Computational prediction of Carica papaya phytocompounds as potential drug agent against RdRp and spike protein of SARS-nCoV2 by molecular docking and dynamics simulation approaches

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Research Article

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Computational prediction of *Carica papaya* phytocompounds as potential drug agent against RdRp and spike protein of SARS-nCoV2 by molecular docking and dynamics simulation approaches

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Abstract:

The emergence of the COVID-19 outbreak caused by SARS-nCoV2 led to mass-scale mortalities worldwide. The need of the hour is to develop strategies and design drugs/vaccines to control this contagion. Current research predicted the promising drug agents from the *Carica papaya* compounds by docking and MD simulations approaches with two major drug target proteins of spike receptor-binding domain and RNA-dependent RNA polymerase of SARS-nCoV2. MOE & PyRx softwares were used for ligand-protein interactions and docking scores predictions. Additionally, MD simulation analysis was performed on the ligands with the lowest binding energies using the NAMD/VMD softwares to compute the stability of the best complexes. Furthermore, SwissADME analysis was also performed to check Lipinski's physiochemical parameters of ligands. Our docking results showed the best binding energies of Lutein & β-cryptoxanthin ligands with both of the proteins followed by MDS analysis to check the complex stability and physical perturbations to determine the fitness of these complexes as a potential drug agent against COVID-19 infection. The findings of the current study need experimental validations to proceed further for clinical trials.

Keywords: COVID-19, *Carica papaya*, spike protein, RNA-dependent RNA polymerase, ligand-protein docking, molecular dynamic simulations

1 Introduction

COVID-19 has been vigorously spreading around the globe since December 2019. Due to an increase in confirmed cases and mortality rate worldwide, World Health Organization (WHO) declared the pandemic of novel coronavirus (SARS-nCoV2) as a global health emergency [1-5]. This causative agent leads to the disturbance in the respiratory tract and shows symptoms like pneumonia that can damage various body organs in severe conditions [6, 7].

At present, vaccines developed against this viral disease, but no explicit remedies for COVID-19 are available to control this pandemic. [4]. The researchers have identified major target proteins for drug designing, which may be considered: main protease/3C-like protease ($3CL^{pro}$), papain-like protease (PL^{pro}), spike protein, and polymerase protein RdRP. [8-10].

SARS-nCoV2 is an RNA virus having a positive sense single strand of 27-32 kb approximately [11, 12]. Because of its crown-like appearance under an electron microscope, it is named CORONA [13]. This virus is made up of different kinds of structural proteins includes Spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins; among them, S protein provides an attachment site to the host cell [14, 15]. ACE2; angiotensin-converting enzyme 2 is present on the human cell membrane, which works as the receptor for SARS-nCoV2 [16]. Spike protein significantly contributes to the entry of the virus into the host cell, which is concerned with the focus of therapeutic and vaccine design [17]. The other target RNA-dependent RNA Polymerase is also considered the potent source for dealing with this disease. Coronaviruses assemble a multi-RNA-synthesis complex of viral non-structural proteins (nsps), which is accountable for the transcription and replication of the viral genome [12]. Hence, the polymerase is a promising drug target [18]. It infects a wide range of hosts, including humans and other animals as well. Presumably, the transmission of this virus in humans is via droplets that come out of an infected person's mouth after sneezing or coughing along with direct contact [19, 20].

Different web-based or stationary-based software were used for the computational analysis of drug designing. We used Molecular Operating Environment (MOE) software for molecular docking studies. It is a complete package that integrates ligand-protein docking, drug-likeness attributes, molecular and binding interactions, modelling and simulations of structures, and docking scores to interpret the docked compounds' compatibility [21, 22]. Moreover, we used PyRx software for cross-docking authentication of our compounds together with BIOVIA Discovery Studio Visualizer 2020 (DS-2020) and UCSF Chimera for structural preparation of ligands and proteins [23-25]. Additionally, we performed molecular dynamics (MD) simulations analysis on best-docked complexes using NAMD/VMD to check our significant complexes' stability [26-28]. The comprehensive workflow of our computational prediction analysis is summarized in [Figure 1]. Actively, our research is focused on the computational estimation of Carica papaya compounds having anti-inflammatory and immunomodulatory activities as a latent drug target, counter to SARS-nCoV2 RNA-Polymerase RdRP (7BW4) and Spike (7BZ5) protein. This study reassures

scientists and pharmaceutical industries to have in-vitro and in-vivo investigations on those ligands for COVID-19 clinical trials.

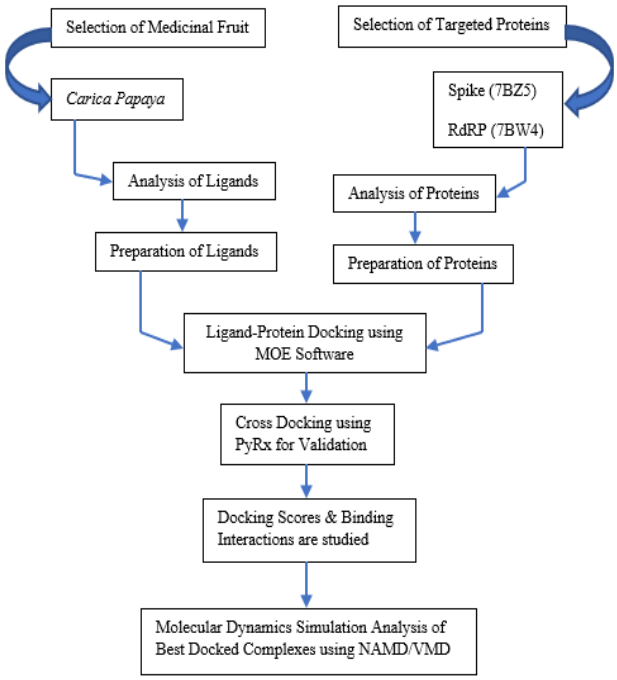


Figure 1: *The comprehensive work stream of computational analysis*

2 Materials and Methods

2.1 Selection of the medicinal fruit

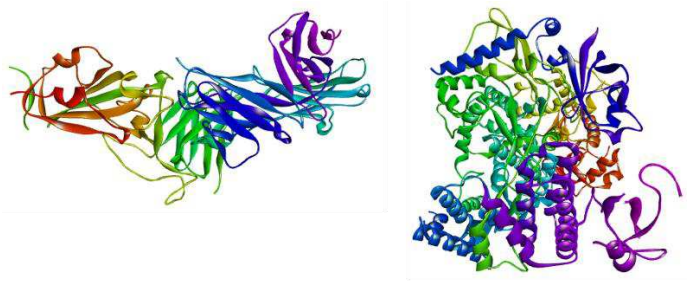
Ample of research was carried out on *Carica papaya,* commonly known as Papaya, renowned for its medicinal properties among different world regions. It can treat various ailments such as asthma, ulcer, diabetes, and cardiovascular diseases [29]. Papaya extracts have significant immunostimulatory, antioxidant, and inflammation-reducing activities [30]. For these significances, scientists may consider Papaya's derived substances for immunotherapeutic purposes against the COVID-19 viral pandemic soon under clinical tests.

2.2 Selection of targeted enzymes

We chose two proteins from the SARS-nCoV2 coronavirus with different natures as the primary drug targets. 3D structures of Spike S1 protein (https://www.rcsb.org/structure/7BZ5) and RdRP; RNA-dependent RNA Polymerase (https://www.rcsb.org/structure/7BW4) are downloaded from Protein Data Bank (RCSB-PDB) under PDB ID of 7BZ5 and 7BW4 respectively. Crystallographic properties of 7BZ5 and 7BW4 are shown in [Table 1]. The structures of proteins are presented in [Figure 2(a, b)].

Table 1: *Crystallographic properties of proteins (enzymes)*

Enzyme	PDB Code	Classification	Organisms	Expression System	Resolution	Method	Total Structure Weight	Chain
Spike (S1)	7BZ5	Viral protein	SARS-nCoV2	Homo sapiens	1.84 Å	X-RAY Diffraction	73.36 kDa	A
RdRp	7BW4	Replication	SARS-nCoV2	Escherichia Coli	3.70 Å	Electron Microscopy	159.03 kDa	A



a) **b)**

Figure 2: *Proteins used for docking studies:* ***(a)*** *Spike; 7BZ5 and* ***(b)*** *RdRP; 7BW4*

2.3 Preparation of ligands

2.3.1 Preparation of ligands using molecular operating environment (MOE)

Firstly, by searching *Carica papaya* compounds from literature, we used a chemical database (e.g., PubChem) to retrieve structures and then using Molecular Operating Environment (MOE) software for the preparation of ligands [30]. Table 2 reports the major chemical-derived compounds of *Carica papaya* collected. Furthermore, we downloaded *Canonical Smiles* of all associated ligands and exported them one by one in the MOE *builder module* to construct fully prepared ligands for molecular docking.

2.3.2 Preparation of ligands using Python Prescription (PyRx) Virtual Screening Tool

For the sake of validation purposes, cross-docking was also being performed using PyRx software [31]. In this software, the ligand's 2D structure in *.sdf* format is downloaded from PubChem. Before performing docking using PyRx, we have to minimize the energy of our ligand using a built-in UFF force field and a conjugate gradient algorithm up to 500 steps [32]. The next step is to convert our ligand in pdbqt format, and our desired ligand is prepared for further execution in this software for molecular docking analysis.

2.4 Preparation of proteins

2.4.1 Preparation of proteins using MOE

Secondly, we selected two proteins of SARS-nCoV2 coronavirus for primary drug targets having different natures [16, 33]. We used *open* command to visualize these structures in the MOE window and created the pure form of protein to make them ready for docking with the desired

ligands. This step was carried out in the following ways. At first, we removed all the water molecules from the protein's surface and already attached inhibitors to avoid hindrance at the interaction region while molecular docking for precise results. Secondly, we made *structure preparation* of selected proteins in MOE to resolve its chains error. After that, we found the active sites in *the Site Finder* tab of MOE to create the space for the attachment site for our ligands. As we were not familiar with the active site residues of the enzyme, we selected the longest chain from the list of possible catalytic sites, appeared on *the Site Finder* window. In addition, dummy sites were created by clicking on *the Dummies* tab. Moreover, on completion of these steps, our protein was fully prepared for docking.

2.4.2 Preparation of proteins using PyRx

We generated our fully prepared selected proteins in Molecular Operating Environment (MOE) for docking studies; the same is done for redocking using PyRx. For this purpose, we used two different software for the preparation of our receptors. Firstly, BIOVIA Discovery Studio Visualizer 2020 (DS-2020) is used. The desired protein is opened in DS, and pre-processing is carried out by removing all the heteroatoms, already bound ligands, and water molecules from the chains of the protein molecule. The only protein part with its particular chain having active sites is left and saved, a pre-processed protein molecule in the computer. Secondly, this pre-processed protein file is loaded in another software UCSF Chimera for the energy minimization process. From the *tools* option, we click on *Dock Prep*, and its window is displayed. The new module is opened by clicking on OK, and hydrogen atoms are added to the protein structure. After this step, the AMBER force field (AMBER ff14SB) is used by default for energy minimization and for adding charges on protein using the Gasteiger package. Now save this protein file in PDB format; this is a fully prepared protein protocol for cross-docking authentication using PyRx software.

2.5 Molecular docking

2.5.1 Molecular docking by MOE

The interaction of our chosen ligands with the active sites of the 7BZ5 and 7BW4 was carried out through *a dock* feature implanted in MOE, using its built-in algorithms and tools. Additionally, the molecular interactions with the binding site of the receptors consumed some time to complete. The docking scores of the ligands are displayed in a tabulated form in a new window after completion. Together with, ligands 2D and 3D interactions were also visible by choosing their options as well. These interactions showed that where the probable hydrogen bonds formed with amino acids. Our main emphasis was on the binding energies or E-Score of this docking process to consider *Carica papaya* compounds as potent drug agents [34].

2.5.2 Molecular docking using PyRx

The molecular docking was performed by uploading our prepared ligand and protein on the PyRx window. After that, from the control command option, select *Vina Wizard* and navigate our ready-to-dock macromolecules. Clicking the *forward* tab on PyRx, the grid box is generated on our

complex. Adjust that box in our desired active sites of protein and ligand molecule for accurate binding of the complex. After sometimes the docking score appears in a new window which is our desired results. These docking results were compared with the docking scores generated by MOE for cross-checking and validation purposes.

2.6 Molecular Dynamics Simulation (MDS)

The best-docked complexes are selected for molecular dynamics simulation (MDS) study. MD simulations were run using nano-scale molecular dynamics (NAMD) software to predict the dynamic behavior of the ligand-protein complexes [35]. Furthermore, Visual Molecular Dynamics VMD is utilized to prepare complexes and their trajectories analysis [36]. The configuration files which are required for MD simulations were generated from the CHARMM-GUI website (https://www.charmm-gui.org/). For ligand parameterization CHARMM General Force Field (CGenFF) web-based tool (http://mackerell.umaryland.edu/~kenno/cgenff/) was used. MD simulation is carried out in the following different steps. The path directory is set for all the manipulation process in our computer.

First of all, the resultant file of the cross-docking process using the PyRx. pdbqt file of our desired complex has to be altered that contains the best binding ligand with our particular protein. The coordinates of the ligand which showed the best binding energy were inserted in the protein file. Now, covert this file format into *.pdb* file format by using *Open Babel-GUI* software for further evaluation. After this *complex. A PDB* file is generated, open it on VMD using windows power shell for creating topologies files using CHARMM-GUI of bound ligand in. mol*2* file format. It will create *a .psf* file of our desired ligand.

Similarly, we have to load protein files in VMD using the built-in functionality of Automatic PSF Builder to create protein topology. Furthermore, a particular *script.tcl* file was used to compile both our ligand and protein to make it ligand-protein complex. Next, we must add a solvation box around our complex for simulations studies by loading our complex in VMD. In addition, our last step for MD simulation was to run the final scripted command by uploading it on NAMD software by adjusting all the parameters like temperature, pressure, time, energy minimization, and periodic boundary conditions of our complex. This simulation will take much time for its completion and simulation for our complex to be done.

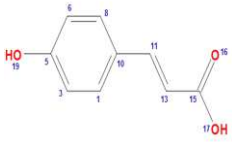
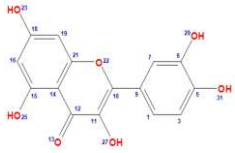
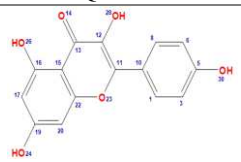
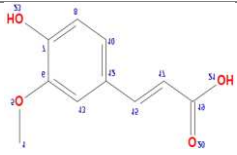
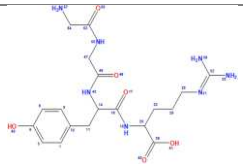
3 Results and Discussion

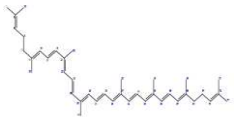
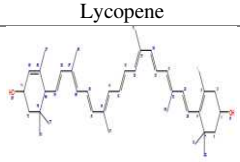
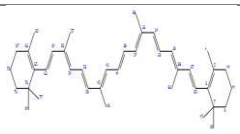
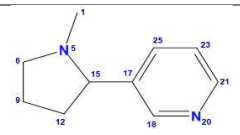
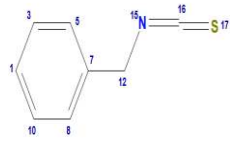
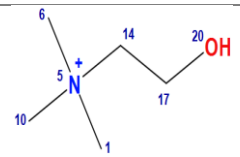
Our docking results and simulation analysis revealed that some compounds of *Carica papaya* extracts have the lowest energies with different natures of proteins 7BZ5 and 7BW4, respectively. Here we deliberated the ligand's properties and their docking scores. Moreover, the medicinal significance, ligand-protein interactions, surface maps of significant compounds (having best docking scores), and MD simulation analysis involve RMSD and RMSF calculations. H-bond interactions of best-docked complexes were also described one by one in detail.

3.1 Ligand's Lipinski's physiochemical properties

Initially, we confer about the ligands, which we selected and prepared for our docking purposes. Chemical structures, their generic names, PubChem ID's and molecular formulas of chemical compounds from *Carica papaya* are reported in [Table 2]. Lipinski's rule key physicochemical parameters were also studied for each ligand (testified in Table 2) along with the violations of this rule by using the SwissADME web tool to evaluate drug likeliness attributes (http://www.swissadme.ch/index.php). There is a total of 12 derived compounds of Papaya, which were studied and observed. Out of which four compounds (Papain, β-Cryptoxanthin, Lycopene, and Lutein) violate Lipinski's rule with two violations each. The rest of the ligands have zero violations and fulfil the properties of this rule. So, all these ligands are acknowledged according to Lipinski's rule of 5 and could be considered potential inhibitors [37]. Many studies also suggested that Lipinski's physiochemical rules do not apply to natural and artificial drugs [38].

Table 2: *Chemical structure of derived substances from Carica papaya with Lipinski's rule*

Pub Chem ID / Molecular Formula	**Ligand's Structure and Names**	**Lipinski's Physiochemical Parameters Rule**	
		Properties	**Value**
637542		MW(g/mol)	164.16
		H-donor	2
		H-acceptor	3
		LogP	1.26
$C_9H_8O_3$		LogS	-2.02
		TPSA (A°)	57.53
	p-Coumaric Acid	Violations	0
5280343		MW(g/mol)	302.24
		H-donor	5
		H-acceptor	7
		LogP	1.23
$C_{15}H_{10}O_7$		LogS	-3.16
		TPSA (A°)	131.36
	Quercetin	Violations	0
5280863		MW(g/mol)	286.24
		H-donor	4
		H-acceptor	6
		LogP	1.58
$C_{15}H_{10}O_6$		LogS	-3.31
		TPSA (A°)	111.13
	Kaempferol	Violations	0
445858		MW(g/mol)	194.18
		H-donor	2
		H-acceptor	4
		LogP	1.36
$C_{10}H_{10}O_4$		LogS	-2.11
		TPSA (A°)	66.76
	Ferulic Acid	Violations	0
3705436		MW(g/mol)	451.48
		H-donor	8
		H-acceptor	8
		LogP	-2.09
$C_{19}H_{29}N_7O_6$		LogS	1.13
		TPSA (A°)	235.25
	Papain	Violations	2

5281235		MW(g/mol)	552.87
		H-donor	1
		H-acceptor	1
$C_{40}H_{56}O$		LogP	10.20
		LogS	-10.33
		TPSA (A°)	20.23
	β-Cryptoxanthin	Violations	2
446925		MW(g/mol)	536.87
		H-donor	0
		H-acceptor	0
$C_{40}H_{56}$		LogP	11.90
		LogS	-11.92
		TPSA (A°)	0.00
	Lycopene	Violations	2
5281243		MW(g/mol)	568.87
		H-donor	2
		H-acceptor	2
$C_{40}H_{56}O_2$		LogP	9.21
		LogS	-9.64
		TPSA (A°)	40.46
	Lutein	Violations	2
5280489		MW(g/mol)	536.87
		H-donor	0
		H-acceptor	0
$C_{40}H_{56}$		LogP	11.11
		LogS	-11.04
		TPSA (A°)	0.00
	β-Carotene	Violations	0
89594		MW(g/mol)	162.23
		H-donor	0
		H-acceptor	2
$C_{10}H_{14}N_2$		LogP	1.50
		LogS	-1.89
		TPSA (A°)	16.13
	Nicotine	Violations	0
2346		MW(g/mol)	149.21
		H-donor	0
		H-acceptor	1
C_8H_7NS		LogP	2.91
		LogS	-3.07
		TPSA (A°)	44.45
	Benzyl Isothiocyanate	Violations	0
305		MW(g/mol)	104.17
		H-donor	1
		H-acceptor	1
$C_5H_{14}NO^+$		LogP	-1.38
		LogS	-0.10
		TPSA (A°)	20.23
	Choline	Violations	0

3.2 Docking Scores

Table 3 reports E-score (Kcal/mol) or binding energies obtained from MOE docking algorithms of *Carica papaya* compounds with 7BZ5 and 7BW4, and cross-docking scores are validated using PyRx software. Acquired results showed that Lutein gave the lowest binding energy, i.e., -8.9013

Kcal/mol in complex with spike receptor-binding domain (7BZ5) protein from MOE and by using PyRx β-Cryptoxanthin gave the lowest binding affinity, i.e., -9.3, which is the best score in our research compared to other docked compounds. In addition, Lycopene gave the lowest energy, i.e., -8.7148 Kcal/mol in complex with our second selected protein RNA-Dependent RNA polymerase (7BW4) from MOE and with redocking using PyRx, Lutein has the lowest docking score, i.e., -8.3, which is another best score among all docked compounds. These scores are the reckoning-based analysis in the search for scheming the drugs against COVID-19.

Table 3: *Docking results of Carica papaya compounds with 7BZ5 and 7BW4*

Ligands	Scores (Kcal/mol) by MOE		Scores (Kcal/mol) by PyRx	
	7BZ5	**7BW4**	**7BZ5**	**7BW4**
p-Coumaric Acid	-4.8987	-4.3025	-6.6	-6.0
Quercetin	-6.3272	-5.5544	-7.9	-8.0
Kaempferol	-5.4416	-5.5174	-6.1	-7.3
Ferulic Acid	-5.0683	-5.0404	-6.5	-5.9
Papain	-8.6222	-7.9099	-7.3	-7.3
β-Cryptoxanthin	-8.6993	-8.5152	-9.3	-8.1
Lycopene	-8.3588	-8.7148	-6.4	-7.4
Lutein	-8.9013	-8.4875	-8.7	-8.3
β-Carotene	-8.7844	-8.1668	-8.6	-7.8
Nicotine	-4.9437	-5.0314	-5.0	-5.3
Benzyl Isothiocyanate	-4.6074	-4.4378	-4.9	-4.1
Choline	-4.2034	-4.2146	-3.4	-3.5

3.3 Medicinal significance of ligands

The significant compounds that gave the best docking score and lower energies were the therapeutic drugs used against the causative agent (SARS-nCoV2). Their medicinal significance was studied and described as follows: the first significant compound is Papain, having properties like immunomodulatory and antioxidant, and is used to treat inflammation [39]. It has enzymatic activities, which act as an inhibitor as well. The second primary compound having a good docking score is β-Cryptoxanthin which belongs to the carotenoid family. (https://www.phytochemicals.info/phytochemicals/beta-cryptoxanthin.php) The antioxidant property of this compound prevents cells and their machinery from making free radicals which otherwise can damage the cells and tissues [40]. The third major compound, which has a maximum docking score with RdRP protein (7BW4) of -8.7148 Kcal/mol, is Lycopene. It is a naturally occurring carotenoid, a potent antioxidant molecule that scavenges ROS, reactive oxygen species [41]. It has potential health benefits in many biological activities in humans. It also uncovered a critical antiviral impact and anticytotoxic movement against HSV-1 contamination. The fourth major compound is Lutein which gave the maximum docking score with Spike protein (7BZ5) of -8.9013 Kcal/mol. It is rich in antioxidants, which play an anti-inflammatory role as per literature studies [42]. It also protects cells against the harmful effects of free radicals. In the preceding, the fifth primary compound is β-Carotene which is a potent antioxidant, a human metabolite having

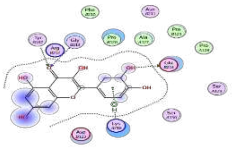
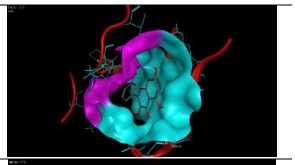
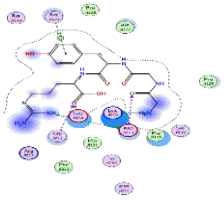
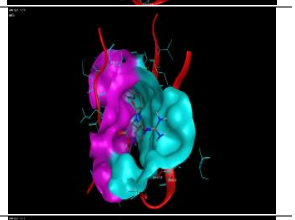
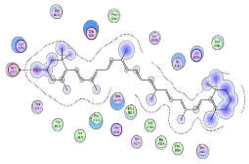
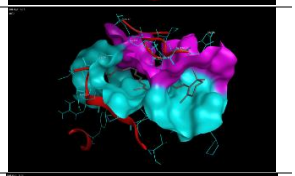
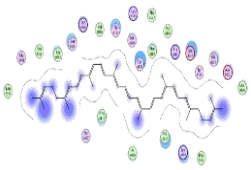
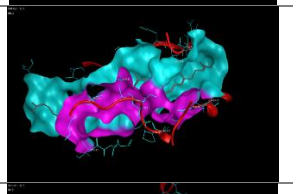
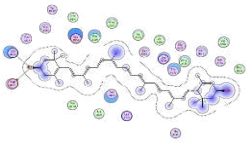
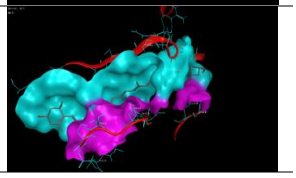
chemo preventive activities. It stimulates the immune system by releasing killer cells, lymphocytes, and phagocytes [43]. All these ligands have been scientifically impactful against the damage caused by the foreign agents of viral infection and act as the immunomodulator in cellular events.

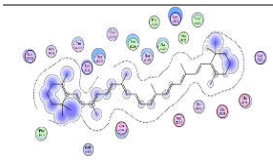
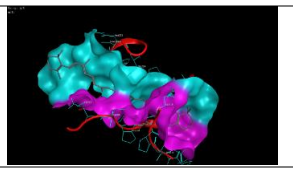
3.4 Ligands interactions with targeted proteins

3.4.1 Ligands and spike receptor-binding domain protein complexes

We got the results of all possible ligand-protein interactions by using MOE software after completing the docking process. All the observable interactions of the 7BZ5 complex with the major compounds of *Carica papaya* having the lower binding energies are discussed in table 4. Firstly, the ligand interactions with the active site of spike protein (7BZ5) can be easily observed. Secondly, the probable hydrogen interactions with the amino acids, type of bonding, their required energies (in Kcal/mol), and their distances in Angstrom (Å) are mentioned. Thirdly, the surface maps are graphically represented with the attachment sites of ligands in Spike protein. Besides, some compounds have the best docking scores with non-perceptible hydrogen interactions.

Table 4: *2D and 3D interactions of the major compounds of Carica papaya with 7BZ5*

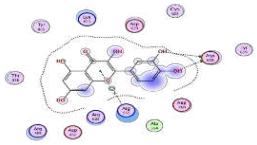
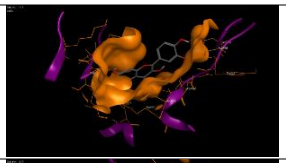
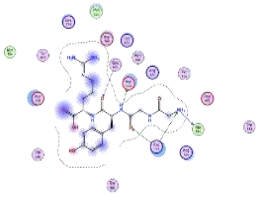
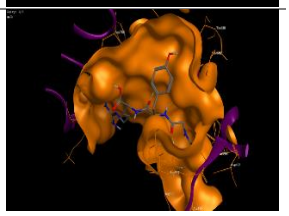
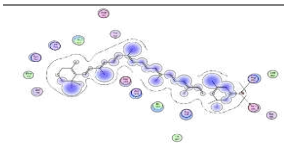
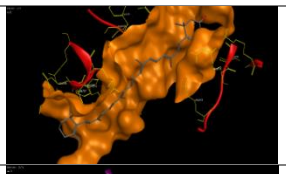
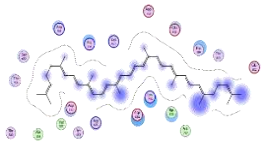
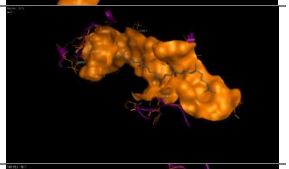
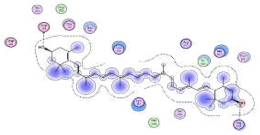
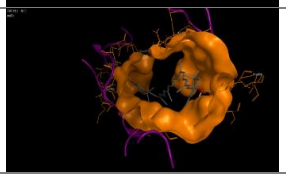
2D-Interactions/Ligand Bonding	Possible Interactions and Name				3D- Interactions/Surface Maps
	Amino Acid	Type	Distance (Å)	Energy (Kcal/mol)	
	GLU-214	H-don	2.78	-7.2	
	TYR-130	H-acce	3.02	-1.1	
	GLY-213	H-acce	2.92	-1.5	
	LYS-216	π-H	3.99	-0.6	
	Quercetin				
	ASP-123	H-don	3.10	-2.5	
	GLY-213	H-acce	3.16	-1.0	
	GLU-214	H-acce	3.27	-1.9	
	ASP-123	H-acce	3.02	-4.4	
	LYS-216	H-acce	3.10	-8.0	
	SER-129	π-H	3.83	-0.6	
	Papain				
	ASP-123	H-don	2.72	-2.9	
	β-Cryptoxanthin				
	There are non-observable interactions; only Van der Waals (electrostatics) interactions are evident.				
	Lycopene				
	ASP-123	H-don	3.30	-1.0	
	LYS-216	H-acce	3.08	-0.6	
	Lutein				

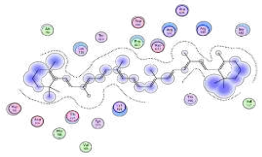
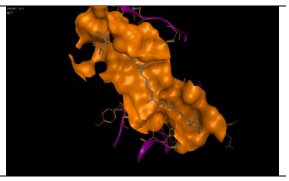
	There are non-observable interactions; only Van der Waals (electrostatics) interactions are evident. **β-Carotene**	

3.4.2 *Ligands with RdRp protein complexes*

After completing the docking process, we got the results of all possible ligand-protein interactions of significant compounds of *Carica papaya* (having the best docking scores) with 7BW4, as shown in table 5. The 2D interactions of ligands with the active sites of (7BW4) RNA-dependent RNA polymerase (RdRp) is primarily displayed. Furthermore, the noticeable hydrogen interactions with the amino acids, type of bonding, distances in Angstrom (Å), binding energies (Kcal/mol), and 3D interactions are mentioned. Moreover, there are two compounds (Lycopene and β-Carotene) that have non-perceptible hydrogen interactions as well.

Table 5: *2D and 3D interactions of the major compounds of Carica papaya with 7BW4*

2D-Interactions/Ligand Bonding	Possible Interactions and Name				3D- Interactions/Surface Maps
	Amino Acid	Type	Distance (Å)	Energy (Kcal/mol)	
	ASP-618	H-don	2.78	-2.6	
	ASP-618	H-don	2.89	-1.4	
	ASP-618	H-don	3.40	-0.8	
	ARG-553	π-cation	3.44	-0.6	
	Quercetin				
	ASP-623	H-don	2.98	-0.9	
	ALA-554	H-don	3.12	-1.1	
	CYS-622	H-acce	3.35	-2.6	
	ARG-553	H-acce	2.95	-2.8	
	ARG-553	H-acce	2.91	-3.2	
	ARG-553	H-acce	2.92	-4.1	
	Papain				
	ASP-865	H-don	2.72	-2.5	
	ARG-858	H-acce	2.88	-1.7	
	β-Cryptoxanthin				
	There are non-observable interactions; only Van der Waals (electrostatics) interactions are evident.				
	Lycopene				
	ASP-164	H-don	2.90	-2.9	
	ARG-858	H-acce	3.24	-1.7	
	Lutein				
	There are non-observable interactions; only Van der Waals (electrostatics) interactions are evident.				

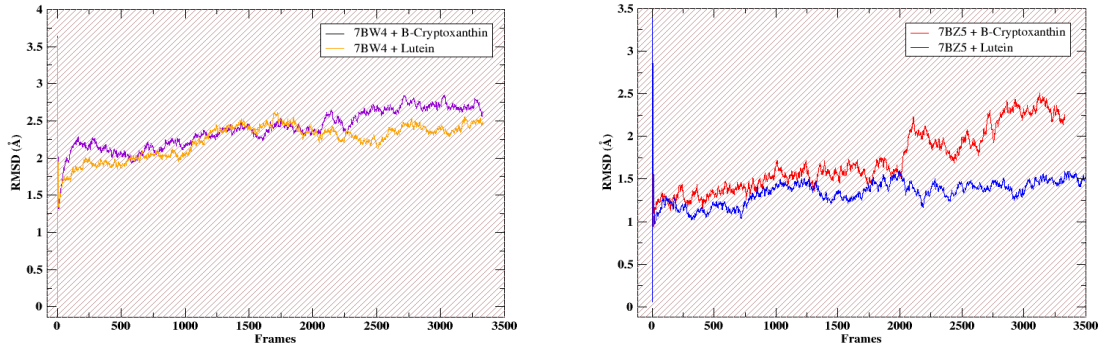
	**β-Carotene**	

3.5 Molecular dynamics simulations trajectories

In the present study, the MD simulation analysis was carried out on ligands which provided the best binding energies with docking and cross-docking results. It was performed to evaluate the stability of our conformers. By cross-checking the results of docking studies from MOE and PyRx, we selected two major ligands of *Carica papaya* (Lutein and β-cryptoxanthin) with both of the targeted proteins Spike; 7BZ5 and RdRp; 7BW4 for simulation prediction analysis. Moreover, simulation of 1ns was achieved for evaluating the stability of our complexes. Root-mean-square deviation (RMSD) values, root-mean-square fluctuation (RMSF) values, and H-bonds interactions were calculated from MD trajectories.

3.5.1 RMSD graphs calculations

The simulation trajectories were analyzed using the VMD tool. Overall fluctuations in the conformation of the protein-ligand complexes were first studied in terms of Root Mean Square Deviation (RMSD) in the coordinates of the protein backbone and ligands from the coordinates in the initial docked pose. These calculations graph plots were constructed using qt-Grace software. As depicted in the [figure 3a], RMSD plots of RdRp protein (7BW4) along with β-Cryptoxanthin and Lutein remained stable within the range of 1.5Å to 2.5Å from 250 frames till 2000 frames and then slightly fluctuated till 3300 frames of both the complexes. Furthermore, the results of spike protein (7BZ5) along with β-Cryptoxanthin and Lutein persisted stable from the start from 100 frames to 700 frames and then slightly deviate and keep on its stability with the conformer till 2000 frames at 1Å to 1.6Å. After that, both complexes lose stability and fluctuate in opposite directions; the complex of Lutein gained constancy until the last 3500 frames at under 1.5Å. The graph plot is illustrated in [figure 3b]. On the other hand, the complex 7BZ5 plus β-Cryptoxanthin was unstable from 1.5Å to 2.5Å throughout the simulations process. Therefore, the Lutein and β-Cryptoxanthin complexed RdRP are more stable than the 7BZ5 complexed structures.

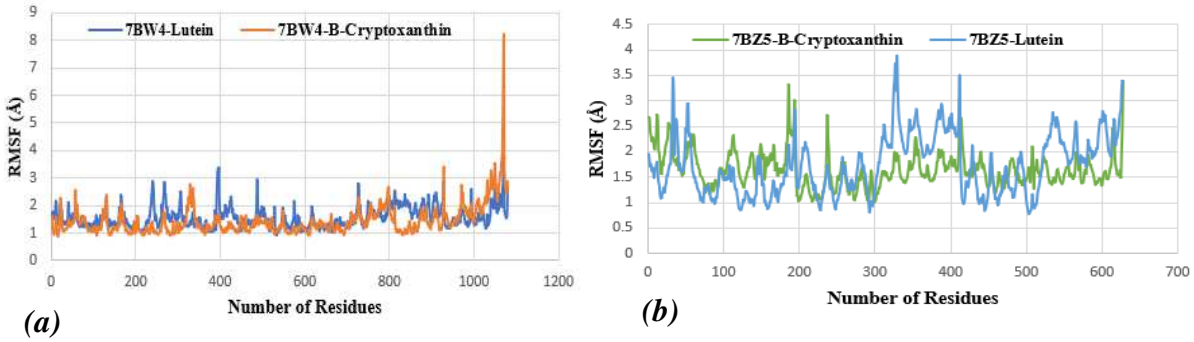


(a) ***(b)***

Figure 3: ***(a)*** *RMSD of 7BW4-complexes,* ***(b)*** *RMSD of 7BZ5-complexes*

3.5.2 RMSF plots

A thorough study of the Root Mean Square Fluctuation (RMSF) curves of the Spike and RdRP and its complexes was calculated using the VMD tool using the RMSF *script. TCL* file. [Figure 4(a-b)] showed residue-wise RMSF values of both complexes during the simulations. The RMSF plots of 7BW4 with Lutein and β-Cryptoxanthin showed that all amino acids located in the enzyme's active site had RMSF fluctuations between 0.8Å and 3Å, which indicated that they were indicated that the studied compound kept close contact with their binding pockets during the MD simulations. However, Spike protein (7BZ5)-Lutein and β-Cryptoxanthin depicted higher variations for residues 20 - 100, 170 - 230, and 300 - 420, and this observation is consistent throughout the complex within 1Å to 3.5Å. The higher fluctuations were observed in RMSF profiles of the spike protein with its conformers. Consequently, the RMSF plots of RdRP complexes depicted that the residues lining the substrate-binding pocket were interacting quite stably with the bound ligands, as indicated by the low fluctuating values compared to the complexes of spike (7BZ5) protein.



(a) ***(b)***

Figure 4: *. **(a)** RMSF plot of 7BW4-complexes,* ***(b)*** *RMSF plots of 7BZ5-complexes*

3.5.3 Hydrogen bonding analysis

Hydrogen bonding is the predominant contributor to the specificity of molecular recognition. Hence, we looked into the H-bonds patterns of complexes over the entire 1ns simulation trajectory

using the built-in function in VMD. As shown in [figure 5(a-d)], the spike complex with β-Cryptoxanthin was making more hydrogen bonds than all other conformers. 7bz5 plus β-Cryptoxanthin complex formed four hydrogen bonds, including TYR-178 side chain of an enzyme act as a donor with our primary ligand as an acceptor with an occupancy of 0.03%. The second hydrogen bond is formed between LEU-172 primary (donor) as ligand as an acceptor with the occupancy of 1.56%. The third hydrogen bond where ALA-170 main act as an acceptor with 5.55% occupancy and the fourth one with TYR-178 side with 7.53% occupancy rate. 7BZ5-Lutein complex formed two hydrogen bonds here PRO-96 main act as an acceptor with the occupancy rate of 53.05%, and GLY-41 main act as a donor with the occupancy rate of 13.89% with our ligand molecule.

On the other hand, 7BW4 complexes have different patterns of H-bonds during simulations pathways. The complex 7BW4 plus β-Cryptoxanthin showed zero hydrogen bonding. In contrast, we found six hydrogen bonds between the 7BW4-lutein complex in which many amino acids residues are involved with different percentages of occupancy rate. The residues involved are LYS-47 main, ASP-221 central, and ASN-713 side having both the donor and acceptor behavior was observed with occupancy ranges from 0.06% to 1.17% at the course of MD simulation trajectory. This H-bond analysis taking our inhibitors as a reference molecule suggested that Lutein has a good affinity towards the substrate-binding pocket of SARS-CoV-2 Spike (7BZ5) and RdRP (7BW4) and could probably be the natural and readily available drugs for the inhibition of COVID-19 functional activity.

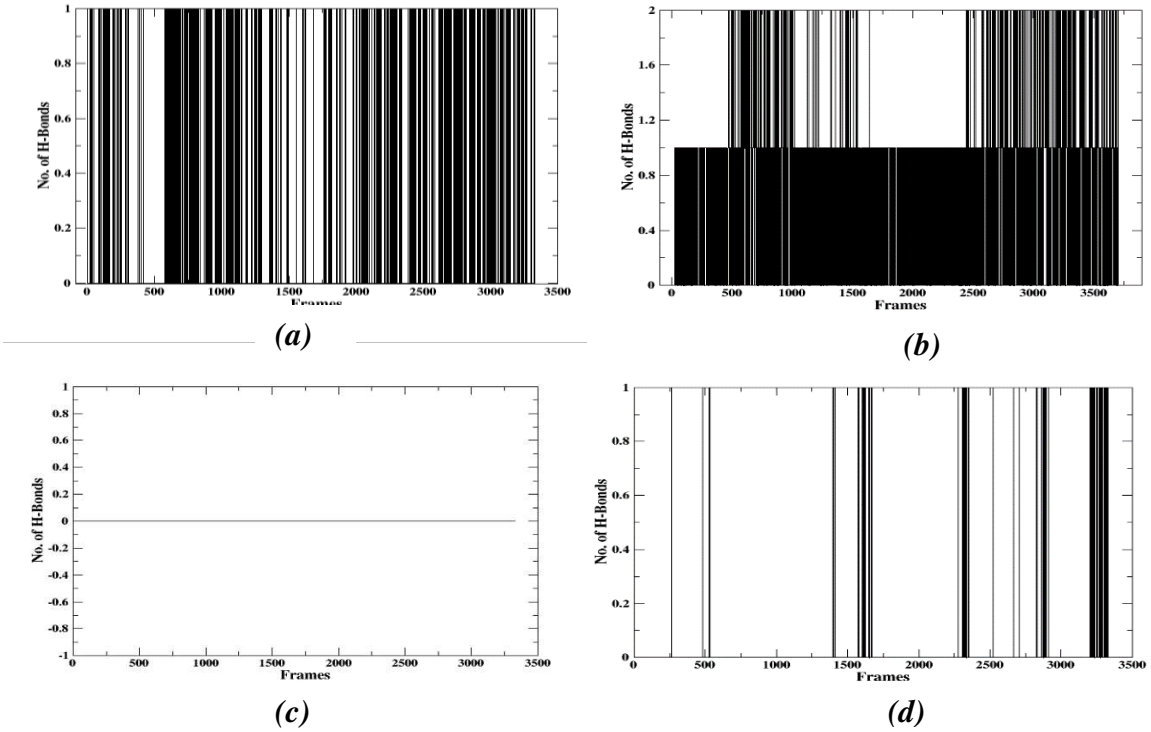


Figure 5: *Hydrogen bonds formed between complexes,* ***(a)*** *7BZ5-β-cryptoxanthin,* ***(b)*** *7BZ5-Lutein,* ***(c)*** *7BW4-β-cryptoxanthin,* ***(d)*** *7BW4-Lutein*

Conclusion

Current study has provided us insights of computational prediction of the drug agents from *Carica papaya* compounds which may be beneficial against SARS-nCoV2 infection. The two main targets of viral proteins (Spike & RdRp) are tested and predicted as evident drug agents because of their molecular binding, redocking scores and MD simulation trajectories analysis with the selected ligands. Therefore, the best compounds from *Carica papaya* are Lutein & β-Cryptoxanthin which gave us the lowest binding affinities and depicted stability in complex formation on the course of simulations, have more potential to inhibit COVID-19 infection as compared to other drugs used at present for the treatment of this disease. Consequently, these results encourage further in vivo and in-vitro investigations with more computational power as a potential drug against SARS-nCoV2.

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Author Contributions

Rashid Saif (RS) envisaged the idea, involved in critical thinking, data analysis, editing, proofreading, and correspondence with the journal. Muhammad Osama Zafar (MOZ) is involved in software features, data analysis and initial write-up. Muhammad Hassan Raza (MHR) helped in data analysis, software features and initial write-up. Saeeda Zia (SZ) helped in software algorithms understanding and redocking insight. Abdul Rasheed Qureshi (ARQ) helped in proof reading and editing of the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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