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Research Article

# Identification of Lead Molecules against Multi-target of SARS-CoV-2 from *Carica papaya* L. through *In-silico* Method

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ABSTRACT

The efficacy of currently used vaccines against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is questionable since the virus is rapidly mutating. A single drug that can simultaneously act on multi-target at different stages of disease causing pathways is the best option to fight against it. The multi-target inhibitory activities of many phytochemicals have been reported. The present investigation was aimed to screen the multi-target inhibitory activity of 215 phytochemicals from *Carica papaya* L. against three targets of SARS-CoV-2 viz spike protein (SP), main protease ($M^{pro}$), RNA-dependent RNA-polymerase (RdRp) and a target from host, angiotensin-converting enzyme 2 (ACE-2) using the docking tool, AutoDock Vina in PyRx 0.8. The docked results with free energy of binding ≤ -6 kcal/mol were considered active/hit molecules. Of the 215 phytochemicals, 48 have binding energy ≤ -6 kcal/mol against all the targets. Further molecular interaction between the ligand and targets, pharmacokinetics and ADMET analysis of the top ranked five hits obtained against each target revealed that the compound hesperidin can be selected as the best lead since it has the least binding energy, admissible ADMET and a better binding score than the control drugs. Hesperidin has been used as an approved drug to treat vascular disease. Overall, results revealed that *C. papaya* is a rich source of phytochemicals with activity on multi-target of SARS-CoV-2 infection and multiplication in the human host.

## Introduction

At present, COVID-19 related cases are ever-increasing, and as of June 15, 2022, worldwide, over 534,495,291 confirmed cases and 6,311,088 deaths were reported.[1] It is caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2), which is a rapidly mutating virus species that creates uncertainty in the vaccination process. SARS-CoV-2 is an enveloped, positive-sense single-stranded RNA virus. Its genome is~30 kbp RNA genome comprising 14 open reading frames (ORFs); two-thirds encode non-structural proteins, and the rest, one-third, encode structural and accessory proteins.[2] Spike protein is the first structural protein that interacts with the host cell receptor angiotensin-converting enzyme 2 (ACE2) and mediates viral entry. The presence of conserved residues in the receptor-binding domain (RBD) within the spike protein makes it a promising target for drug discovery.[3] The $M^{pro}$ of SARS-CoV-2 is another enzyme that plays a key role in processing polyproteins into non-structural proteins. Two major factors make it an attractive target: they share less similarity with human proteases and play a significant role in viral replication/transcription.[4] Another important enzyme is RNA-dependent RNA polymerase (RdRp), a multi-domain protein that plays a key role in viral replication. RdRp is the most conserved enzyme among different viral species, including CoVs, and thus is considered a primary target for therapeutics.[5] Since SARS-CoV-2 has been rapidly mutating, the discovery of phytochemicals having biological activity on multi-targets of SARS-CoV-2 may be the best sustainable option to prevent this viral infection.

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*Carica papaya* L., commonly known as papaya, is an important tropical fruit tree belonging to the family Caricacea. It has been used as an antiviral agent in traditional treatment systems. It has immunomodulatory, anti-cytokine storm, and anti-thrombocytopenic properties and possesses antimicrobial, antifungal, antiparasitic, anticancer, antioxidant, contraceptive, anti-sickling, and antidiabetic effects.[6] The anti-dengue[7] and anti-dengue with immunomodulatory effects of papaya leaf extract were reported.[8-10] Papaya pulp possesses anti-ZIKV virus activity.[11] Its anti-dengue and anti-chikungunya activities were also reported.[12] The silver nanoparticles prepared using *C. papaya* leaves showed activities against chikungunya[13] and dengue type 2 virus.[14] Clinical studies have reported the benefits of papaya leaf extracts for dengue patients.[15] Its extract has been prescribed by physicians as an anti-dengue medicament under various trade names, such as caripill tablet and zandu capsule, particularly for enhancing blood platelet count in dengue patients. *In-silico* studies have been conducted and identified phytochemicals with anti-dengue, anti-chikungunya, anti-influenza[16] and anti-SARS-CoV-2[17] activities from papaya. However, evaluation of anti-SARS-CoV-2 activity of all the phytochemicals from *C. papaya* and identification of lead molecules with multi-target activity has not been tested yet.

The significance of the multi-target activity of plant-derived chemical molecules has been well-reviewed.[18,19] Recently, the common practice "one disease-one target-one drug" approach has been transformed into a polypharmacology or multi-target drug discovery approach, since it is a potential solution for diseases of complex etiology and drug-resistance problems.[20] It is well demonstrated that plant-derived molecules have biological activity on multi-targets. Many such drug molecules discovered in modern medicine by serendipity were later repurposed for other diseases. For example, the compound salicylic acid first isolated from the willow tree based on traditional knowledge was modified into aspirin. Which has been used as a painkiller in modern medicine and later found to be a medicament for many other diseases such as coronary artery disease, heart attack, stroke, etc., and now aspirin is considered a wonder drug.

Similarly, many drugs such as remdesivir, favipiravir, hydroxychloroquine, azithromycin, lopinavir/ritonavir, and nafamostat mesylate have been repurposed to treat chronic COVID-19 patients.[21] When compared to synthetic drugs, plant-derived drugs are safer, stable, and have unpredictable activity on multi-target since these phytochemicals evolved within the living system in accordance with various stimuli, and such molecules are repeatedly tested and modified by the complex network of the biological system, which a synthetic chemist can't do. A single molecule that can simultaneously bind to multiple proteins of SARS-CoV-2 can inhibit the viral infection at different phases of disease-causing pathways such as attachment, fusion, entry, replication, multiplication, assembly, and egress, thus unraveling an effective treatment strategy against the emerging viral variants. Many authors have reported that this may be the future treatment scenario for COVID-19.[22] The *in-silico* screening of phytochemicals from *Punica granatum* against tuberculosis[23] and *Syzgium aromaticum* against SARS-CoV-2[24] revealed that several phytochemicals in these plants have inhibitory activity on multi-targets. In these backdrops, the present investigation aimed to evaluate the anti-SARS-CoV-2 activity of all the phytochemicals reported from *C. papaya* and identify leads that can simultaneously act on multi-targets of the COVID-19 pathogenicity in humans through the *in-silico* method.

## Materials And Methods

### Selection and Preparation of Macromolecules

Spike protein (SP PDB ID: 6M0J), angiotensin-converting enzyme 2 (ACE-2 PDB ID: 1R4L) receptor from humans, main protease ($M^{pro}$ PDB ID: 7BUY), and RNA-dependent RNA-polymerase (RdRp PDB ID: 7BV2) from SARS-CoV-2 were selected as targets. Its structures were downloaded from the RCSB protein data bank and prepared for docking, i.e., removed ligand complexed with the targets, water, or hetatoms and saved the protein in pdb form at using AutoDock 4.2.[25] The active site residues of the proteins were detected using the PDBsum tool.

### Selection and Preparation of Ligands

A total of 215 phytochemicals from *C. papaya* were used as the ligands. The 3D structure of each ligand was downloaded from the PubChem database in sdf format and was then minimized using universal force field (uff) with conjugate gradient as the algorithm and converted to pdbqt format using Open Babel software in PyRx 0.8 virtual screening tool.[26]

### Molecular Docking

Molecular docking was performed using AutoDock Vina in PyRx 0.8 software implying the Lamarckian Genetic Algorithm and Empirical Free Energy Scoring Function.[27] The proteins were loaded as rigid and ligands as flexible and docking were performed using a grid whose centre was assigned as follows. Spike protein X= -36.10, Y= 27.91 Z= 8.24; ACE2 X= 39.25, Y= 4.68 Z= 25.94; $M^{pro}$ X= -13.90, Y= 23.06 Z= 65.67; and RdRp X= 94.71, Y= 94.03 Z= 100.32, respectively. The top five ligands with the least binding energy were further analyzed. The protein-ligand interaction was studied using Pymol[28] and Discovery studio visualizer.

### Physiochemical, Pharmacokinetic, and Toxicity Predictions

The pharmacokinetic properties were predicted using Swiss ADME,[29] Molsoft LLC,[30] and Pro-Tox II web servers.[31]

### Similarity Screening

The lead compounds were checked using the SWISS similarity tool to select antiviral drugs that FDA has already approved.

## Results

### Structure of Target Proteins

The spike protein of SARS-CoV-2 has 1273 amino acid residues, including N-terminal signal peptide, S1 subunit for receptor binding, and S2 subunit for membrane fusion. S1 subunit contains a receptor-binding domain that forms antiparallel β sheets (β1, β2, β3, β4, and β7). Between β4, and β7 sheets is a core containing receptor binding motif, the spike protein region directly interacting with the ACE2 receptor.[3] The humanACE2 receptor, a key regulator of renin angiotensin aldosterone system (RAAS) is an 805 amino acid protein that includes a zinc metallopeptidase catalytic domain (19–611 residues), composed of 20 α-helixes and nine $3_{10}$ helical strands and six short β-pleated sheets which play a vital role in RAAS by counterbalancing the adverse effects of ACE/RAAS pathway.[32] $M^{pro}$ is a 33.8 kDa homodimer, made up of two protomers A and B, each protomer further divided into three domains: I (8–101 residues), II (102–184 residues) composed of antiparallel β barrels, and III (201–303 residues) composed of α-helices.[33] RdRp (RNA-dependent RNA polymerase) is a 240–450 kDa enzyme containing an N-terminal β-hairpin (31–50 residues), a nucleotidyltransferase domain (115–250 residues) composed of seven α-helixes and three β-sheets, an interface domain (251–365 residues) composed of three α-helixes and five β-sheets and RdRp domain (366–920 residues).[34]

### Molecular Docking

A total of 215 phytochemicals derived from *C. papaya* were docked against spike protein, human ACE2, $M^{pro,}$ and RdRp of SARS-CoV-2. Out of the 215 phytochemicals, 48 showed binding energy ≤ -6 kcal/mol against all four targets (Table 1), and these molecules were considered active/hits as reported earlier.[23,35] The number of hit molecules (ΔG ≤ -6 kcal/mol) obtained against each target in the order of merit was ACE2 94, $M^{pro}$ 58, RdRp 58, and spike protein 48, respectively. The top-ranked five hits obtained against spike protein were hesperidin (-8.4 kcal/mol), violaxanthin (-7.8 kcal/mol), stigmasterol glucoside (-7.8 kcal/mol), zeaxanthin (-7.7 kcal/mol) and all-trans-neoxanthin (-7.6 kcal/mol). The protein-ligand interaction studies revealed that the compound hesperidin established seven H-bonds with spike protein involving active site residues Lys417, Gln493, and compound violaxanthin formed 2 H-bonds connected to catalytic residue Asn501. Similarly, compounds all-trans-neoxanthin, stigmasterol glucoside, and zeaxanthin exhibited a single H-bond interaction. The top-ranked leads against ACE2 were hesperidin (-11.3 kcal/mol), naringin (-11.2 kcal/mol), β-cryptoxanthin (-10.9 kcal/mol), violaxanthin (-10.7 kcal/mol) and zeaxanthin (-10.7 kcal/mol). The compound hesperidin exhibited nine H-bond interactions involving Tyr515 catalytic residue and compound naringin formed four H-bonds connected with active site residues Arg273 and Thr371. The compound zeaxanthin has three H-bonds but β-cryptoxanthin and violaxanthin have only hydrophobic interactions. The top-ranked five hits obtained against $M^{pro}$ were stigmasterol glucoside (-8.9 kcal/mol), dicumarol (-8.7 kcal/mol), rutin (-8.5 kcal/mol), naringin (-8.4 kcal/mol) and hesperidin (-8.1 kcal/mol). The compounds stigmasterol glucoside and naringin showed two H-bonds, hesperidin formed seven H-bonds involving active site residues Gly143, Ser144, and Cys145. Similarly, compounds dicumarol and rutin established four H-bonds with $M^{pro}$. The compounds hesperidin, stigmasterol glucoside, pseudocarpaine, naringin, and dehydrocarpaine II showed the least binding energy against RdRp with binding scores of -8.6 kcal/mol, -8.6 kcal/mol, -8.6 kcal/mol, -8.4 kcal/mol and -8.4 kcal/mol respectively. The compound hesperidin formed eight H-bond interactions, and pseudocarpaine and naringin formed three H-bonds with RdRp. Similarly, stigmasterol glucoside and dehydrocarpaine II showed two and one H-bond against RdRp. Among the top five leads obtained against the selected targets, the compound hesperidin showed a good binding affinity with all four targets (Table 2 and Fig. 1).

Molecular property analysis of leads showed that compounds β-cryptoxanthin, hesperidin, naringin, rutin, stigmasterol glucoside, and violaxanthin have good druggability index. In physicochemical properties, except dehydrocarpaine II, dicumarol, and pseudocarpaine all the lead molecules showed violation to Lipinski's rule of five (Table 3). Lipinski's rule of five (RO5) postulates the criteria for a drug molecule, which is molecular weight < 500 Da, hydrogen bond acceptors < 10, hydrogen bond donors < 5, and LogP < 5.[36]

The ADMET property analysis results are depicted in Table 3. The absorption profile showed that the compounds dehydrocarpaine II, dicumarol pseudocarpaine, and stigmasterol glucoside have good intestinal absorption, and all the lead molecules except dicumarol and pseudocarpaine act as P-glycoprotein substrates. BBB permeability indicates the ability of a drug to cross the brain membrane and all the lead molecules showed no BBB permeation. Inhibition of various cytochrome P450 isoforms like CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 affects the drug metabolism and excretion. All the lead molecules showed similarity in cytochrome metabolism except dicumarol which inhibits CYP1A2. All the lead molecules are non-mutagenic, non-hepatotoxic, and non-cytotoxic except all-trans-neoxanthin, hesperidin,

**Table 1:** List of phytochemicals from *C. papaya* with inhibitory activity (binding energy ≤ -6 kcal/mol) on all the selected targets.

| *S. No.* | *Phytochemical* | *Binding energy (kcal/mol)* | | | |
|---|---|---|---|---|---|
| | | *Spike* | *ACE2* | *$M^{pro}$* | *RdRp* |
| 1 | 1-Coumaroylquinic acid | -6.3 | -8.1 | -7.3 | -6.8 |
| 2 | 24-Methylenecycloartanol | -6.3 | -9.9 | -7.2 | -7.1 |
| 3 | 3-Hydroxyflavone | -6.7 | -8.4 | -6.6 | -6.3 |
| 4 | 5-Dehydro-avenasterol | -6.3 | -9.4 | -6.6 | -7.4 |
| 5 | 5-p-Nitrobenzoyl gentisic acid | -6.1 | -8.6 | -7.5 | -6.7 |
| 6 | all-trans-Neoxanthin | -7.6 | -10.5 | -7.1 | -8.2 |
| 7 | Antheraxanthin | -6.7 | -10.7 | -7.2 | -8.1 |
| 8 | Apigenin | -7.1 | -8.5 | -7.5 | -7.7 |
| 9 | Benzyl glucosinolate | -6.7 | -8.4 | -7 | -6.2 |
| 10 | beta-Carotene-5,6-epoxide | -7.4 | -10.5 | -7.2 | -7.8 |
| 11 | beta-Cryptoxanthin | -7.1 | -10.9 | -7.2 | -8 |
| 12 | Campesterol | -6.3 | -9.2 | -6.3 | -7.2 |
| 13 | Carpaine | -6.9 | -9.5 | -7.2 | -8.3 |
| 14 | Chlorogenic acid | -6.5 | -9 | -7.5 | -7 |
| 15 | Cianidanol | -6.6 | -9.1 | -7.4 | -7.5 |
| 16 | Clausamine G | -6.1 | -8.9 | -6.7 | -7 |
| 17 | Cycloartenol | -6.7 | -9.9 | -7.3 | -7.6 |
| 18 | Dehydrocarpaine I | -7 | -10 | -7.2 | -8.4 |
| 19 | Dehydrocarpaine II | -7.4 | -10.2 | -7.8 | -8.4 |
| 20 | delta7-Avenasterol | -6.3 | -9.1 | -6.7 | -7.3 |
| 21 | Dicumarol | -7.1 | -9.1 | -8.7 | -7.3 |
| 22 | epsilon-Carotene | -7 | -10.5 | -7.1 | -7.7 |
| 23 | Ethinylestradiol | -6.6 | -9.4 | -6.7 | -6.7 |
| 24 | Flavone | -6.5 | -8.3 | -7.6 | -6.8 |
| 25 | gamma-Carotene | -6.6 | -10.6 | -6.6 | -7.4 |
| 26 | Genistein | -6.2 | -8.5 | -6.8 | -7.2 |
| 27 | Glucotropaeolin | -7 | -8.3 | -7.1 | -6.7 |
| 28 | Hesperidin | -8.4 | -11.3 | -8.1 | -8.6 |
| 29 | Kaempferol | -6.3 | -8.5 | -7.1 | -6.8 |
| 30 | Luteolin | -7.5 | -8.9 | -7.7 | -7.8 |
| 31 | Lycopene | -6.7 | -9.7 | -6.7 | -7 |
| 32 | Myricetin | -7 | -8.6 | -7.7 | -7.7 |
| 33 | Naringenin | -7 | -8.5 | -7.2 | -7.7 |
| 34 | Naringin | -7.3 | -11.2 | -8.4 | -8.4 |
| 35 | Olean-12-ene | -7.2 | -9.7 | -7.1 | -8.2 |
| 36 | Prunasin | -6.1 | -7.7 | -6.8 | -6.3 |
| 37 | Pseudocarpaine | -7.4 | -9.9 | -8.1 | -8.6 |
| 38 | Quercetin | -6.8 | -9.2 | -7.6 | -7.7 |
| 39 | Quinine | -6.2 | -8.1 | -6.3 | -6.4 |
| 40 | Reserpine | -6.7 | -9.8 | -8 | -7.8 |
| 41 | Rutin | -7.5 | -10.5 | -8.5 | -8.1 |

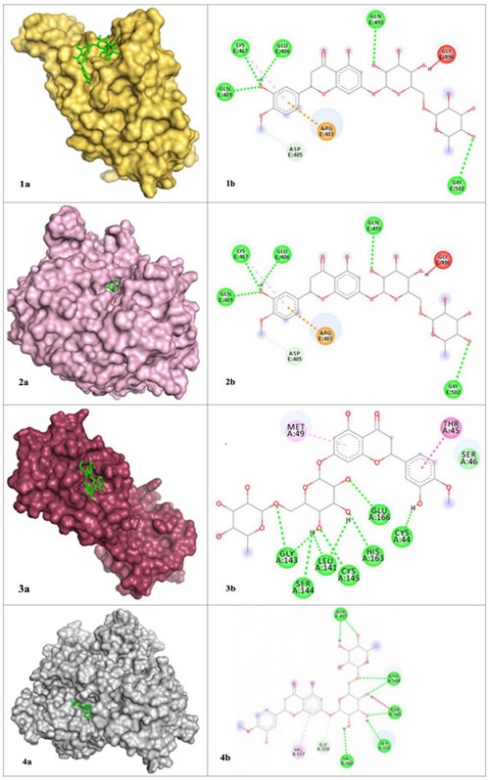

**Fig. 1:** Docking between target proteins and the best lead molecule hesperidin (a) 3D view and (b) 2D view. 1 (a&b) Spike protein and hesperidin, 2 (a&b) ACE2 and hesperidin, 3 (a&b) $M^{pro}$ and hesperidin, 4 (a&b) RdRp and hesperidin

naringin, rutin, and stigmasterol glucoside, which are immunotoxic.

In pharmacokinetic properties, ADMET parameters of the drug molecule are studied (Table 3). ADMET (absorption, distribution, metabolism, excretion, toxicity) properties play an important role in drug design since it accounts for the majority of drug failures in clinical trials. It determines whether a drug molecule will reach its active site, how long it will stay in the bloodstream, how well it gets distributed in tissues and the adverse effect of the molecule on the body.[37]

## Discussion

The effectiveness of vaccines, therapeutic medicines, and diagnostic tools is a great challenge to treat COVID-19 since the pathogen, SARS-CoV-2, is rapidly mutating.[38] Recent reports have unequivocally demonstrated that multi-target treatment approaches are a viable strategy to address this menace.[39] Several studies have reported the efficacy of phytomolecules with biological activity on multi-targets that effectively prevent or control the pathogenicity of mutating viruses.[40] *C. papaya* L. has been effectively used against dengue, HIV, ZIKA, and chikungunya viruses.[41] It may be due to the presence of a wide range of secondary metabolites such as alkaloids, fatty acids, sterols, and triterpenoids as major components in papaya.[17] The alkaloid carpaine, isolated from papaya

| | | | | | |
|---|---|---|---|---|---|
| 42 | Sapogenin A | -7 | -10.7 | -7.1 | -8.1 |
| 43 | Stigmasterol | -6.6 | -9.4 | -6.9 | -7.6 |
| 44 | Stigmasterol glucoside | -7.8 | -10.1 | -8.9 | -8.6 |
| 45 | Tiron free acid | -6 | -6.7 | -6.9 | -6.1 |
| 46 | Violaxanthin | -7.8 | -10.7 | -7.5 | -8.3 |
| 47 | Zeaxanthin | -7.7 | -10.7 | -6.9 | -7.9 |
| 48* | Remdesivir | -7.3 | -8 | -6.9 | -7.4 |
| 49* | Lopinavir | -6.7 | -9.7 | -6.7 | -7.5 |
| 50* | Hydroxychloroquine | -5.4 | -7.1 | -6.1 | -5.3 |

*Reference drug molecule.

leaf, has antiprotozoal, anthelmintic, antiplasmodial, and antithrombocytopenic activities.[16,42,43] The *in-vitro* cytotoxicity, anti-viral, and selective indices of papaya leaf n-butanol, ethyl acetate, and n-hexane fractions indicated that the n-hexane fraction was the most selective to the viral cells without inducing toxicity in the normal cells. Thirteen compounds were annotated from the foregoing three fractions of papaya leaf extracts, and those compounds were docked with multi-targets of SARS-CoV-2 and found to have significant anti-SARS-CoV-2 activity.[17] There are about 215 phytochemical constituents reported from *C. papaya* and all these compounds were not subjected to *in-silico* or *in-vitro* screening for the identification of the best lead against SARS-CoV-2. The *in-silico* approach is the primary option for rapid screening of phytochemicals to determine their druggability with the least economic input and generating clear-cut theoretical insights.

**Table 2:** Binding interaction of the lead molecule hesperidin with the selected targets

| *Hit Molecules* | *PDB ID* | *BE* | *Active Site Residues* | | |
|---|---|---|---|---|---|
| | | *(kcal/mol)* | *H-Bond* | *Bond Length ($A^0$)* | *Hydrophobic Interactions* |
| Spike protein | 6M0J | -8.4 | Glu406:OE2--H:Lig | 2.35 | Arg403 |
| | | | Asp405:O---C:Lig | 3.52 | Lys417 |
| | | | Gln409:HE21--O:Lig | 2.73 | |
| | | | Lys417:HN---O:Lig | 2.87 | |
| | | | Gln493:OE1--O:Lig | 3.02 | |
| | | | Gln493:HE21--O:Lig | 2.21 | |
| | | | Gly502:HN---O:Lig | 2.81 | |
| ACE2 | 1R4L | -11.3 | Pro346:CD---O:Lig | 3.38 | Arg273 |
| | | | Lys363:HZ3---O:Lig | 2.69 | Phe274 |
| | | | Asp367:OD1---H:Lig | 2.3 | Pro346 |
| | | | Asp368:OD1---H:Lig | 2.79 | Glu375 |
| | | | His374:NE2--H:Lig | 2.5 | |
| | | | Glu402:OE1--H:Lig | 2.23 | |
| | | | Thr445:OG1---H:Lig | 2.82 | |
| | | | Tyr515:HH---O:Lig | 2.15 | |
| | | | Arg518:HH11--O:Lig | 2.63 | |
| $M^{pro}$ | 7BUY | -8.1 | Cys44:O--H:Lig | 1.75 | |
| | | | Leu141:O--H:Lig | 2.17,2.87 | Thr45 |
| | | | Gly143:HN--O:Lig | 2.48 | Met49 |
| | | | Ser144:OG--H:Lig | 2.34 | |
| | | | Cys145:HN1--O:Lig | 2.25 | |
| | | | His163:HE2--O:Lig | 2.32 | |
| | | | Glu166:HN--O:Lig | 2.21 | |
| RdRp | 7BV2 | -8.6 | Asn497:HN---O:Lig | 2 | Val557 |
| | | | Thr565:HG1--O:Lig | 2.08 | |
| | | | Arg569:HH22-O:Lig | 2.66,2.70 | |
| | | | Val560:O----H:Lig | 2.69 | |
| | | | Asn497:O----H:Lig | 2.04 | |
| | | | Ser501:O----H:Lig | 2.93 | |
| | | | Gly559:CA----O:Lig | 3.00 | |

**Table 3:** Pharmacokinetic analysis of selected hit molecules

| | | β-cryptoxanthin | All-trans-neoxanthin | Dehydrocarpaine II | Dicumarol | Hesperidin | Naringin | Pseudocarpaine | Rutin | Stigmasterol glucoside | Violaxanthin | Zeaxanthin |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Physiochemical properties | Molecular weight | 552.8 | 600.8 | 474.6 | 336.2 | 610.5 | 580.5 | 478.7 | 610.5 | 574.8 | 600.8 | 568.8 |
| | HBA | 1 | 4 | 6 | 6 | 15 | 14 | 6 | 16 | 6 | 4 | 2 |
| | HBD | 1 | 3 | 0 | 2 | 8 | 8 | 2 | 10 | 4 | 2 | 2 |
| | MolLogP | 12.25 | 8.74 | 5.66 | 2.07 | -0.14 | -0.44 | 6.29 | -0.33 | 5.6 | 9.76 | 10.91 |
| | Drug-likeness score | 0.78 | -0.78 | -1.17 | -0.02 | 0.94 | 1.05 | -1.49 | 0.91 | 0.33 | 0.29 | -0.18 |
| Pharmacokinetics | GI absorption | Low | Low | High | High | Low | Low | High | Low | High | Low | Low |
| | BBB permeability N | | N | N | N | N | N | N | N | N | N | N |
| | P-gp substrate | Y | Y | Y | N | Y | Y | N | Y | Y | Y | Y |
| | CYP1A2 inhibitor | N | N | N | Y | N | N | N | N | N | N | N |
| | CYP2C19 inhibitor | N | N | N | N | N | N | N | N | N | N | N |
| | CYP2C9 inhibitor | N | N | N | N | N | N | N | N | N | N | N |
| | CYP2D6 inhibitor | N | N | N | N | N | N | N | N | N | N | N |
| | CYP3A4 inhibitor | N | Y | N | N | N | N | N | N | N | N | N |
| Toxicity | Hepatotoxicity | N | N | N | N | N | N | N | N | N | N | N |
| | Carcinogenicity | N | N | N | N | N | N | N | N | N | N | N |
| | Immunotoxicity | N | Y | N | N | Y | Y | N | Y | Y | N | N |
| | Mutagenicity | N | N | N | N | N | N | N | N | N | N | N |
| | Cytotoxicity | N | N | N | N | N | N | N | N | N | N | N |

The best way to eradicate the disease is to prevent viral entry and multiplication in the host body. For this purpose, the selection of the right targets in the pathogenicity system is of paramount importance. SARS-CoV-2 enters the human body by the interaction between the viral structural proteins, namely spike protein and the human receptor protein, ACE2. Inactivation of these two proteins prevents the entry of the virus into human host cells, and therefore these two proteins were selected as the targets. Once the virus enters the human cells, its replication should be effectively blocked. $M^{pro}$ and RdRp are the viral proteins that play pivotal roles in viral replication[33,34] and therefore, these two viral proteins were also selected as the targets. The selection of multi-targets involved in the two main stages of infection and replication and the discovery of a single drug that can simultaneously inhibit all these multi-target involved at different stages of pathogenicity can effectively overcome the challenge of the mutating behavior of the virus. Out of 215 phytochemicals screened, 48 have binding energy ≤ -6 kcal/mol against all four targets, *i.e.,* 22.32% of the phytochemicals have inhibitory activity on multi-target (Table 1), and the majority of them belong to flavonoids, carotenoid, and phytosterol classes. Among these, the compound hesperidin showed the least binding energy with all the targets, such as spike protein (-8.4 kcal/mol), human ACE2 (-11.3 kcal/mol), $M^{pro}$ (-8.1kcal/mol), and RdRp (-8.6 kcal/mol), and showed a better binding score when compared with the control standard drugs remdesivir, hydroxychloroquine, and lopinavir.[44] Although hesperidin showed a violation of Lipinski's rule of five, it has a promising druggability index and is an FDA-approved drug (DrugBank accession number: DB04703) used to treat vascular diseases. Lipinski stated that his rule of five does not apply to natural products.[45] The significant inhibitory activity of hesperidin at

multiple targets of SARS-CoV-2, such as spike protein, ACE2, and proteases ($PL^{pro}$ and $M^{pro}$), was demonstrated through docking and its mode of inhibition was also well discussed.[46] The present docking results were in line with the earlier reports[46] in which the compound, hesperidin, was mostly derived from citrus fruits. Papaya is a rich source of flavonoids, carotenoids, and sterols. Besides hesperidin, the flavonoids naringin and rutin, carotenoid β-cryptoxanthin, stigmasterol glucoside, and violaxanthin also showed good druggability indexes, physicochemical properties, and ADMET. These molecules are widely accepted dietary antioxidants with multiple pharmacological activities. So the wide range of antiviral activity, particularly against SARS-CoV-2, of papaya is not only induced by a single compound, hesperidin, but also by the synergistic and cumulative effects of different classes of the foregoing phytochemicals.

The multiple pharmacological effects of hesperidin, such as antiviral, antioxidant, antidiabetic, antihypertensive, anti-inflammatory, and cardioprotective effects, were reported.[46] It can bind to multi-targets in complex diseases like Alzheimer's disease.[47] The safety profile of hesperidin was confirmed by FASEB (the Federation of American Societies of Experimental Biology), and a clinical trial with more than 2850 patients administered with hesperidin for almost a year showed no toxicity.[48] Tablet daflon 500 mg combines hesperidin and diosmin to treat vascular diseases.[49] Also, the FDA-approved drug ouabain showed similarity with hesperidin with scores of 0.467, which was used to treat COVID-19 patients with cardiovascular diseases.[50] Therefore, hesperidin can be recommended as a promising multi-target drug candidate against COVID-19.

## Conclusion

Overall, results indicated that *C. papaya* is a rich source of phytochemicals with activity on muti-targets involved at different stages of SARS-CoV-2 infection and multiplication in the human host. Among these, the compound hesperidin was selected as the best lead since it showed the least binding energy, admissible ADMET, and a better binding score than the control drugs. Moreover, it is already used as an approved drug to treat vascular disease. However, *in-vitro* and *in-vivo* experiments are to be required to validate the activity in the live system.

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