IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
September 2021 Vol.:22, Issue:2
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Identification of Potential Phytochemicals from *Carica papaya* Against COVID -19 Activity

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INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
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ISSN 2349-7203
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Submitted: 25 August 2021
Accepted: 31 August 2021
Published: 30 September 2021

HUMAN JOURNALS
www.ijppr.humanjournals.com

Keywords: Coronavirus, COVID-19, Envelope protein, Molecular docking, *Carica papaya, in-silico study,* ADMET, Phytocompounds

ABSTRACT

Coronaviruses belong to the family of Ribonucleic Acid viruses that causes diseases that vary from mild to severe diseases such as Middle East Respiratory Syndrome and Severe Acute Respiratory Syndrome. The WHO has named the disease "COVID-19". Covid becomes a serious global health concern because of the high death rate and the unavailability of proper treatment. The treatment in hospitals for COVID patients consists of symptomatic management with ventilation and for mild cases, they prefer antipyretics and antibiotics. Several antiviral drugs being actively tested, but none has been approved. As a solution, Researchers bring up a medicinal plant "*Carica papaya*" commonly known as papaya. Studies say extracts from *Carica papaya* contain 30 phytocompounds. There is no scientific evidence for its activity against COVID-19. Hence, researchers tried to investigate the anti-viral activity of phytocompounds in *Carica papaya* against envelope protein (6LZG) of COVID-19. Molecular docking and ADMET have been used in this study and the result reveals that 6 compounds have high binding capacity towards the receptor site. These findings conclude that the six phytocompounds can act as antiviral drugs than standard Hydroxychloroquine for COVID-19.

INTRODUCTION

Coronaviruses belong to the family of Ribonucleic Acid viruses that causes diseases that vary from the cold to severe diseases such as Middle East Respiratory Syndrome and Severe Respiratory Syndrome. [1] In December 2019, the new variant of the corona virus was identified in Wuhan city, Hubei province of China. It has been named by ICTV as SARS-CoV-2. [2] This organization has identified that SARS-CoV-2 is the same as the SARS-CoV beta variant.[3-4] The WHO has named the disease "COVID-19". This name was chosen because it was identified in 2019. The source of COVID 19 transmission remains unidentified. The Genetic and Epidemiological data suggest that SARS-CoV-2 may be a zoonotic pathogen that spread between animals and humans directly from wildlife or via animal products. [5] The main symptoms are fever and respiratory symptoms like coughing and shortness of breathing; while some other cases are mild. When an individual with (COVID19) coughs or exhales, here leases droplets of fluid infected with the disease, and those droplets fall on surfaces and nearby objects such as staircases, tablets, or phones.[6] COVID-19 is transmitted when people touch their eyes, mouth, or face after they get in contact with those contaminated substances. In the meanwhile, numerous ways can relieve the symptoms both at home as well as at the hospital. There are mainly 3 different types of covid-19 tests are available: Molecular (RT-PCR) test, Antibody test, Antigen test.[7] Many scientists across the world are trying to generate new medicines to prevent the spread of the corona virus or to treat COVID-19.

The GC-MS study of the methanol extraction of *C. Papaya* has shown 30 peaks representing 30 phytocompounds present in *C. Papaya* which belongs to the family Caricaceae and is commonly known as papaya. [8-11] Molecular docking and ADMET analysis have been used to determine the Docker energy and ADMET properties of the phytochemicals present in *C. papaya* to analyze whether these phytocompounds can act as an antiviral drug for COVID-19.

MATERIALS AND METHODS

1. Materials and methods

In our present study, *in silico* molecular docking studies were carried out using BIOVIA Discovery Studio (DS) 2017 software.

1.1. Preparation of Protein

The X-ray diffraction-based crystal structure of COVID-19 main protease in complex with its receptor ACE2 (PDB ID: 6LZG) with a resolution of 2.5 Å was selected for this study. Hydrogen was added to the protein 6LZG by applied the force field algorithm subsequently the energy of protein was minimized using CHARM force field in DS.

1.2. Ligand Preparation

The standard Hydroxychloroquine drug, Mol. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, and 14 were drawn in ChemDraw software, subsequently, energy was minimized and saved in SDF file format for docking studies.

1.3. Docking studies

A molecular docking study was performed, to evaluate the most preferred geometry of protein-ligand complex. Computational docking study was used to analyze structural complexes of the 6LZG with Hydroxychloroquine drug, Mol. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, and 14 to understand the structural basis of these target proteins. Possible binding modes between the ligands and this target protein were studied by CDOCKER (CHARMm-based DOCKER) protocol incorporated within DS. The parameter to run the CDOCKER was tabularized in Table 1. The algorithm offers full ligand flexibly and employs CHARMm force fields. Ligand binding affinity was calculated using CDOCKER energy, CDOCKER Interaction energy, Hydrogen bonds, binding energies, protein energy, and ligand-protein complex energy. The CDOCKER energy is mentioned in negative values. More negative value energy indicated a higher binding affinity of the ligands with the target protein.

Table No. 1: Parameter of C-DOCKER Protocol

Input Receptor	Input/6LZG.dsv
Input Ligands	/Input/Total_min_ligands.sd
Input Site Sphere	-23.9454, 29.2003, 7.29961,9
Top Hits	1
Random Conformations	10
Random Conformations Dynamics Steps	1000
Random Conformations Dynamics Target Temperature	1000
Include Electrostatic Interactions	True
Orientations to Refine	10
Maximum Bad Orientations	800
Orientation vdW Energy Threshold	300
Simulated Annealing	True
Heating Steps	2000
Heating Target Temperature	700
Cooling Steps	5000
Cooling Target Temperature	300
Force field	CHARMm
Use Full Potential	Yes
Grid Extension	8.0
Ligand Partial Charge Method	CHARMm
Random Number Seed	314159

Final Minimization	Full Potential
Final Minimization Gradient Tolerance	0
Parallel Processing	False
Parallel Processing Batch Size	25
Parallel Processing Server	Localhost
Parallel Processing Server Processes	2
Parallel Processing Preserve Order	True
Random Dynamics Time Step	0.002

1.4. ADMET Prediction

Absorption, distribution, metabolism, elimination, and toxicity (ADMET) properties were predicted using ADMET descriptors in Discovery Studio (Accelrys, San Diego, CA, USA). [12-13] The module uses six mathematical models, to quantitatively predict properties by a set of rules/keys that specify threshold ADMET characteristics for the chemical structure of the molecules based on the available drug information: ADMET absorption predicts human intestinal absorption (HIA) after oral administration. The model was developed using standard drug data's in the training set based on the calculations AlogP (ADMET AlogP98) and 2D polar surface area (PSA 2D). The absorption levels of the HIA model are defined by 95% and 99% confidence ellipses in the ADMET PSA 2D, ADMET AlogP98 plane.[13]

RESULTS AND DISCUSSION

1.1 MOLECULAR DOCKING RESULTS:

The secondary structure of the target protein of novel coronavirus spike with active receptor site of sphere shape (Radius 9) was depicted in Figure 1. The crystal structures were refined by removing water molecules and repeating coordinates. Hydrogen atoms were added and charges were assigned to the protein atoms. In the molecular docking study, all the molecules were successfully interacted with the target novel coronavirus spike receptor and shows CDOCKER binding energy (Table 2).

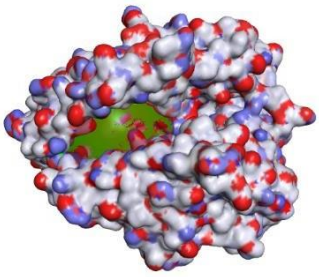

Figure No. 1: Secondary Structure of the Novel Corona Virus Spike Protein

Table No. 2: CDOCKER Energy of Phytocompounds Present in *Carica papaya*

Mol. No	Molecule Name	C-DOCKER Energy
1.	Hexadecanoic acid	-44.5037
2.	Tetradecanoic acid methyl ester	-44.2457
3.	Phthalic acid-hept-4-yl-isobutyl-ester	-43.9122
4.	1-Dodecanol	-36.7561
5.	N-Methyl-D-aspartic acid	-34.8245
6.	Formic acid-3-methylhept-3-yl-ester	-34.7725
7.	Butanoicacid-3-(trimethylsilyl)oxy trimethylsilylester	-32.8471
8.	5-Heptadecene	-28.9351
9.	2-Allyl-5-t-butylhydroquinone	-23.2377
10.	2-Methoxy-4-vinylphenol	-22.0815
11.	Naphtho-2,3-b-furan-4,9-dione	-19.0342
12.	(3S,5S)-3,5-Dihydroxy-3,5-dimethyl-1-phenyloct-7-en-4-one	-18.5532
13.	Hydroxychloroquine	-16.0464
14.	2,6-di(t-butyl)-4-hydroxy-4-methyl-2,5cyclohexadien-1-one	-0.844725

Amongthese14 phytocompounds present in the *C. papaya*, Hexadecanoic acid, Tetradecanoic acid methyl ester, Phthalic acid-hept-4-yl-isobutyl-ester, 1-Dodecanol, N-Methyl-D-aspartic, Formic-acid-3-methylhept-3-yl-ester and Hydroxychloroquine (standard drug), acid have high docker energy.

Table No. 3: Six phytocompounds that act as an antiviral drug for COVID-19 are:

PHYTOCOMPOUNDS	C.DOCKER ENERGY
Hexadecanoic Acid	-44.5037
Tetradecanoic acid-methyl ester	-44.2457
Phthalic-acid-hept-4-yl-isobutyl-ester	-43.9122
1-Dodecanol	-36.7561
N-Methyl-D-aspartic-acid	-34.8245
Formic-acid-3-methylhept-3-yl-ester	-34.7725

a) Hexadecanoic Acid: Has Docker energy of about -44.2457 kcal/mol^{-1}. This compound experiences weak Vander Waals, conventional hydrogen bond, the carbon-hydrogen bond between its amino groups. From the docking results, the molecule forms one strong hydrogen bond interaction with Arg403. His34, Glu37, and Tyr449 interacted with the Pi-Alkyl bond. Most of the hydrophobic nature groups show only van der Waals interaction.

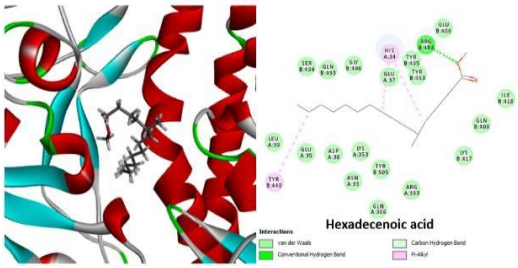

Figure No. 2: Depicts the docking result of hexadecanoic acid (Mol.1) with the presence of amino groups

b) Tetradecanoic acid methyl ester: Docker energy is about -44.2457kcal/mol^{-1}. Tyr 449 and His34 are interacted by 4 pi-alkyl bonds. 3 strong hydrogen bond interaction is experienced for amino groups like Asp30 and Leu29. This compound experiences vanderwaals, C-H bonds, and pi-alkyl bonds.

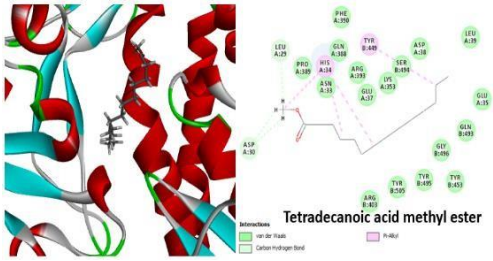

Figure No. 3: Depicts the docking result of Tetradecanoic acid methyl ester (Mol. 2) with the presence of amino groups

c) Phthalic acid hept-4-yl-isobutyl ester: In this molecule, it shows CDocker energy of -43.9122 kcal/mol^{-1}. The benzene group of this molecule shows Pi-Pi stacked binding interaction with Tyr453 residue. Similarly, the benzene group and C=O group of the Mol. 3 forms two strong pi donor hydrogen bond interactions with Arg403.

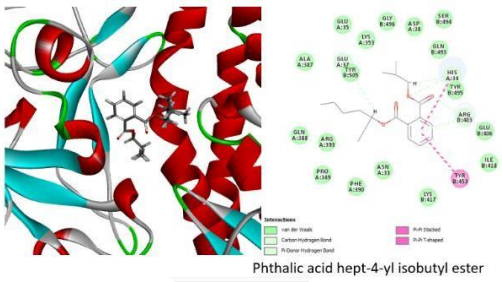

Figure No. 4: Depicts the docking result of Phthalic acid hept-4-yl-isobutyl ester (Mol. 3) with the presence of amino groups

d) 1-Dodecanol: CDocker energy of Mol. 4 is -36.7561 kcal/mol^{-1}. The hydrogen group of this molecule shows 3 strong hydrogen interactions with Tyr505, Asn501, and Lys353 residue. This molecule shows Pi-Alkyl binding interaction with Tyr449 residue. Most of the hydrophobic nature groups show only vanderwaals interaction.

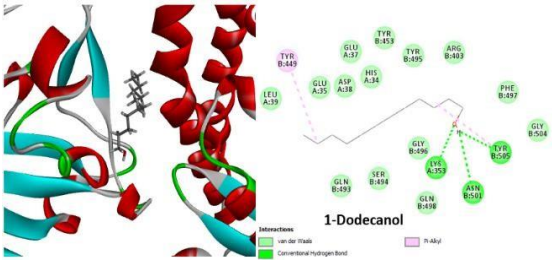

Figure No. 5: Depicts the docking result of 1-Dodecanol (Mol 4)

e) N-Methyl-D-aspartic-acid: CDocker Energy is of about 34.8245 kcal/mol^{-1}. The active functional group -COOH group of (Mol 5) interacted with Lys353, Asp38, and Gly496 by 3 hydrogen bonds. The -NH group of this molecule forms a hydrogen bond with Tyr453 residue. The other active residue in Figure 6 shows Vander Waals interactions.

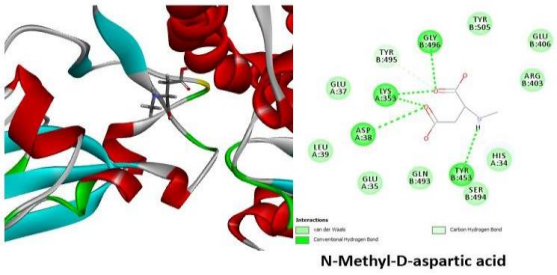

Figure No. 6: Depicts the docking result of N-Methyl-D-aspartic (Mol 5)

f) Hydroxychloroquine: Has Docker energy of about -16.0464 kcal/mol^{-1}. The standard Hydroxychloroquine interacted with less binding energy compared to other molecules. The -NH group of this molecule forms a hydrogen bond with Glu37 residue and His34 residue interacts with the pyridine group in the molecule forms Pi-Pi T-shaped interactions.

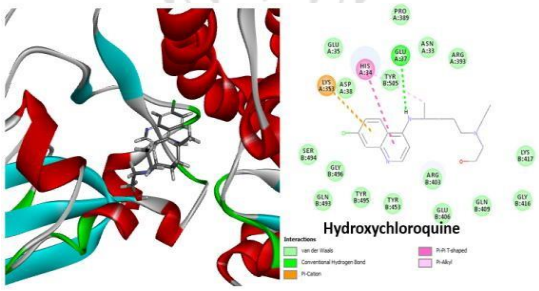

Figure No. 7: Depicts the docking result of Hydroxychloroquine

1.2 ADMET Results:

The ADMET result of Mol 1-6 (Mol 6 is Formic acid-3-methylhept-3-yl-ester) have shown in Table 5 and the plot of polar surface area (2D PSA) versus AlogP of these compounds was depicted in Figure 8. The intestinal absorption and blood-brain barrier (BBB) penetration were predicted by 2D PSA and AlogP that also include 95% and 99% confidence ellipses in the ADMET study. The region of ellipses defines, where the compounds are expected as well-absorbed. The absorption level (human intestinal absorption-HIA) of all the molecules doesn't show a good absorption value but Mol 2 and 4 show extremely good absorption value. Mol 6 shows good value. The absorption levels of

the HIA model are defined by 95% and 99% confidence ellipses in the ADMET. If absorption levels are defined by 95% then it denotes by the red line whereas 99% denotes by the green line (figure 8). Similarly, Mol 2 and 4 shows extremely good solubility level and Mol 5 and 6 show a good level of solubility. BBB protects against circulating toxins that could cause brain infections from drugs. All the molecules experience very low BBB. PPB-Plasma protein binding capability is less than 90% for all the molecules. Plasma protein is an essential part of blood so the drugs shouldn't bind with protein. All the molecules were found to be satisfactory concerning CYP4502D6 liver enzyme, suggesting that the derivatives molecules were non-inhibitors of the metabolic enzyme. All the compounds showed polar surface area (PSA) < 140 $Å^2$. Since the AlogP98 criteria, all the compounds had AlogP98 value <5. From the result of ADMET, we found that the molecules have drug-likeness properties, and also it will be useful as a potent new drug for COVID- 19 instead of Hydroxychloroquine.

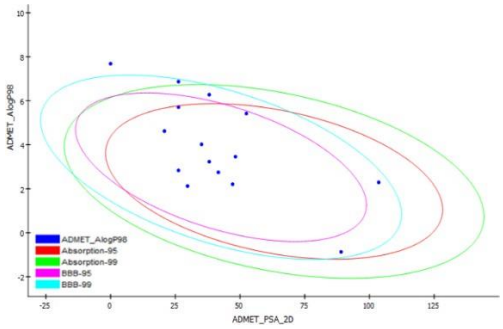

Figure No. 8: Plot of polar surface area (PSA) versus AlogP for Hydroxychloroquine and 6 molecules showing the 95% and 99% confidence limit ellipses corresponding to the blood-brain barrier (BBB) and intestinal absorption.

Table No. 4: ADMET Properties of the Molecule

Mol. No.	Absorption level	Solubility level	BBB level	PPB level	Hepatotoxic level	CYP 2D6	PSA 2D	AlogP98
1.	Low	Low	Low	<90%	No	No	25.47	4.16
2.	Extremely good	Extremely good	Low	<90%	No	No	53.89	5.19
3.	Very low	Low	Low	<90%	No	No	55.48	4.52
4.	Extremely good	Extremely good	Low	<90%	No	No	57.67	5.14
5.	Low	Good	Low	<90%	No	No	67.1	4.27
6.	Good	Good	Low	<90%	No	No	86.12	3.98

CONCLUSION

Molecular docking analysis found that all 6 molecules were exhibiting excellent binding affinity towards the protein (6LZG). A computational docking study was used to analyze the structural complexes of the 6LZG with standard Hydroxychloroquine drug and all other 5 phytochemicals. HCQ tablets have gained importance during the corona period but the serious side effects with long-term use make us think about an alternative for it. Moreover, HCQ can interact with other drugs that have been consumed for certain health issues and it causes liver damage. So as an alternative for HCQ, these results explain that 5 phytochemicals that present in *C. papaya* show no hepatotoxicity, low BB level, and <90% PPB level. ADMET findings indicate that the properties of the molecules are drug-like nature. These 6 molecules can help in the discovery of COVID-19 anti-viral drugs based on the observation of the docking score and ADMET results.

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