Letters in Applied NanoBioScience
Open-Access Journal (ISSN: 2284-6808)

Article
Volume 12, Issue 4, 2023, 108
https://doi.org/10.33263/LIANBS124.108

Comprehensive *In silico* Investigation Validates Two Flavonoid Compounds of *Derris robusta* (Roxb. ex DC.) Benth. to Supplant Remdesivir as Natural Therapeutic Remedy Against a Range of Coronaviruses

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Received: 7.05.2022; Accepted: 23.06.2022; Published: 18.09.2022

Abstract: Diversified Coronaviruses like MERS-CoV, SARS-CoV-1, SARS-CoV-2, etc., have badly affected human life by causing various respiratory syndromes. Natural bioactive products like flavonoids are well-known for their anti-viral property. *Derris robusta* (Roxb. ex DC.) Benth. is a reservoir of flavonoids, which encouraged the *in silico* study of the signature flavonoid compounds in it towards investigating the possible inhibitory effect of those flavonoid compounds against the viral replication of MERS-CoV, SARS-CoV-1, and SARS-CoV-2. Therefore, investigating the treatment methodology for alleviating these types of diseases is the ultimate priority for public health. In our *in silico* study, Flavonoids like Isosinensetin and Retusin inhibited the replication of all three viruses with greater binding affinity compared to the control drug Remdesivir in multiple instances. Physicochemical characterization of the compounds by following Lipinski's rule of five and the ADMET study have helped recognize these compounds as a probable natural therapeutic drug against Coronaviruses. The present study exhibits the potential of alternative drug molecules as anti-viral compounds against these three types of Coronaviruses. Further *in vitro* and *in vivo* evaluation followed by clinical trials for developing and successfully implementing these two compounds as an effective inhibitory agents against Coronaviruses can be initiated.

Keywords: molecular docking; ADME study; coronavirus; Isosinesetin; Retusin.

1. Introduction

A broad and diverse group of enveloped viruses with non-segmented, positive-stranded RNA belonging to the Coronaviridae family are called Coronaviruses (CoVs). They cause various diseases, including neurological disorders, bronchial, hepatic, pulmonary, enteric diseases, etc., in humans and animals, with mild to lethal effects leading to death. The size of the viruses generally varies from 80-220nm in diameter, and their genome size ranges between 26-32 kilobases [1]. Host cytoplasm plays a crucial role in their lifecycle as the entire replication cycle occurs there. Among the Coronaviruses, Human Coronavirus 229E, NL63, OC43, HKU1, etc., causes respiratory infections. In the last two decades, three new Coronaviruses named severe acute respiratory syndrome CoV (SARS-CoV-1), severe acute respiratory syndrome CoV (SARS-CoV-2), and Middle East respiratory syndrome CoV

(MERS-CoV) have spread rapidly across the globe causing immense loss to human life. These three types of Coronaviruses belong to the genus Betacoronavirus.

SARS-CoV-2 has emerged as a pandemic and severely damaged the global social, economic, and health sectors [2]. In 2003, Pneumonia, a disease that started to spread across Shunde, a city of Guangdong province, China, was identified as a causative agent of SARS, and WHO named it SARS-CoV. After a gap of nine years, in 2012, a new type of coronavirus was identified in Jeddah, a city in the Hejaz region of Saudi Arabia, which was named MERS-CoV by WHO. Just after seven years, in the month of December, the citizens of Wuhan, China, were badly affected by the Pneumonia disease, which finally came out as a novel strain of another Coronavirus named SARS-CoV-2 by the International Committee on Taxonomy of Viruses (https://en.wikipedia.org/wiki/Coronavirus). The symptoms of SARS-CoV and MERS-CoV are almost similar to headache, muscle pain, fever, cough, Pneumonia, dyspnea, etc., with a reported fatality rate of 11% and 34.3%, respectively. Symptoms of SARS-CoV-2 are usually fever, cough, breathlessness, sore throat, fatigue, etc., with mild effects in some and severe effects in the elderly and patients with comorbidities leading to multi-organ failure. The fatality rate of this disease is between 2-3% [3]. Several vaccines like Adenovirus vector vaccines, mRNA vaccines, inactivated virus vaccines, subunit vaccines, and intranasal vaccines are already available as a preventive measure, but no specific treatment or medicines are available which could treat this disease (https://en.wikipedia.org/wiki/COVID-19_vaccine). Bioactive compounds derived from various herbs, spices, and medicinal plants were also tested against the virus, with some positive effects. This outcome encouraged researchers to explore more natural molecules for treatment and gain immunity since plant-derived bioactive molecules are easily tolerable to the human body compared to their synthetic counterparts. Bioactive groups like flavonoids, tannins, alkaloids, phenols, etc., were useful against different diseases [4].

Flavonoids are one of the most important plant secondary metabolites which act as pigments to attract a pollinator, help in symbiotic nitrogen fixation, may regulate the physiological process, cell cycle inhibitor (https://en.wikipedia.org/wiki/Flavonoid), and antioxidant with multi-enzyme inhibition capability [5]. *Derris robusta*, a leguminous plant belonging to Fabaceae, contains several flavonoids that can be used against many ailments. So, this research was intended to perceive the efficacy of two major flavonoids- Isosinensetin and Retusin as a possible candidate to cure or prevent coronavirus-related disease as detected by a gas chromatography-mass spectroscopic technique employing *in silico* approaches.

2. Materials and Methods

2.1. Collection, extraction, and GC-MS analysis of plant samples.

Fresh leaves of *Derris robusta* were collected from the tea plantation of North Bengal University. Collected plant samples were air dried and turned to form a powder. Being a semi-polar solvent, powdered samples were dissolved in acetone for 48 hours. After filtration, GC-MS analysis was conducted by following the protocol of Ghosh *et al*. [6].

2.2. Evaluation of molecular properties and drug-likeness features.

An e-server resource entitled Molinspiration (http://www.molinspiration.com/) was used to compute the molecular attributes and drug-likeness features of the major query

flavonoid biomolecules identified in *Derris robusta* in the form of parameters like miLogP (partition coefficient), TPSA (topological polar surface area), n-atoms (number of atoms), MW (molecular weight), nON (number of hydrogen bond acceptor), nOHNH (number of hydrogen bond donor), nrotb (number of rotatable bonds) and molecular volume along with calculation of bioactivity scores in respect of being effective as a G-protein coupled receptor (GPCR) ligand; ion channel modulator; kinase inhibitor; nuclear receptor ligand; protease and enzyme inhibitor [7]. The query compounds were subjected to further *in silico* exploration employing another e-server named Molsoft program (http://www.molsoft.com/), which is supposed to provide a drug-likeness model score of the respective test molecules along with a graphical representation of biomolecular properties.

2.3. Selection and modeling of protein and ligand structures.

The major flavonoid compounds detected in *Derris robusta* by GC-MS were considered for molecular docking analysis against a range of Coronavirus (SARS-CoV, MERS-CoV, and SARS-CoV-2) proteins as a probable target for promising drug development, namely, two RNA binding domain nucleocapsid proteins (N protein), main protease of SARS-CoV; N-terminal domain, two receptors binding domain spike protein of MERS-CoV and two nonstructural proteins, 3CL protease (3cl pro) of SARS-CoV-2. The high-resolution X-ray diffraction crystal structures of the two RNA binding domain of the SARS-CoV Nucleocapsid protein (PDB ID: 2OFZ; 1.17 Å resolution, 2OG3; 1.85 Å resolution) and main protease (Mpro) (PDB ID: 2HOB; 1.95 Å resolution), N-terminal domain of MERS-CoV spike protein or S1-NTD protein (PDB ID: 6PXH; 2.30 Å resolution); S1-RBD protein (PDB ID: 4L72; 3.00 Å resolution) and RDB of spike glycoprotein (PDB: 4L3N; 2.13 Å resolution), two nonstructural ribosomal proteins of SARS-CoV-2 (PDB ID:7K5I; 2.90 Å resolution, 7K3N; 1.65 Å resolution) and 3CL protease or main protease (6M2N; 2.20 Å resolution) were selected and downloaded from Protein data bank. To have a smooth interaction, water molecules were deleted from PDB files, and polar hydrogen and Kollman charges were added in AutoDock tools. The 3D structure of the selected flavonoid compounds was extracted from NCBI PubChem (https://pubchem.ncbi.nlm.nih.gov/). Molecular docking was performed using AutoDock Vina, utilizing the software Ligplot+ for interaction studies.

2.4. Evaluation of ADME-Tox status.

The ADME (absorption, digestion, metabolism, and excretion) and toxicity (mutagenic, tumorigenic, and irritant) features of query drug molecules can be correlated with biological efficiency and metabolic output. Therefore, evaluating ADME-Tox properties would decipher a clear idea of the anticipated side effects and the associated health impact. ADMET analysis was done by employing vNN-ADMET (Variable Nearest Neighbor ADMET) (https://vnnadmet.bhsai.org/vnnadmet/home.xhtml) about the methodology of 8. Schyman *et al*. [8] predict the range of liver toxicity, metabolic function, membrane transport features, and minimum recommended therapeutic dose of the concerned test molecules.

3. Results and Discussion

3.1. Investigation of metabolites through gas chromatography-mass spectrometry (GC-MS).

This study aimed to identify bioactive compounds found in *Derris robusta* by using GC-MS. GC-MS analysis detected a total of 28 compounds with 30 different peaks, of which two flavonoids (Isosinensetin and Retusin) were abundant with a major peak area of 16.66% and 19.41%, respectively. Molecular docking analysis was done with the two compounds as a ligand against the selected MERS-CoV, SARS-CoV, and SARS-CoV-2 proteins. Isosinensetin and Retusin were previously reported from a fungus (*Cordyceps militaris*) and higher plants [9,10]. These compounds have antioxidant, anticancer, anti-viral, anti-inflammatory, bacteriostatic, anti-HIV, and antimicrobial activity [11-13]. Several fatty acids (linoleic acid; 9-octadecanoic acid; 9,12,15-Octadecatrienoic acid, methyl ester, (9Z,12Z,15Z)-), alkane (nonacosane; docosane; undecane) and terpenoids (α-Bisabolol oxide; α-tocospiro-B; β amyrin; neophytadien, etc.) were also detected in this study, which has various bio-activities [6,14,15].

3.2. Physicochemical properties of test drug molecules.

The pharmaceutical and physiochemical traits of query compounds, namely Isosinensetin and Retusin, including the positive control drug Remdesivir were scrutinized through *in silico* technique for evaluation of miLogP (Octanol-water partition coefficient) value, molecular weight, count of Hydrogen bond acceptors and donors in addition to the number of rotatable bonds. These manifold features were assessed in accordance with Lipinski's rule of five, which envisages the drug-likeness characteristics of the prospective anti-COVID molecules, with the corresponding numerical values being depicted in multiples of five. Lipinski's rule of five affirms that potent compounds anticipated to be effective as drug molecules should possess superior plasma membrane permeability with a measure of LogP≤5, molecular weight≤500, number of hydrogen bond acceptors≤10 along with a numeral count of hydrogen bond donors≤5 to be effective in cell function modulation. Molecules violating more than one of these parameters may be considered less likely than drug targets.

The compounds Isosinensetin and Retusin detected in *Derris robusta* were evaluated against diverse parameters after adjudging them as tentative drug targets. Isosinensetin and Retusin exhibited no instance of Lipinski's rule violation, while the standard drug Remdesivir violated Lipinski's rule in two instances owing to higher molecular weight and surplus hydrogen bond acceptor count (Table 1). The biophysical scores of the query molecules are as with partition coefficient (miLogP) value ranging between 3.11-3.19, molecular weight (MW) lying between 358.35-372.37 KDa, topological polar surface area (TPSA) value ranging between 76.38-87.38 Å, numerical count of hydrogen bond donor and acceptor (noHNH and nON) lying between 1-7, number of rotatable bonds (RB) being investigated to be in between 5-6 in addition to the molecular volume being scrutinized to be less than 327.72 g/mol. These results show Isosinensetin and Retusin to be comparatively superior to Remdesivir (Table 1). The number of rotatable bonds is a critical factor for determining conformational flexibility in drug compounds that affects the binding mechanism of ligand molecules to respective receptor proteins. Priya *et al*. [16] earlier reported that in order to qualify the drug for the oral bioavailability criterion, the count of rotatable bonds in a drug target should be ≤10. Our tested molecules showed exactly similar results.

The drug-likeness characteristics of the prospective test compounds through calculation and study of bioactivity scores related to its efficacy as GPCR (G-protein coupled receptor) ligand; ion channel modulator; kinase inhibitor; nuclear receptor ligand; protease and enzyme inhibitor too portrayed analogous results concerning the positive control drug Remdesivir. Bioactivity scores concerning these features can be considered significant for positive values exceeding +0.2, with scores higher than +0.5 depicting extremely noteworthy results, whereas negative value depicts insignificant output. GPCR ligand, ion channel modulator, and protease inhibitor parameter were investigated to exhibit negative scores for both the test molecules Isosinensetin and Retusin, while Remdesivir displayed negative values for ion channel modulation activity and nuclear receptor ligand feature. Higher degrees of nuclear receptor ligand potential, including kinase and enzyme inhibition ability, were noted in retusin compared to isosinensetin, validating their effectiveness as potential drug molecules. Remdesivir exhibited a significant GPCR ligand score in addition to high protease and enzyme inhibition potential (Table 1).

Table 1. Physicochemical characterization of query drug targets.

Drug evaluation		**Isosinensetin**	**Retusin**	**Remdesivir**
Molecular physicochemical property	miLogP	3.19	3.11	2.82
	TPSA (Å)	76.38	87.38	203.57
	n-atoms	27	26	42
	MW(g/mol)	372.37	358.35	602.59*
	Number of HBA	7	7	>10*
	Number of HBD	0	1	5
	n-violations	0	0	2
	Number of rotatable bonds	6	5	14
	Volume (g/mol)	327.72	310.20	523.04
Bioactivity scan	GPCR ligand	-0.12	-0.13	0.27
	Ion channel modulator	-0.07	-0.22	-0.35
	Kinase inhibitor	0.11	0.12	0.20
	Nuclear receptor ligand	0.04	0.13	-0.48
	Protease inhibitor	-0.23	-0.25	0.49
	Enzyme inhibitor	0.09	0.14	0.38
Drug- likeness	MolLogP	3.57	2.88	2.44
	MolLogS	-3.96	-3.17	-2.68
	MolPSA	59.71A2	67.49A2	160.14A2
	MolVol	384.66A3	366.93A3	559.60A3
	pK_a of most Basic/Acidic group	<0. /32.01	<0. / 10.27	4.35/14.59
	BBB score	3.65	2.95	1.02
	Number of stereo centers	0	0	5
	Drug-likeness model score	0.18	0.23	0.13

*Violation of Lipinski's rule of five

3.3. Study of Molsoft computed molecular properties of query drug compounds.

Figure 1 portrays the drug-likeness model scores besides the graphical representation of the test molecules, including the positive control drug Remdesivir calculated through the Molsoft software program. The Blue colored line demonstrates drug-like behavioral features, and the green colored line indicates non-drug-like traits. Drug-like molecules usually show positive values in the drug-likeness model score, while non-drug-like molecules display zero or negative values [17]. The query compounds were found to possess positive drug-likeness model scores, with Retusin (0.23) exhibiting a higher score than Isosinensetin (0.18). Both the test drug molecules portrayed higher drug-likeness model scores compared to Remdesivir (0.13), which estimates their potential as effective anti-COVID molecules. *Derris robusta*, a reservoir of these natural products, has prospects of validation through *in vitro* and *in vivo* assays. The remaining parameters were similar to the results computed through the

Molinspiration program, in addition to the blood-brain barrier (BBB) score for the query compounds being in the medium range and comparatively higher than the standard positive control drug Remdesivir. The BBB score denotes the likeness of the drug molecule to cross the blood-brain barrier border and bring about therapeutic modifications.

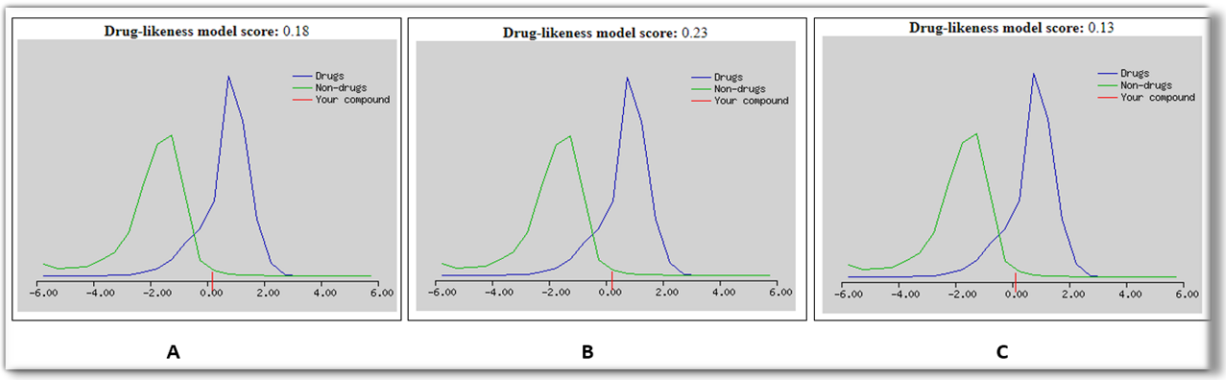

Figure 1. Drug-likeness model score of A=Isosinesetin; B= Retusin; C=Remdesivir

3.4. Molecular docking of Remdesivir, Isosinensetin, and Retusin against MERS-CoV proteins.

Furin, a protease enzyme, activates the MERS-CoV, which helps merge with the cell membranes and enter the host body. (https://news.cornell.edu/stories/2014/10/study-reveals-how-deadly-MERS-COV-virus-enters-human-cells). The entry of MERS-CoV to the host body happens through type I transmembrane glycoprotein (S protein) [18]. Molecular docking of Remdesivir, Isosinensetin, and Retusin was executed at the active site of the N-terminal domain, and two receptors binding domain spike protein of MERS-CoV (PDB ID: 6PXH; 4L72; 4L3N). The whole binding energy scores of two phytocompounds and one control compound have been provided in Table 2. Isosinensetin exhibited the best binding energy score of -6.5kcal/mol and -7.1kcal/mol against NTD-S protein (PDB ID: 6PXH) and RBD-S protein (PDB ID: 4L3N), respectively compared to control compound Remdesivir. A detailed investigation showed that Isosinensetin interacted with residues of the spike proteins of Leu273(A), Trp629(A), and Thr489(A) with NTD and RBD, respectively. Molecular interactions of Retusin tangled the residues Arg62(A), Gln78(B), Asn66(A), Nag1(E) of NTD-S; Try540(B) of RBD, and Leu495(B), Thr533(B) of RBD. Whereas the interaction of the control compound Remdesivir involved with the residues Nag1(E), His 81(B) of NTD; Glu206(A), Try662(A), Asn710(A) of RBD, and Edo1000(B) of RBD of the MERS-CoV proteins (Table 3). In this study, Remdesivir was chosen as a control compound because it is being used as an anti-viral drug [19]. Remdesivir showed a binding energy score of -6.5 kcal/mol, -8.0 kcal/mol, and -6.9kcal/mol with the RBD of the MERS-CoV proteins. It is important to observe that the compound Isosinensetin and Retusin showed almost similar or higher binding affinity compared to Remdesivir [(Table 2, Table 3, Figure 2, Figure 3) and (Figure S1 and Figure S4)].

3.5. Molecular docking of Remdesivir, Isosinensetin, and Retusin against SARS-CoV-1 proteins.

The Spike protein of SARS-CoV-1 binds with the human angiotensin-converting enzyme 2 (ACE2) [20]. Suitable inhibition of SARS-CoV could be possible when the

functioning of viral Mpro and N-protein is stopped, thereby blocking viral replication [21]. Our molecular docking study of two compounds disclosed exciting information about their inhibitory properties. A comprehensive data of the binding energy scores are listed in Table 2. Both compounds show binding energy scores greater than -6.4 kcal/mol. Isosinensetin and Retusin have been found as good inhibitors against the active binding site of RBD of nucleocapsid protein (PDB ID: 2OFZ; 2OG3) and main protease (PDB ID: 2HOB) with a corresponding binding energy score of Isosinensetin being -8.0 kcal/mol, -6.9 kcal/mol and -6.4 kcal/mol besides binding affinity of Retusin being deciphered to be -7.7 kcal/mol, -7.0 kcal/mol and -6.7 kcal/mol against the respective proteins (Table 2). Interestingly the control compound Remdesivir showed either less or similar binding affinity against the two nucleocapsid proteins 2OFZ (-7.3 kcal/mol) and 2OG3(-7.0 kcal/mol) in comparison to the two query molecules.

Compound Isosinensetin interacted with viral nucleocapsid proteins 2OFZ and 2OG3 via binding to Lys128(A) and Arg69(A) amino acid residue, respectively. Retusin interacted with the constituting amino acid residues of N-protein through binding with Arg66(A), Phe67(A), and Try124(A) of 2OFZ and Arg66(A) of 2OG3 (Table 3). The binding efficacy of the anti-viral drug, Remdesivir was also predicted by molecular docking, and the binding affinity values were found to be -7.3 kcal/mol; -7.0 kcal/mol, and -8.1 kcal/mol against the active pocket of the two nucleocapsid proteins (2OFZ and 2OG3) and Mpro with corresponding interaction with the residues His-1(A) and Phe67(A) of 2OFZ; Gly130(A), Ile131(A) and Phe67(A) of 2OG3 including Asp295(A), Asn151(A) and Thr111(A) of 2HOB [(Table 2, Table 3, Figure 2, Figure 3) and (Figure S2 and Figure S5)].

3.6. Molecular docking of Remdesivir, Isosinensetin, and Retusin against SARS-CoV-2 proteins.

The attachment of SARS-CoV-2 initiates through the binding of its spike protein with the receptor-binding domain (RBD) of the human receptor angiotensin-converting enzyme 2 (ACE2) [21]. The binding energy scores of the two compounds, namely Isosinensetin and Retusin, to the active binding site of SARS-CoV-2 proteins have been summarized in Table 2, with equivalent binding energy scores being estimated to be greater than 5.6 kcal/mol depicting the remarkable binding potential of the query molecules to the concerned viral peptides. The calculated binding energy scores of Isosinensetin against ribosomal protein targets 7K5I and 7K3N besides main protease 6M2N were -8.8 kcal/mol; -5.6 kcal/mol, and -6.8 kcal/mol, respectively; whereas, Retusin displayed binding affinity values of -9.0 kcal/mol and -5.6 kcal/mol against corresponding ribosomal proteins 7K5I and 7K3N along with a portrayed binding energy score of -7.0 kcal/mol against Mpro (6M2N) enzyme of SARS-CoV-2 (Table 2). Isosinesetin was found to be associated with the residues a670(2) and a664(2) of 7K5I; Arg15(A), Gln 54(A), and Gln 13(A) of 7K3N in addition to Ser121(C) of 6M2N. Retusin was involved in interaction with the residues g6(2) and c14(2) of 7K5I; Arg15(A), and Glu46(A) of 7K3N besides binding to Thr26(D) residue of 6M2N (Table 3). Remdesivir, employed as an anti-viral drug, can inhibit viral growth and replication [19]. The binding technique of this standard drug with SARS-CoV-2 concerned proteins was compared to the binding pattern of the two studied flavonoid compounds in *Derris robusta*. It showed distinguishable binding affinity against the ribosomal proteins, although near the scores of -10.2 kcal/mol in interaction with g6(2), c14(2), u5(2), and g16(2) of 7K5I including -5.8 kcal/mol on account of interaction with Thr94(A), Lys63(A), Gln87(A) of 7K3N, in addition to an estimated binding score of -

8.3 kcal/mol by interacting with the residues Asp295(A), Thr292(A), Thr111(A), Asn151(A) of Mpro (6M2N) enzyme respectively [(Table 2, Table 3, Figure 2, Figure 3) and (Figure S3 and Figure S6)].

Table 2. Binding energy scores (kcal/mol) of the bioactive compounds with MERS-CoV, SARS-CoV-1, and SARS-CoV-2 proteins.

Diseases	Proteins	Remdesivir	Isosinensetin	Retusin
MERS-CoV	6PXH	-6.5	-6.5	-6.3
	4L72	-8.0	-7.0	-7.5
	4L3N	-6.9	-7.1	-6.8
SARS-CoV-1	2OFZ	-7.3	-8.0	-7.7
	2OG3	-7.0	-6.9	-7.0
	2HOB	-8.1	-6.4	-6.7
SARS-CoV-2	7K5I	-10.2	-8.8	-9.0
	7K3N	-5.8	-5.6	-5.6
	6M2N	-8.3	-6.8	-7.0

Table 3. Interaction profiling of concerned compounds with MERS-CoV, SARS-CoV-1, and SARS-CoV-2 proteins.

		Remdesivir			Isosinensetin			Retusin		
Disease	**Protein**	**No. of H-bond**	**Interaction with**	**No. of hydrophobic bond**	**No. of H-bond**	**Interaction with**	**No. of hydrophobic bond**	**No. of H-bond**	**Interaction with**	**No. of hydrophobic bond**
MERS-CoV	**6PXH**	3	Nag1(E), His81(B)	8	1	Leu273(A)	10	4	Arg62(A), Gln78(B), Asn66(A), Nag1(E)	3
	4L72	4	Glu206(A), Try662(A), Asn710(A)	9	1	Trp629(A)	8	2	Try540(B)	10
	4L3N	1	Edo1000(B)	13	1	Thr489(A)	14	3	Leu495(B), Thr533(B)	8
SARS-CoV-1	**2OFZ**	4	His-1(A), Phe67(A)	11	1	Lys128(A)	10	3	Arg66(A), Phe67(A), Try124(A)	7
	2OG3	5	Gly130(A), Ile131(A), Phe67(A)	12	1	Arg69(A)	10	3	Arg66(A)	11
	2HOB	4	Asp295(A), Asn151(A), Thr111(A)	13	----	----	9	----	----	12
SARS-CoV-2	**7K5I**	6	g6(2), c14(2), u5(2), g16(2)	10	2	a670(2), a664(2)	11	2	g6(2), c14(2)	9
	7K3N	4	Thr94(A), Lys63(A), Gln87(A)	8	5	Arg15(A), Gln 54(A), Gln 13(A)	6	4	Arg15(A), Glu46(A)	9
	6M2N	5	Asp295(A), Thr292(A), Thr111(A), Asn151(A)	12	2	Ser121(C)	6	1	Thr26(D)	6

3.7. Characterization of ADMET properties of test molecules.

The pharmacokinetic attributes and safety profile of the query compounds, along with the positive control drug Remdesivir in the form of ADME-Tox predictions, were estimated using the nearest neighbor computational methodology. ADMET, the abbreviated form of absorption, distribution, metabolism, excretion, and toxicity, influences the quantity of drug requirement and drug exposure kinetics of the apprehensive tissue system. ADMET prediction denotes Isosinensetin (showing six instances of negative output) to be a better drug molecule than Retusin (showing seven instances of negative output), with Remdesivir depicting five instances of negative output.

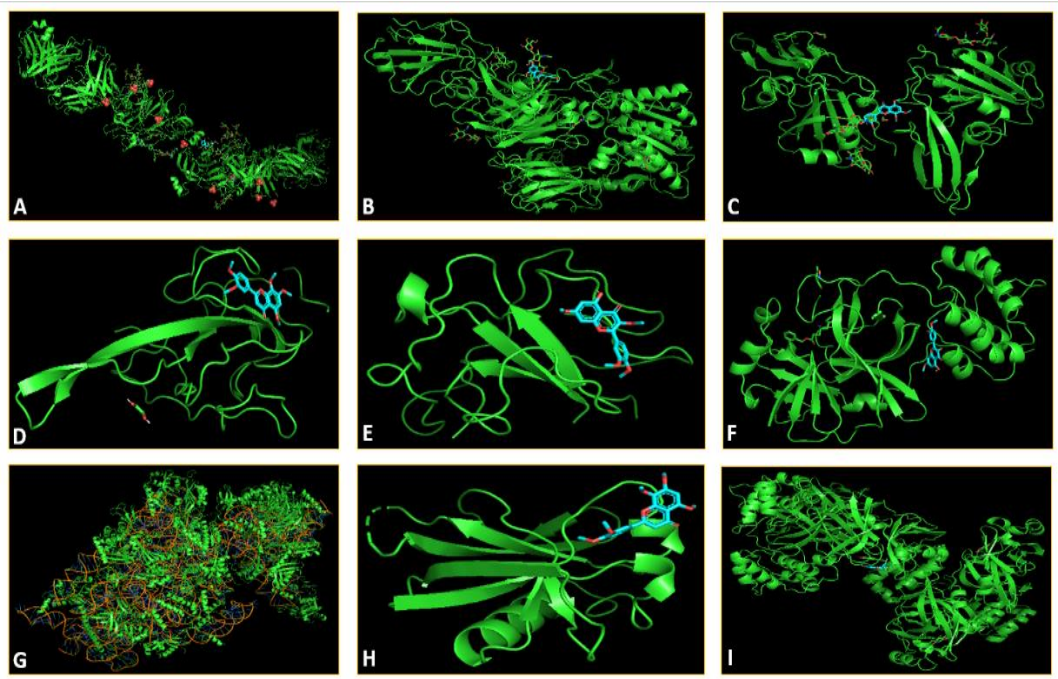

Figure 2. Molecular docking of Isosinensetin and Retusin with concerned proteins of MERS-CoV, SARS-CoV-1, and SARS-CoV-2. A=Isosensetin against 6PXH; B=Retusin against 4L72; C=Isosinesetin against 4L3N of MERS-CoV, D=Isosinesetin against 2OFZ; E=Retusin against 2OG3; F=Retusin against 2HOB of SARS-CoV-1, G= Retusin against 7K5I; H=Isosinesetin against 7K3N; I=Retusin.

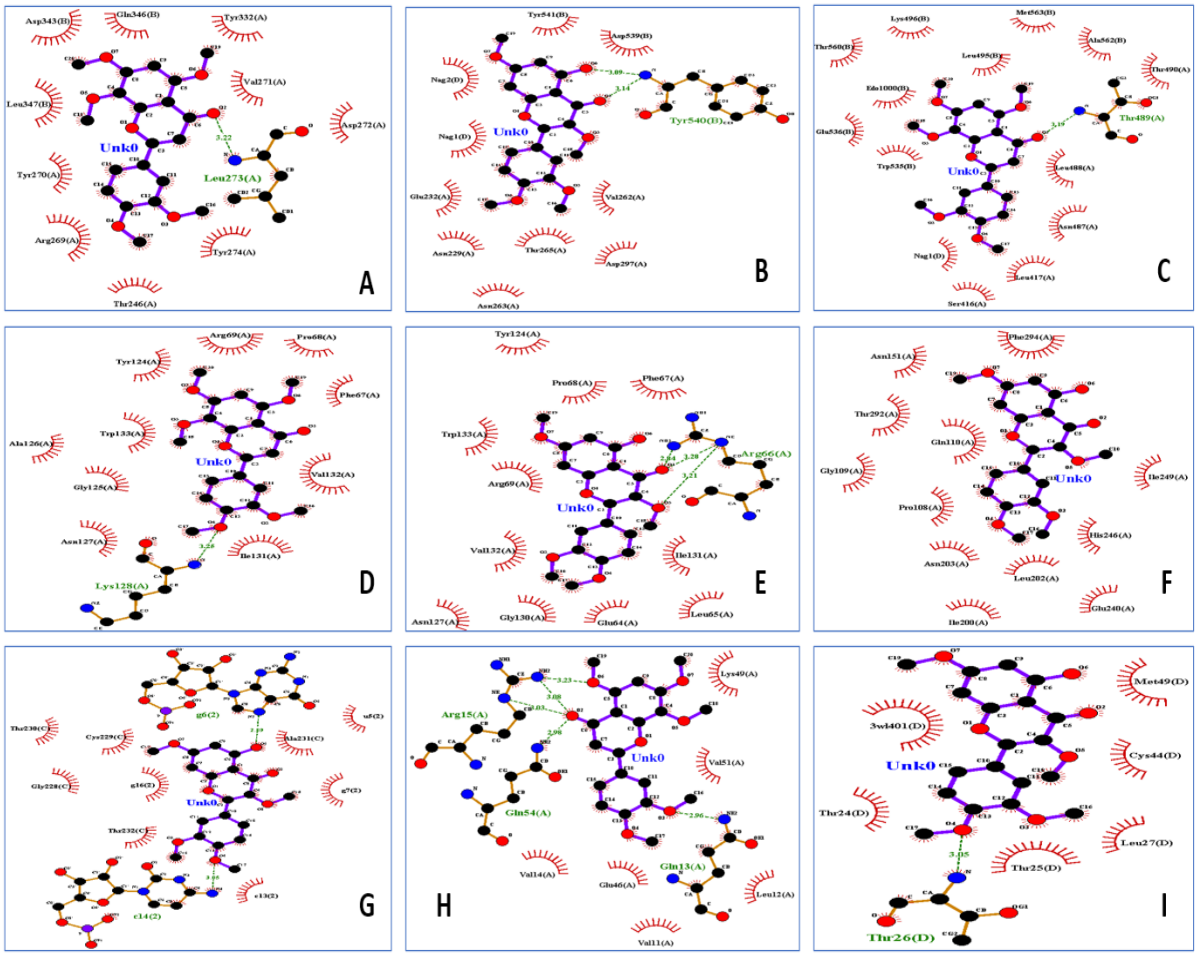

Figure 3. Interaction profile of Isosinensetin and Retusin with concerned proteins of MERS-CoV, SARS-CoV-1, and SARS-CoV-2. A=Isosensetin against 6PXH; B=Retusin against 4L72; C=Isosinesetin against 4L3N of MERS-CoV, D=Isosinesetin against 2OFZ; E=Retusin against 2OG3; F=Retusin against 2HOB of SARS-COV-1, G= Retusin against 7K5I; H=Isosinesetin against 7K3N; I=Retusin against 6M2N of SARS-CoV-2.

Isosinensetin depicted drug-induced liver injury (DILI), including metabolically functioning as Cytochrome P450 (Cyp) inhibitors for Human liver microsomal (HLM), 1A2 and 2C19 enzymes in addition to acting as membrane transporters via P-glycoprotein (P-gp) substrate and inhibitory activity. Retusin is predicted to modulate metabolism by functioning as Cytochrome P450 (Cyp) inhibitors for Human liver microsomal (HLM), 1A2, and 3A4 enzymes, in addition to acting as membrane transporters via P-glycoprotein (P-gp) substrate and inhibitory activity along with being anticipated to be human ether-a-go-go-related gene (hERG) blocker and positive inducer in AMES test. Remdesivir depicted drug-induced liver injury (DILI) and hepatic cytotoxicity, including metabolically functioning as Cytochrome P450 (Cyp) inhibitors for Human liver microsomal (HLM) enzyme in addition to acting as membrane transporters via P-glycoprotein (P-gp) substrate and inhibitory activity. The maximum recommended therapeutic dose (MRTD) was significantly lower in the case of Remdesivir (229 mg/day), followed by Retusin (311 mg/day) and Isosinensetin (3971 mg/day) (Table 4).

So, our present study delivers the chances of further research through *in vivo* and *in vitro* methods to determine the inhibitory activity of those two compounds against SARS-CoV-1, MERS-CoV, and SARS-CoV-2.

Table 4. ADME and T study of test drug compounds.

ADMET study parameter		Isosinensetin	Retusin	Remdesivir
Liver toxicity	DILI	Yes	No	Yes
	Cytotoxicity	No	No	Yes
Metabolism (Cyp inhibitors for)	HLM	Yes	Yes	Yes
	1A2	Yes	Yes	No
	3A4	No	Yes	No
	2D6	No	No	No
	2C9	No	No	No
	2C19	Yes	No	No
Membrane transporters	BBB	No	No	No
	P-gp inhibitor	Yes	Yes	Yes
	P-gp substrate	Yes	Yes	Yes
Others	hERG blocker	No	Yes	No
	MMP*	No	No	No
	AMES	No	Yes	No
	MRTD (mg/day)	3971	311	229

4. Conclusions

Coronavirus diseases have affected human beings in many ways throughout the world. There is a high demand for drugs for treating these diseases, especially against SARS-CoV-1, MERS-CoV, and SARS-CoV-2. Compounds from natural sources will always be important for the treatment of various kinds of diseases. But unless we protect and conserve our environment, the opportunity to discover new natural drugs for medicinal and biological purposes will be closed. This study aimed to explore the inhibitory properties of the flavonoid compounds from *Derris robusta*, and exposed that these two compounds could effectively work against the SARS-CoV-1, MERS-CoV, and SARS-CoV-2 types of Coronaviruses of their investigated binding energy scores along with the characterization of lower or similar cytotoxicity in comparison to synthetic anti-viral drug Remdesivir. Physicochemical characterization also revealed that the test compounds Isosinensetin and Retusin exhibit more drug-like characteristics than the control drug. Interestingly, we have found that both the compounds are major compounds being revealed from the same plant, so if we can find out the genes behind the biosynthesis of these compounds, then with the help of bio-engineering technique, we can

halt the breakdown or promote upregulation and boost metabologenesis of these compounds. Isolation of these drug molecules can help in the betterment of medication facilities for the treatment of this deadly pandemic disease. The results also affirm these two compounds collectively work better as a combination drug since the cumulative binding energy score was always higher than Remdesivir against this range of Coronaviruses. However, further *in vitro* and *in vitro* trials are required to conclude their effectiveness as an anti-coronavirus drug.

Funding

This research received no external funding.

Acknowledgments

This research has no acknowledgment.

Conflicts of Interest

The authors declare no conflict of interest. MB conceived the idea. AG and SM conducted extraction and GC-MS studies while AG and SC carried out *in silico* experiments and wrote the draft manuscript. All authors read and approved the final manuscript.

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Supplementary material

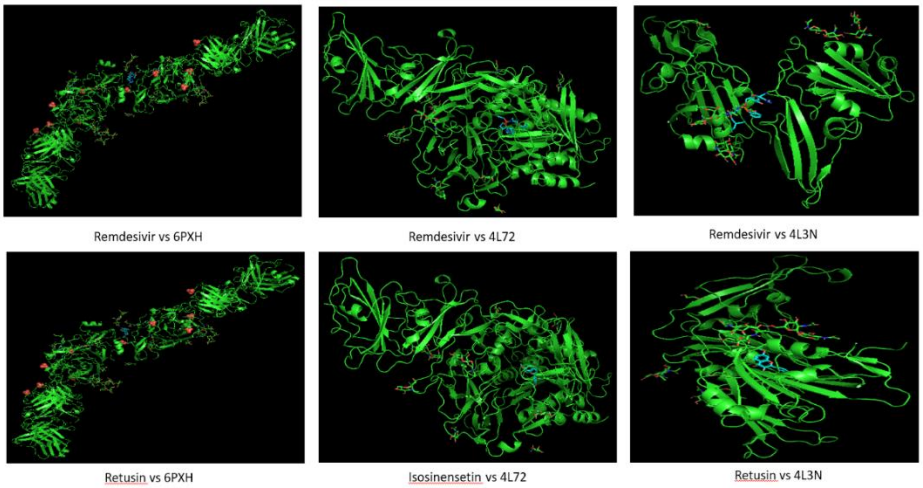

Figure S1. Molecular docking of Remdesivir, Isosinensetin, and Retusin with the MERS-COV proteins.

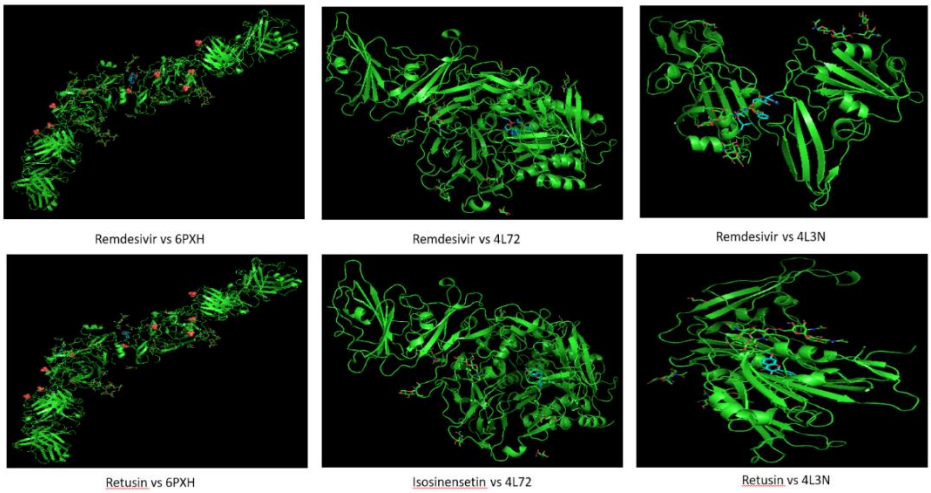

Figure S2. Molecular docking of Remdesivir, Isosinensetin, and Retusin with the SARS-COV-1 proteins.

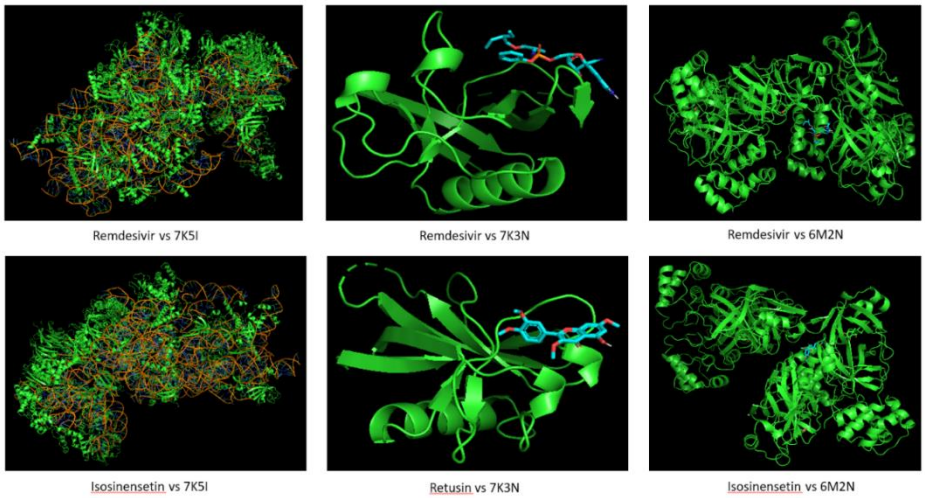

Figure S3. Molecular docking of Remdesivir, Isosinensetin, and Retusin with the SARS-COV-2 proteins.

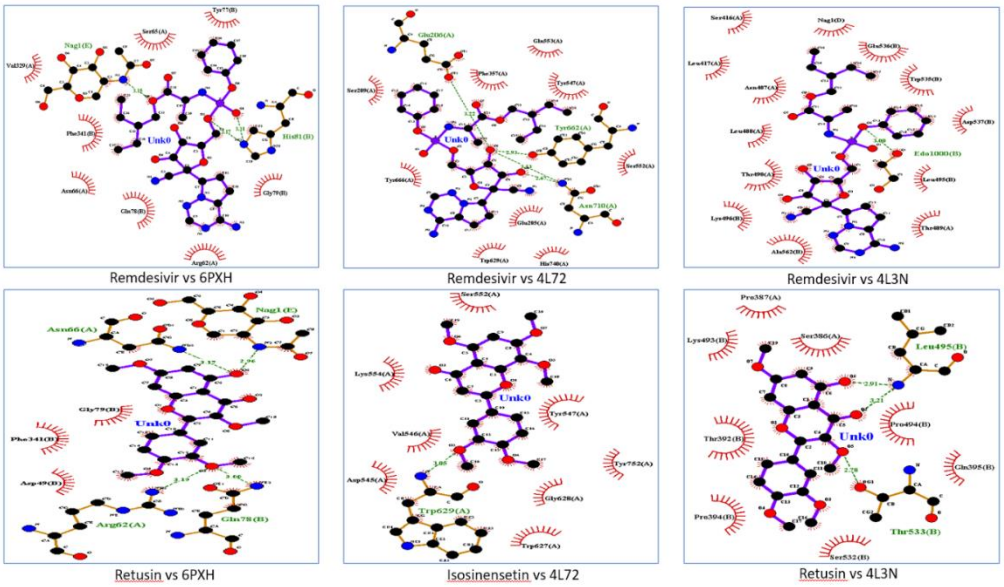

Figure S4. Graphical representation of the interaction between MERS-COV proteins and concerned compounds.

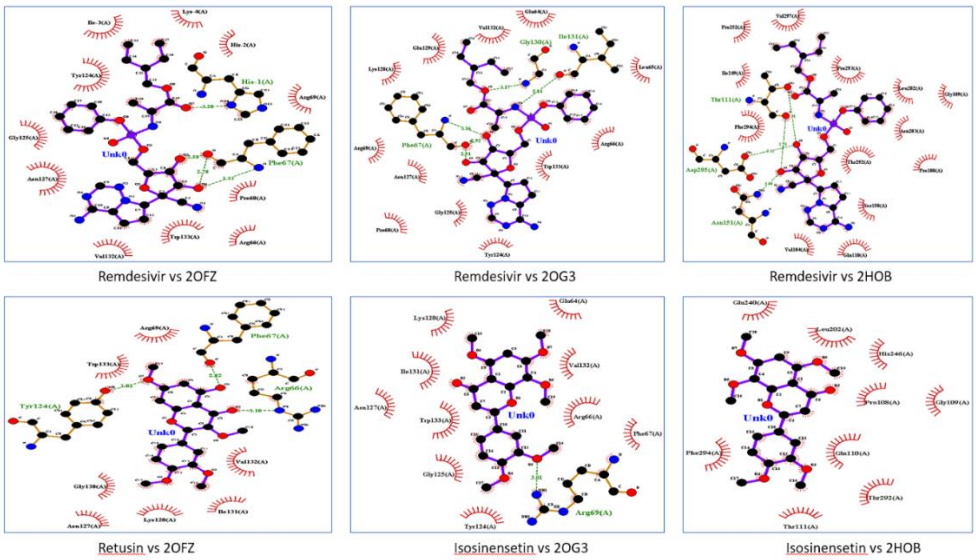

Figure S5. Graphical representation of the interaction between SARS-COV-1 proteins and concerned compounds.

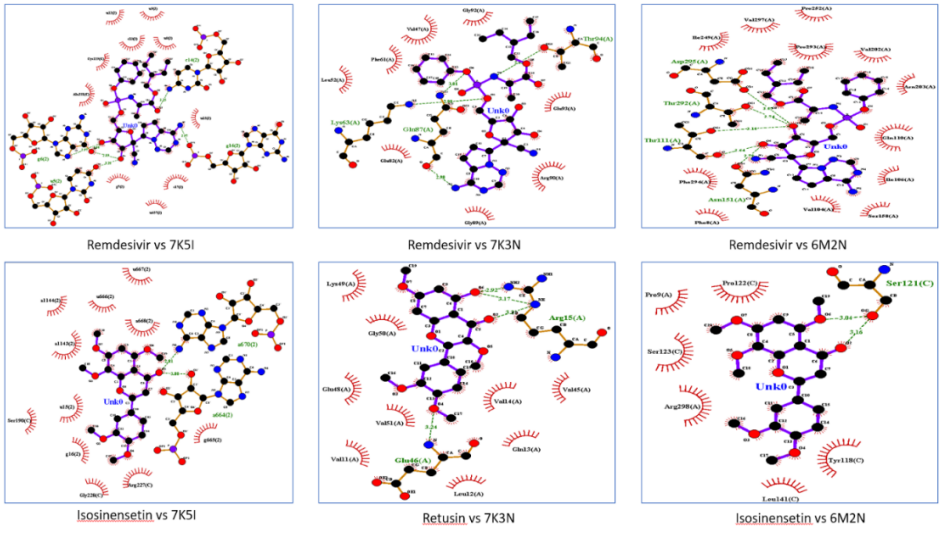

Figure S6. Graphical representation of the interaction between MERS-COV proteins and concerned compounds.