2]

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Sidechain	Donor Atom	Acceptor Atom
5	143A	GLY	2.59	3.10	112.68	✔	✖	1214 [Nam]	93 [O3]
6	144A	SER	2.21	2.90	125.35	✔	✖	1218 [Nam]	93 [O3]
7	145A	CYS	2.47	3.15	125.94	✔	✖	1224 [Nam]	93 [O3]
8	166A	GLU	2.80	3.41	121.08	✔	✖	1390 [Nam]	94 [O3]
9	166A	GLU	3.12	3.85	136.61	✔	✔	1397 [O3]	97 [O3]
10	166A	GLU	2.02	2.92	152.27	✖	✔	96 [O3]	1397 [O3]
11	188A	ARG	2.77	3.65	150.72	✖	✖	99 [O3]	1558 [O2]
12	190A	THR	3.41	3.63	105.79	✖	✖	52 [O3]	1578 [O2]

Table 4. Details of H-Bonding between residues in the binding pocket of MPRO with Procyanidin b2.

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Sidechain	Donor Atom	Acceptor Atom
1	41A	HIS	2.63	3.08	108.73	✖	✔	2391 [O3]	312 [N2]
2	141A	LEU	2.33	3.25	158.02	✖	✖	2394 [O3]	1092 [O2]
3	143A	GLY	2.15	2.90	131.46	✔	✖	1105 [Nam]	2392 [O3]
4	144A	SER	2.33	3.01	125.33	✔	✖	1109	2393 [O3]

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Sidechain	Donor Atom	Acceptor Atom
								[Nam]	
5	145A	CYS	2.52	3.19	125.29	✔	✖	1115 [Nam]	2393 [O3]
6	164A	HIS	2.99	3.86	150.24	✖	✖	2395 [O2]	1266 [O2]
7	189A	GLN	3.11	3.80	129.43	✖	✔	2389 [O3]	1465 [O2]
8	190A	THR	2.51	3.18	125.52	✔	✖	1466 [Nam]	2397 [O2]
9	190A	THR	1.76	2.70	160.48	✖	✖	2397 [O2]	1469 [O2]
10	192A	GLN	2.32	3.08	133.02	✔	✔	1485 [Nam]	2397 [O2]

Table 5. Details of H-Bonding between residues in the binding pocket of MPRO with azithromycin.

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Sidechain	Donor Atom	Acceptor Atom
1	142A	ASN	2.13	3.02	150.05	✔	✔	1103 [Nam]	2403 [O3]
2	142A	ASN	2.24	3.16	156.26	✖	✔	2403 [O3]	1104 [O2]
3	143A	GLY	1.99	2.91	154.48	✔	✖	1105 [Nam]	2407 [O2]
4	144A	SER	3.52	4.03	114.55	✔	✖	1109 [Nam]	2407 [O2]
5	166A	GLU	2.61	3.17	116.32	✔	✖	1281 [Nam]	2406 [O3]
6	166A	GLU	1.87	2.80	159.55	✖	✔	2405 [O3]	1289 [O-]
7	166A	GLU	2.38	2.76	104.23	✔	✔	1288 [O3]	2409 [O3]
8	189A	GL	1.96	2.92	169.14	✖	✔	2401 [O2]	1465 [O2]

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Sidechain	Donor Atom	Acceptor Atom
		N							
9	189A	GLN	2.11	2.97	145.01	✔	✔	1464 [Nam]	2402 [O3]

3.4 Drug-Likeness Prediction Studies

A good drug candidate is absorbed in required time and well distributed throughout the system for its effective metabolism and action. Toxicity is another very important factor which often overshadows the ADME behaviour. Failure of drugs at clinical trial stage due to adverse effects generated because of their toxicity proves very expensive and detrimental in the drug development process. The drug-likeness properties of test compounds with least binding energies predicted using OSIRIS Property Explorer is shown in Table 6. The OSIRIS tool measures the log value (logarithm of compound's partition coefficient between octanol and water) which is a well-established measure of the compound's hydrophilicity.

Table 6. Drug-likeness prediction through OSIRIS Property Explorer

S.N.	Ligand	C log P	log S	Mol.Wt.	TPSA	Drug Score
1	Azithromycin	1.66	-3.09	748	180	0.48
2	Mangiferin	-0.43	-3.16	422	197.3	0.24
3	Procyanidin b2	2.3	-3.06	578	220.7	0.33

Higher log value indicates lower hydrophilicity and, thus, poor absorption and permeation while low log value indicates higher solubility which would enhance the absorption. A lower molecular weight would again enhance the absorption rate and thus most of the drugs are tried to be kept at the lowest possible molecular weight. TPSA or Topological Polar Surface Area indicates the surface belonging to polar atoms in the compound. An increased TPSA is associated with diminished membrane permeability while lower TPSA was favorable for drug-like property. It was also predicted that a molecule with better CNS penetration should have lower TPSA value [27]. Of all the toxicological features predicted; like mutagenicity, tumorigenicity, irritability, and reproductive toxicity, all three i.e azithromycin, mangiferin and procyanidin b2 were found to be free of other advert properties like reproductive toxicity, carcinogenicity. All the test ligands of this study were shown to comply with these properties used to predict drug-likeness but azithromycin due to high molecular weight was not a suitable drug candidate.

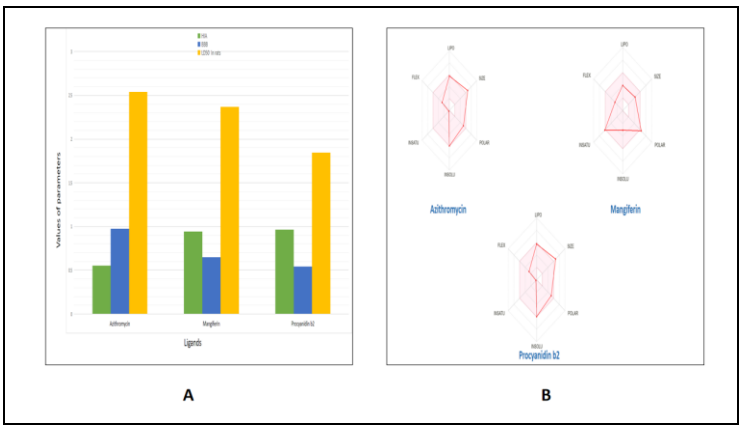
3.5 ADMET Prediction

ADMET properties studied using admetSAR server, revealed that mangiferin and procyanidin b2 had better Human Intestinal Absorption (HIA) score than azithromycin. Greater HIA denotes that the compound could be better absorbed from the intestinal tract upon oral administration. The penetration through the Blood-Brain Barrier (BBB) came out to be best for azithromycin and followed by mangiferin. When it comes to predicting the efflux; azithromycin comes to be substrate and non inhibitor whereas other two were nonsubstrate and noninhibitor. A noninhibitor of CYP450 means that the molecule will not hamper the biotransformation of drugs metabolized by CYP450 enzyme. AMES toxicity test is employed to know whether a compound is mutagenic or not. All the test ligands displayed negative AMES toxicity test which means that the ligands are nonmutagenic. Carcinogenic profile also revealed ligands to be noncarcinogenic.

Important information obtained from admetSAR server was the computed LD50 dose in rat model. Comparing the LD50 doses, a compound with lower dose is more lethal than the compound having higher LD50. From our observations, we found that azithromycin and mangiferin had higher LD50, procyanidin had the lowest LD50 of 1.8446 and was most toxic among the test ligands. Various ADMET parameters obtained from admetSAR tool are shown in Table 7. Fig.6A. depicts the comparative HIA, BBB, and LD50 values of the test ligands.

Table 7. ADMET profile of ligands

Compound	HIA	BBB	CYP inhibition/substrate	AMES toxicity	Carcinogenicity	LD50 in rats
Azithromycin	0.5518	0.9739	Substrate/noninhibitor	Nontoxic	Noncarcinogenic	2.5423
Mangiferin	0.9442	0.6472	Nonsubstrate/noninhibitor	Nontoxic	Noncarcinogenic	2.3664
Procyanidin b2	0.9617	0.5434	Nonsubstrate/noninhibitor	Nontoxic	Noncarcinogenic	1.8446



A B

Fig.6.(A) Comparative HIA, BBB, and LD50 of the test ligands.**(B)** Bioavailability radar (pink area exhibits optimal range of particular property) for studied molecules azithromycin, mangiferin and procyanidin b2 (LIPO = lipophilicity as XLOGP3; SIZE = size as molecular weight; POLAR = polarity as TPSA (topological polar surface area); INSOLU = insolubility in water by log S scale; INSATU = insaturation as per fraction of carbons in the sp3 hybridization and FLEX = flexibility as per rotatable bonds)

The drug-likeness of a particular ligand is shown in a radar-like representation (Fig.6B). The ideal drug candidate must have physicochemical and pharmacochemical values (as depicted by solid red lines) inside the pink-colored hexagonal boundary. Mangiferin has all the drug-likeness parameters, as its values stay inside the pink-colored hexagonal shape. Thus, it has the potential to be developed as a drug candidate. Azithromycin due to high molecular weight is exceeding the size, based on those molecular properties.

3.6 Molecular Dynamic Simulations

All three ligand-enzyme complex were subjected to MD simulation for 20 ns to check the stability of the ligand-enzyme complexes using UNRES web server. All three ligand-enzyme complexes showed fluctuations below 2Å. For validation, MPRO with its natural inhibitor was also subject to MD simulations and RMS fluctuations which also fell under 2Å (Fig.7) providing the evidence of stability of azithromycin, mangiferin and procyanidin b2 docked to MPRO.

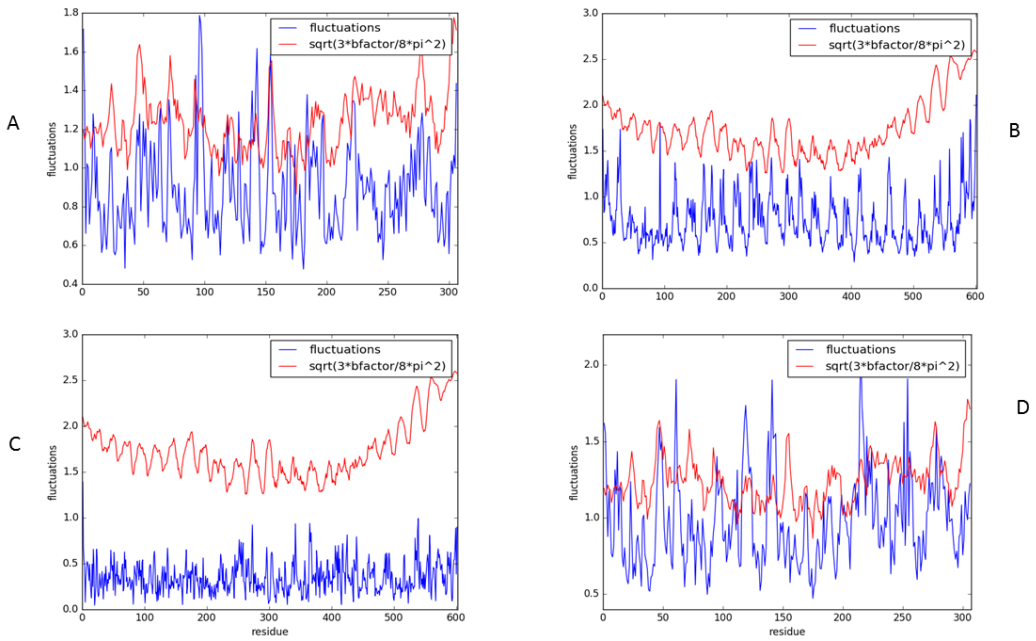


Fig.7. Predicted MD simulation fluctuations and fluctuations derived from B-factors. Left panels show the values of fluctuations in Å. Blue, Fluctuations from MD trajectories; Red line, fluctuations computed from B-factors. (A)Azithromycin-MPRO complex; (B)Mangiferin-MPRO complex; (C) Procyanidin b2-MPRO complex; (D) MPRO with its natural inhibitor N3.

4. DISCUSSION

Newly emerged SARS-CoV-2 at the end of 2019 presented a major threat to human health. After awaited efficacious vaccines additional alternative specific clinical therapeutics are under development for the treatment of SARS-CoV-2-mediated infections. To provide natural scaffolds for drug development, we have screened flavonoids against novel drug target, MPRO.

It has been previously reported that flavonoids exert their antiviral effects via blockage of cellular receptors, inhibiting viral antigenic determinants, loss of enzymatic functions, and/or inhibition of particle biosynthesis. Furthermore, the antiviral activity of many flavonoids has been reported previously against various viral strains [28]. Antiviral natural product-based medicines have also been used for two previous coronavirus outbreaks of SARS-CoV and MERS-CoV which suggest that nature has tremendous potential to provide treatment for the ongoing pandemic of COVID-19. Moreover, flavonoids rutin, baicalin and baicalein, have recently been identified as the novel, natural product inhibitors in COVID-19 *in vitro* [29]. Azithromycin, in our study showed a high affinity of binding to MPRO. While initial studies after the disease outbreak indicated that azithromycin had synergistic effects with other antimalarial drugs in reducing the virus load and bringing about clinical iMPROvement, the concern on its use for the treatment of COVID-19 is now gaining pace [30]. However, a recent study conducted on 11 patients in France showed no rapid anti-viral clearance or clinical benefits in patients with severe infection [31]. Another study [32] showed the addition of azithromycin in combination with antimalarial drugs may induce heart failure and cardiovascular mortality. The use of natural products as a therapeutic for COVID-19 can provide a safer alternative.

Our results indicate that two highly potential flavonoids; mangiferin and procyanidin b2 may show better outcome in treatment of COVID-19. Mangiferin (1,3,6,7-tetrahydroxyxanthone-C2-β-D-glucoside) is a bioactive ingredient predominantly isolated from the mango tree and Procyanidin B2 is found in *Cinchona pubescens*, *Cinnamomum verum*, *Crataegus monogyna*, *Uncaria guianensis*, *Vitis vinifera*, *Litchi chinensis*, apple, and in *Ecdysanthera utilis*. These two flavonoids may have the potential to combat the emergence of COVID-19.

Mangiferin showed polar contacts with His41, Gln192, Asn 142, Ser144, Cys145, Gly143, Leu141, Thr190, Arg188 residues in the binding pocket of MPRO (Table 3). Residues Met165, Glu166 and Gln189 were responsible for the hydrophobic binding of the mangiferin with the binding site. Procyanidin bound to the binding site by residues Gln189, His41, Glu166, Gly143, Asn142, Cys145 (Table 4) and there were hydrophobic interactions through Met165 and Gln189 with distance 3.61 and 3.86 respectively. Met165 and Gln189 form a hydrophobic pocket. Both flavonoids form multiple hydrogen bonds with the main chain of the residues in the substrate-binding pocket, which also helps lock the inhibitor inside the substrate-binding pocket (Fig.8). In azithromycin, binding

residues were Tyr54, Asp187, Gly143, Glu166. In previous studies, it has been seen that the most variable regions were the helical domain III and surface loops, but the substrate-binding pockets located in a cleft between domains I and II are highly conserved among all MPRO of CoV family, suggesting the antiviral inhibitors targeting this site should have wide-spectrum anti-CoV activity.

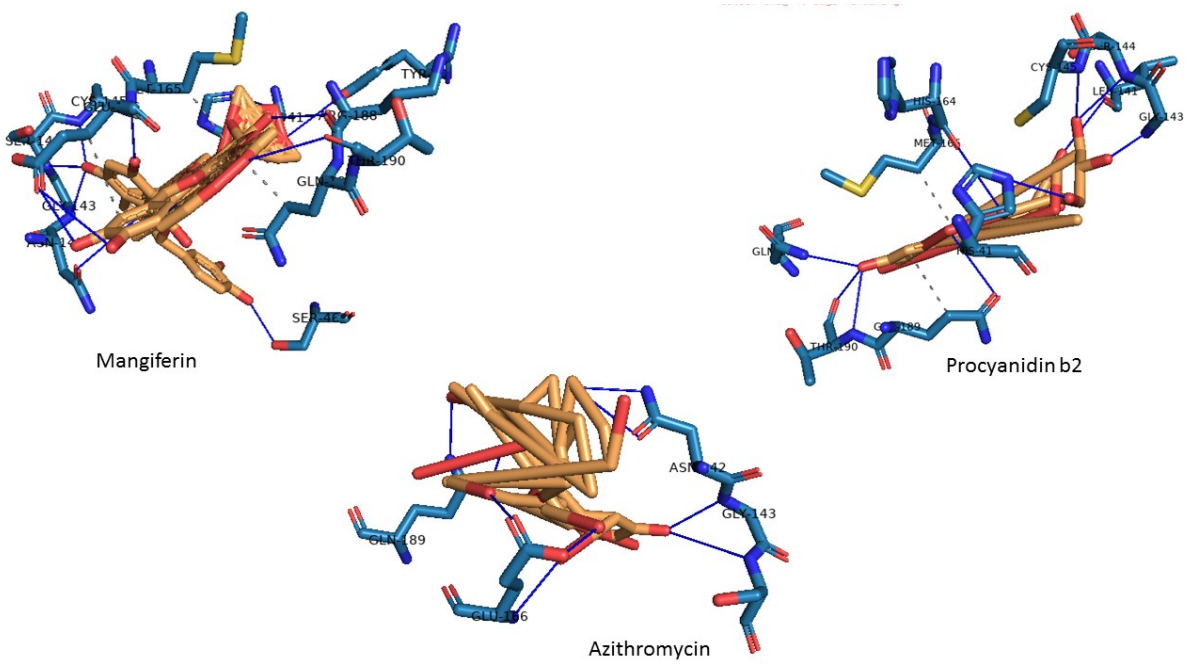


Fig.8. Interactions of mangiferin, procyanidin and azithromycin with MPRO. Blue solid lines indicate H-bonding and black dotted lines show hydrophobic bonding.

To study the stability of these flavonoids, MD simulations were run for 20ns and 1000 steps under 300K temperature condition using Coarse grained force field. All the docked complexes were compared with the fluctuations of the naturally existing enzyme. Two factors were considered for the fluctuation studies; MD fluctuations, B-factor derived fluctuations. The B-factor also termed as temperature factor measures the mobility of atoms. The B-factor reflects dynamic motion, the static disorder of the atom. For both the factors, fluctuations of all three were comparable to those of naturally bound inhibitor and were less than 2Å. However, further studies are needed as fluctuations predicted by B-factors are an accurate measure for stable parts of proteins, but significantly underestimate motion in flexible regions.

5. CONCLUSION

Our results propose that flavonoids such as procyanidin b2 and mangiferin have a better binding affinity to MPRO of COVID-19 than hydroxyhloroquine, favipiravir and remdesivir. Both these compounds bearing good binding potency are components of dietary foods that suggest their biologically safe profile; supporting the potential of these compounds as starting points for therapeutics against COVID-19.

However, further studies should be conducted for the validation of these compounds using *in vitro* and *in vivo* models to pave a way for these compounds in drug discovery.

6. ABBREVIATIONS

SARS-CoV-2- Severe acute respiratory syndrome coronavirus 2

HIV- Human Immunodeficiency Syndrome

ADV- Adenoviruses

HSV- Herpes simplex virus

HCV- Hepatitis C virus

ADMET- Chemical absorption, distribution, metabolism, excretion, and toxicity

TPSA- Topological Polar Surface Area

HIA- Human Intestinal Absorption

BBB- Blood Brain Barrier

7. DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

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CITE THIS ARTICLE AS

Seema Kalra, Anita Chauhan. Identification of potent COVID-19 main protease (MPRO) inhibitors from flavonoids: an in-silico approach. *J of Ayurveda and Hol Med (JAHM)*. 2023;11(2):32-48

Conflict of interest: None

Source of support: None