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Identifying Potential Inhibitors of SARS-CoV-2 from Three Medicinal Plants: An *in silico* Study

Olugbenga Morebise [a++*] and Sharvari Kulkarni [b#]

[a] Department of Molecular and Cellular Sciences, All Saints University School of Medicine, Dominica, West Indies.
[b] Department of Biotechnology, Amity University, Mumbai, India.

Authors' contributions

Both authors collaborated to carry out the study. Author OM designed the study. Both authors read and approved the final manuscript.

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ABSTRACT

Covid-19, caused by SARS-Cov-2, almost brought the world to a standstill due to its transmission from person to person, thereby leading to abrupt changes globally. The virus has utilized different mechanisms to get access into host tissues in order to enact its virulence. One of such is the ligation of its viral spike glycoproteins to the host's angiotensin converting enzyme-2 (ACE-2) by transmembrane serine protease. Inhibitors of the ACE-2 have been reported to be useful in curtailing the spread of the virus. Medicinal plants have been reported to be used in different communities to fight the Covid disease. In this study, the inhibitory actions of 23 ligands selected from *Stachytarpheta jamaicensis, Artemisia annua* and *Andrographis paniculata* on ACE-2 were investigated using computer aided drug designing techniques. Grazoprevir was used as a reference ligand. The 3-D structures of the 24 ligands were retrieved from the PubChem database in their Structure Data Format (SDF). ACE-2 was retrieved in its Protein Data Bank (PDB) format. The

[++] Professor of Biochemistry;
[#] Research Scientist;
**Corresponding author: E-mail: olugbenga.morebise@allsaintsuniversity.org;*

protein and ligands were prepared and loaded for molecular docking algorithm. The reference drug and many ligands, especially from *A. paniculata*, exhibited good docking properties. 5-hydroxy-7, 2', 6'trimethoxyflavone (CID 5318369) from *A. paniculata,* displayed binding energies of -7.4kcal/mol and 2 H bonds with Asn394 residue of the ACE-2 protein, and was thereafter subjected to molecular dynamics simulation at 70ns. After simulation, prominent H bonds were seen for Asn394, Gly395, Lys562 and Asn103. Phe40, Trp69, Leu120 and Phe390 showed hydrophobic interactions. The overall protein, ligand and complex dynamicity and conformational stability suggest that the interaction with the protein binding site region is highly preferable for the desired activity. In conclusion, this study demonstrated that the ligands from *A. paniculata* exhibited great docking properties against ACE-2. In particular, 5-Hydroxy-7, 2', 6'trimethoxyflavone (CID 5318369) displayed good docking and molecular dynamics simulation results and is therefore recommended for clinical trials.

Keywords: SARS-Cov-2; Covid-19; ACE-2; docking; molecular dynamics simulation; medicinal plants.

1. INTRODUCTION

Among diseases currently plaguing the world is the Covid-19, caused by SARS-Cov-2, a coronavirus of the subfamily orthocoronavirinae [1,2]. This virus brought the world to almost standstill due to its transmission from person to person, thereby leading to abrupt changes globally [1-3]. The virus has utilized different mechanisms to get access into host tissues in order to enact its virulence. One of such is the ligation of its viral spike glycoproteins to the host's angiotensin converting enzyme-2 (ACE-2) by transmembrane serine protease. Inhibitors of the ACE-2 have been reported to be useful in curtailing the spread of the virus [1,2].

Among pharmaceutical agents being utilized as targets for the ACE-2 are drugs which have been used in treating other ailments and which are now being repurposed for the SARS-Cov-2. Behera et.al [2] reported the repurposing study of Grazoprevir, a drug approved for treating the Hepatitis C virus, on the SARS-Cov-2 by targeting the host ACE-2. In another study [1], the authors reported the use of 56 commercially available antiviral drugs for repurposing studies on the SARS-Cov-2, and they got promising results.

Medicinal plants have been used in folk treatment of different diseases since ancient times [4-6]. Scientific studies that support ethnomedical uses of different medicinal plants have been reported [6-12]. In addition, notable drugs have been discovered from medicinal plants, among which are vincristine and vinblastine [13].

There are reports that medicinal plants have been employed in different communities across the world for treating or managing the Covid-19 disease. These plants have been used in treating other ailments in those communities, especially respiratory ailments. Among plants being used in different communities to combat the Covid-19 are *Andrographis paniculata, Stachytarpheta jamaicensis and Artemisia annua.*

Andrographis paniculata (Burm. F) Nees is a herbaceous plant found in several parts of Asia. The plant is used by locals to treat respiratory disorders such as asthma and flu. It is also used for treating other disorders such as stroke, arthritis and HIV [14]. Scientific studies have reported the anti-inflammatory, antitumor, antidiabetic and antiviral properties of Andrographolide and its derivatives, obtained from *A. paniculata* [14,15]. During the Covid-19 pandemic, there were reports that extracts from the *A. paniculata* were being administered to prisoners in Thailand who had contacted the virus, with about 99% efficacy [16,17]. Laboratory studies in Thailand also indicated the inhibitory effects of the plant extract against the Covid-19 virus [3].

Stachytarpheta jamaicensis is a plant commonly found in the tropical parts of America and subtropical forests of Africa, Asia and Oceania [4]. The plant is used to treat various disorders, including asthma, cold, flu, bronchitis, ulcers, indigestions, constipations, and other disorders [4]. Scientific studies have reported the antacid, analgesic, anti-inflammatory, hypotensive, antihelminthic, diuretic, laxative, lactagogue, purgative, sedative, spasmogenic, vasodilator, vulnerary, and vermifuge properties of the plant [17]. González-Maldonado et.al. [18] reported the use of *S. jamaicensis* and other plants in treating the Covid-19 disease in Jamaica.

Artemisia annua L (*Asteraceae)* is a plant that has been employed traditionally to treat various ailments, especially malaria. Artemisinin is a drug obtained from *A. annua* and is a popular antimalarial drug used worldwide [19]. A pilot *in vitro* study conducted by Nair et al. [20] indicated that *A. annua* plant extracts exhibited inhibitory activity against the Covid-19 virus.

This study investigated the inhibitory effects of selected ligands from *Andrographis paniculata, Stachytarpheta jamaicensis and Artemisia annua* on the ACE-2, using bioinformatics techniques. Bioinformatics procedures have been widely utilized in identifying and quantifying the bioactivities of drugs, including ligands from medicinal plants [1,2].

2. MATERIALS AND METHODS

Twenty ligands from the three plants were used for this study, using standard bioinformatics protocols. Grazoprevir served as the reference ligand. The 3-D structures of the 24 ligands were retrieved from the National Center for Biotechnology Information (NCBI) PubChem database in their Structure Data Format (SDF) (https://pubchem.ncbi.nlm.nih.gov/). ACE-2 was retrieved in its Protein Data Bank (PDB) format from the NCBI GenBank (https://www.ncbi.nlm.nih.gov/). ACE-2 was cleaned by removal of all nonstandard amino acids and water through the discovery studio (BIOVIA, San Diego, CA, USA). The energy minimization was done by GROMACS software (https://www.gromacs.org/). The ligands and protein were converted to their pdbqt formats through the Open Babel software (https://github.com/openbabel/openbabel). The ACE-2 was docked with the ligands using the Auto-Dock Vina software (https://vina.scripps.edu/). The binding energies and the root mean square deviation values were noted. Pharmacophore modeling were visualized by using the Biovia Discovery Studio Visualizer ((BIOVIA, San Diego, CA, USA). Molecular dynamics simulations were conducted using the desmond maestro software (https://www.schrodinger.com/products/desmond). Simulations were run for 70 ns.

3. RESULTS AND DISCUSSION

3.1 Molecular Docking

Table 1 shows the 24 ligands with their docked binding energies before and after protein energy minimization.

From Table 1, it can be seen that most of the ligands displayed good docking properties before and after energy minimizations. The reference ligand, Grazoprevir, displayed a binding energy of -6.9 kcal/mol before energy minimization. This value is close to what had been reported in the literature (1, 2b), but after the energy minimization, the binding energy rose to a higher negative value (-8.2 kcal/mol).

Fig. 1 shows the pharmacophore modeling of Ligand 5 (5-hydroxy-7, 2', 6'trimethoxyflavone) docking with ACE-2 simulations, as visualized by the Biovia Discovery Studio Visualizer (DVS).

From Fig. 1, it can be seen that 5-hydroxy-7, 2', 6'trimethoxyflavone exhibited two hydrogen bonding with Asn394 residue of the ACE-2. It also exhibited Pi-Pi stacked interactions with the imidazole ring of His401 of the ACE-2 while also exhibiting Pi-Alkyl interaction with the His378 of the protein. This ligand was selected for further tests (simulations) because of its excellent docking attributes.

Molecular docking illustrates the 'best fit' orientation of a ligand to the protein it is docked with, and this has been of great interest in computational biology [2].

3.2 Molecular Dynamics Simulations

Fig. 2 shows the protein (ACE-2) information prior to molecular dynamics simulation.

Fig. 3 gives the molecular dynamics interactions of the ligand and ACE-2, (Protein-Ligand RMSD) studied for 70ns.

From Fig. 3, it can be seen that, within the 70 ns time frame for the MD simulation, the intactness of the protein structure was conserved, with a maximum fluctuation of 4.2 Å at around 20 ns. The fluctuation dropped to ~3.0. Å at 30 ns with further drop to 2.4 Å at 40 ns; furthermore, it was increased to 4.0 Å at 65 to 70 ns (Fig. 3).

The ligand exhibited a similar mode of conformational changes reaching up to ~18 Å as the highest at around 35-50 ns and dropped after 50ns to ~12 Å; further, it increased to 18 Å at 60 to 70 ns (Fig. 3).

At the end frame, the ligand shows a very good binding with protein which indicates that the ligand does not diffuse away from the ACE-2 protein.

Table 1. Ligands with their PubChem CIDs and binding energies

Name	PubChem CID	Plant	Binding Energy before Minimization (kcal/mol)	Binding Energy after Minimization (kcal/mol)
6beta-Hydroxyipolamiide	14137128	*S. jamaicensis*	-6.5	-6.5
Artemisinin	68827	*A. annua*	-6.4	-6.7
5, 7, 2', 3'-tetramethoxyflavone	11772234	*A. paniculata*	-6.6	-6.9
5-hydroxy-7, 2', 3'-trimethoxy flavone	12135219	*A. paniculata*	-6.9	-6.6
5-hydroxy-7, 2', 6'trimethoxyflavone	5318369	*A. paniculata*	-6.8	-7.4
7-O-methyldihydrowogonin	146156496	*A. paniculata*	-7.0	-6.9
7-O-methylwogonin	188316	*A. paniculata*	-6.5	-6.4
5-hydroxy-7, 8, 2', 5'-tetramethoxyflavone	10948318	*A. paniculata*	-6.5	-6.5
Dihydroskullcapflavone	12098358	*A. paniculata*	-6.5	-6.6
Andrographolide	5318517	*A. paniculata*	-6.2	-6.4
Neoandrographolide	9848024	*A. paniculata*	-6.8	-7.4
14-deoxyandrographolide	11624161	*A. paniculata*	-6.5	-6.4
Andrographoside	6439612	*A. paniculata*	-7.2	-7.7
14-deoxy-11, 12-didehydroandrographolide	16121613	*A. paniculata*	-6.0	-7.8
Andrographolactone	44206466	*A. paniculata*	-8.2	-7.2
14-deoxy-15-isopropylidene-11,12-didehydroandrographolide	637300	*A. paniculata*	-6.6	-7.3
Arabinogalactan	24847856	*A. paniculata*	-6.8	-6.6
1,2-dihydroxy-6,8-dimethoxy-xanthone	12443163	*A. paniculata*	-6.9	-6.3
Andrographidoid A	57384307	*A. paniculata*	-7.0	-6.9
Andrographidoid B	57384308	*A. paniculata*	-5.2	-7.0
Andrographidoid C	57384309	*A. paniculata*	-7.6	-7.0
Andrographidoid D	57384563	*A. paniculata*	-7.1	-7.1
Andrographidoid E	57384564	*A. paniculata*	-5.2	-5.2
Grazoprevir	44603531	Reference drug	-6.9	-8.2

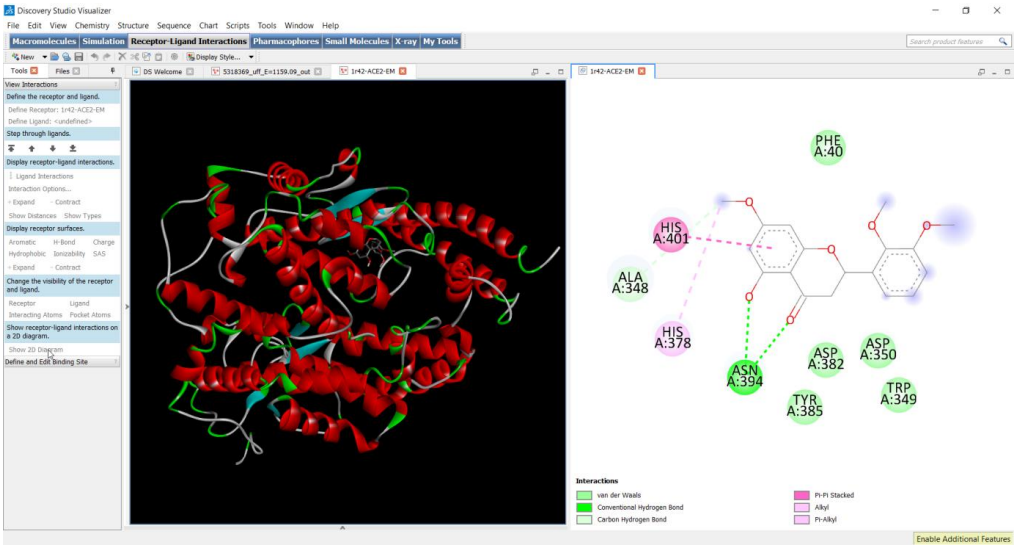

Fig. 1. Pharmacophore Modeling of 5-Hydroxy-7, 2', 6'trimethoxyflavone, CID 5318369 and ACE-2 Pre-Simulation

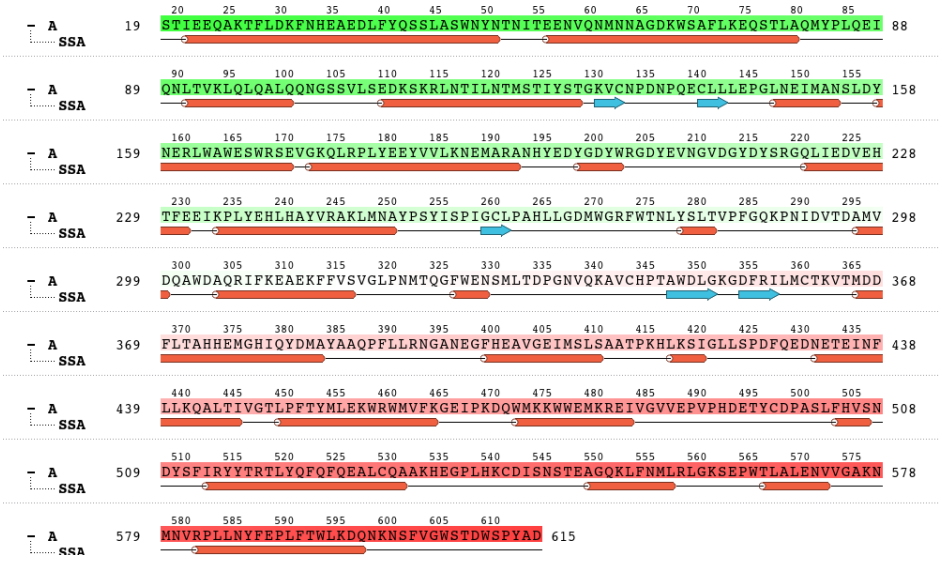

Fig. 2. ACE-2 primary structure information

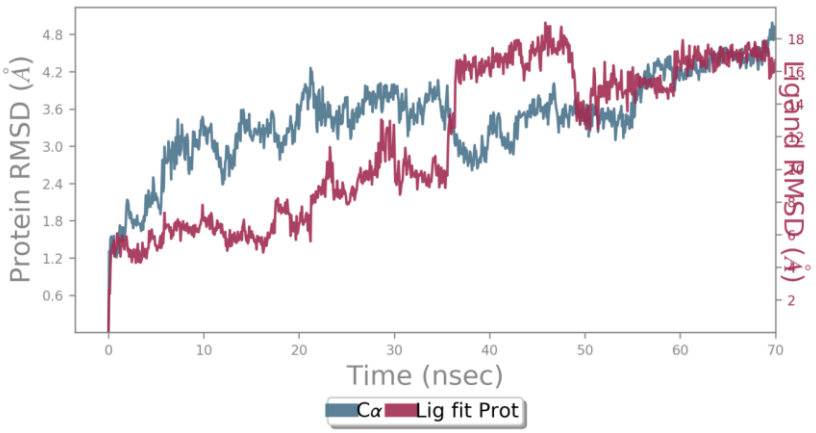

Fig. 3. Molecular dynamics simulation result for ligand-ACE2 studied for 70ns

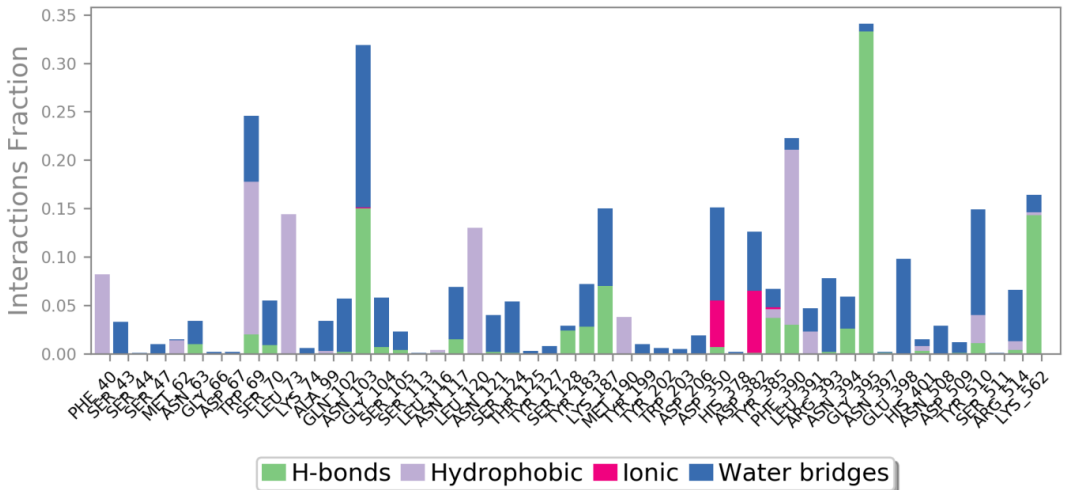

Fig. 4. Protein-ligand interactions post MD simulations

Fig. 4 above shows the protein-ligand interaction post-molecular dynamics (MD) simulation.

Molecular dynamics simulations have been greatly employed in computational biology research to predict and analyze physical movements of atoms and molecules for macromolecular structure-function relationships [2]. The docking results already showed that Asn394 residue of ACE-2 exhibited hydrogen bonding with the ligand (Fig. 1). The MD simulation results shown in Table 1 above indicate that the Asn394 was conserved with the hydrogen bonds in the simulation process whereas the His401 pi bond was converted to hydrophobic interaction.

After simulation, prominent hydrogen bonds were seen for Asn 394, Gly395, Lys562 and Asn103.

Phe40, Trp69, Leu120 and Phe390 showed hydrophobic interactions.

The overall protein, ligand and complex dynamicity and conformational stability suggest that the interaction of the selected ligands with the protein binding site region is highly preferable for the desired activity. This suggests that the observed effects of *A. paniculata* extractsin treating the Covid virus might be through inhibition of the host's ACE-2 protein, thereby curtailing the spread of the virus within the host's tissues.

4. CONCLUSION

This study has supported the medicinal uses of plants in treating the Covid-19 infections. More specifically, 5-Hydroxy-7, 2', 6'trimethoxyflavone, a component of *A. paniculata,* demonstrated very good interactions with the ACE-2 before and after molecular dynamics simulations. This supports previous studies on the *A. paniculata* plant extract as a promising candidate against the Covid-19 virus [3]. Thus, 5-Hydroxy-7, 2', 6'trimethoxyflavone can be taken as a promising drug target against the SARS-CoV-2 and subjected to ADMET: (Absorption, Distribution, Metabolism, Excretion, Toxicity) properties analysis and clinical trials. A positive outcome of the ADMET would make 5-Hydroxy-7, 2', 6'trimethoxyflavone a potential drug to curtail the spread of SARS-CoV-2. Since the plant source has already been administered to SARS-CoV-2 patients with a reportedly good outcome and no reported side effects [3,16,17], using the 5-Hydroxy-7, 2', 6'trimethoxyflavone as a natural product to replace synthetic formulations with obvious side effects would be a major breakthrough.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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