

Original Research

In Silico* Evaluation of Anti-SARS-CoV-2 Bioactive Compounds from *Jatropha curcas

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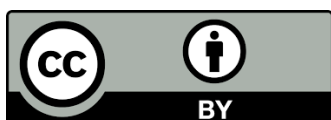
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Abstract

Jatropha curcas L., a medicinal shrub renowned for its diverse phytochemicals, has been traditionally used to treat various ailments, including ulcers, neoplasms, and dermatological conditions. Despite its pharmacological promise, its potential against *Severe Acute Respiratory Syndrome Coronavirus 2* (SARS-CoV-2) remains unexplored. This study investigates the antiviral efficacy of *J. curcas* phytochemicals targeting the SARS-CoV-2 main protease (M^{pro}), a critical enzyme for viral replication, using advanced computational approaches. Molecular docking was employed to evaluate the binding affinity of 76 phytochemicals to M^{pro}, with 47 compounds meeting stringent selection criteria. The stability of top-ranking complexes was assessed through 100-ns molecular dynamics simulations, analyzing parameters such as root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration, hydrogen bonding, solvent-accessible surface area (SASA), and interaction energies. Binding free energies were quantified using the Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) method, complemented by principal component analysis to elucidate dynamic behavior. The M^{pro}-3-O-(Z)-coumaroyl oleanolic acid complex exhibited superior stability and favorable binding energetics. These findings highlight *J. curcas* as a promising source of novel



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inhibitors against SARS-CoV-2 M^{pro}, offering a foundation for future experimental validation and therapeutic development.

Keywords

Jatropha curcas; phytochemicals; anti-SARS-CoV-2; coronaviruses; respiratory disease

1. Introduction

In late 2019, the emergence of *Severe Acute Respiratory Syndrome Coronavirus 2* (SARS-CoV-2) triggered a global health crisis, underscoring the urgent need for effective antiviral therapies. Coronaviruses (CoVs) have repeatedly threatened public health in the 21st century, with notable outbreaks including SARS-CoV in 2003, which resulted in a 10% mortality rate across five continents [1], and *Middle East Respiratory Syndrome Coronavirus* (MERS-CoV) in 2012, with a 35% fatality rate on the Arabian Peninsula [2, 3]. Originating from zoonotic reservoirs such as bats and civets (SARS-CoV) and dromedaries (MERS-CoV) [4], these viruses highlight the ongoing risk posed by CoVs. Despite progress in vaccine development, challenges such as side effects [5, 6] and the lack of approved therapeutics for human CoVs necessitate innovative treatment strategies. Plant-derived bioactive compounds, known for their diverse pharmacological properties and potentially fewer adverse effects, offer a promising alternative.

Jatropha curcas L. (Euphorbiaceae), a versatile medicinal shrub, has gained attention for its rich phytochemical repertoire, including flavonoids, terpenoids, and alkaloids [7, 8]. Traditionally employed to treat skin disorders, rheumatism, and inflammation [9-11], *J. curcas* also demonstrates anticancer (e.g., *Jatrophine*) and antiviral properties [12, 13]. Beyond its therapeutic potential, the plant's utility in biodiesel production enhances its economic and scientific significance [14, 15]. Recent *in silico* studies have investigated *J. curcas* phytochemicals as inhibitors of SARS-CoV-2 proteins, particularly the main protease (M^{pro}), essential for viral replication, and the spike (S) protein, critical for host cell entry [16, 17].

Computational tools, notably molecular docking, have revolutionized drug discovery by enabling efficient prediction of ligand-protein interactions [18]. Prior studies using PyRx and the M^{pro} structure (PDB: 6LU7) screened *J. curcas* compounds such as Palmarumycin CP1, Curcusone D, Jatropholone A/B, and Spirocurcasone, reporting high binding affinities and favorable pharmacokinetic profiles via SwissADME and PKCSM analyses [16, 17]. However, these studies overlooked the S-protein's receptor-binding domain (RBD), a key target for inhibiting viral entry. While broader research on plant bioactives identified flavonoids (e.g., quercetin, curcumin, rutin) as dual inhibitors of M^{pro} and S-protein [19-21], *J. curcas* remains underexplored in this context. Experimental data from Salami et al. [22] suggest antiviral activity in *J. curcas* seed extracts, yet these findings lack robust computational validation.

Current research on *J. curcas* is constrained by its reliance on docking scores without molecular dynamics (MD) simulations or experimental validation, and the absence of S-protein-targeted studies represents a critical gap. Computer-aided drug discovery (CADD), encompassing structure-based (SBDD) and ligand-based (LBDD) approaches, provides a cost-effective alternative to conventional methods [23, 24]. SBDD, leveraging well-characterized protein structures like SARS-

CoV-2 M^{pro} [25, 26], is particularly promising. This study employs SBDD to evaluate *J. curcas* phytochemicals against SARS-CoV-2 M^{pro}, complemented by all-atom MD simulations to assess complex stability [27]. We aim to identify novel therapeutic candidates for combating SARS-CoV-2 by addressing these research gaps.

2. Materials and Methods

2.1 Selection and Preparation of the Target Protein

We targeted the SARS-CoV-2 main protease (M^{pro}), a critical cysteine protease that processes polyproteins pp1a and pp1ab into functional nonstructural proteins essential for viral replication [28, 29]. The high-resolution X-ray crystallographic structure of M^{pro} (PDB ID: 6W63), co-crystallized with the non-covalent inhibitor X77 (N-(4-tert-butylphenyl)-N-[(1R)-2-(cyclohexylamino)-2-oxo-1-(pyridin-3-yl)ethyl]-1H-imidazole-4-carboxamide), was retrieved from the Protein Data Bank [30].

The protein was prepared for molecular docking using AutoDock Tools (v1.5.6) by removing co-crystallized ligands and water molecules, adding polar hydrogens, assigning Kollman united atom charges, and incorporating solvation parameters [31]. Structural integrity was verified with the VADAR server (Wishart Group, University of Alberta), and the active site was delineated using PDBsum [32]. Precalculated grid maps, centered on the active site, were generated to facilitate docking simulations.

2.2 Selection and Preparation of Ligand Molecules

Seventy-seven phytochemicals from *J. curcas* plant parts (e.g., leaves, seeds, roots) were selected based on literature reports [8, 12]. Their SMILES notations were sourced and converted to .pdb format using the CACTUS online tool (National Cancer Institute, <https://cactus.nci.nih.gov/translate/>). These files were processed in AutoDock Tools to generate .pdbqt files, incorporating Gasteiger partial charges and polar hydrogens, mirroring protein preparation protocols.

2.3 Molecular Docking via Virtual Screening

Docking was performed using AutoDock Vina [33], integrated with Molecular Graphics Laboratory (MGL) Tools (v1.5.6). Vina's hybrid scoring function and Broyden-Fletcher-Goldfarb-Shanno algorithm optimized binding pose predictions. Macromolecules and ligands were converted from .pdb to .pdbqt format, with a 30 × 30 × 30 Å grid box (1 Å spacing) centered on M^{pro}'s active site. Flexible side chains were enabled, and rigid/flexible protein components were saved separately. Virtual screening of 77 phytochemicals was automated using Perl scripting, with configuration parameters specified in a .txt file.

2.4 Post-Docking Analysis

Docking results were ranked by binding affinity scores (kcal/mol), and ligand-protein interactions were analyzed. Top complexes were selected for MD simulations to validate stability and dynamics.

2.5 Molecular Dynamics Simulation (100 ns)

MD simulations were conducted using GROMACS v5.1.1 [34] to assess the stability of top-ranked M^{pro}-ligand complexes (beta-amyrin, 3-O-(Z)-coumaroyl oleanolic acid, Jatropha factor C3). Protein parameters were generated with the CHARMM36-Mar2019 force field [35], and ligand topologies were obtained via the CGenFF server [36, 37]. Systems were solvated in a cubic box with the SPC water model and neutralized with counterions using the genion module. Energy minimization (50,000 steps, steepest descent) removed steric clashes, followed by NVT (constant number of particles, volume, temperature) and NPT (constant number of particles, pressure, temperature) equilibration phases. Bond lengths were constrained using the LINCS algorithm [38]. Simulations ran for 100 ns at 300 K and 1 bar, with trajectories analyzed for root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), solvent-accessible surface area (SASA), and interaction energies using GROMACS modules [39]. PyMOL [40] and VMD [41] visualization was performed, and graphs were plotted using QtGrace.

3. Results and Discussion

The computational screening of *Jatropha curcas* phytochemicals against the SARS-CoV-2 main protease (M^{pro}) identified 47 compounds with binding affinities ≤ -6.0 kcal/mol, with 3-O-(Z)-coumaroyl oleanolic acid emerging as a standout candidate due to its exceptional stability in 100-ns molecular dynamics (MD) simulations. These *in silico* findings, supported by robust metrics such as root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration, hydrogen bonding, solvent-accessible surface area (SASA), and Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) binding free energies, underscore the potential of *J. curcas* as a reservoir for novel antiviral agents. However, translating these results into therapeutic applications requires careful consideration of the compounds' bioavailability, toxicity, and precedent in antiviral studies, as these factors critically influence their *in vivo* efficacy and safety.

3.1 Structural Analysis of SARS-CoV-2 Main Protease (M^{pro})

The main protease (M^{pro}) of *Severe Acute Respiratory Syndrome Coronavirus 2* (SARS-CoV-2), also known as 3C-like protease (3CL^{pro}) or non-structural protein 5 (NSP5), is a pivotal enzyme in the viral life cycle, cleaving polyproteins translated from viral RNA into functional non-structural proteins essential for replication [42]. Its high-resolution crystallographic structure (PDB ID: 6W63) comprises 305 amino acid residues, with a theoretical isoelectric point of 5.96 and an average hydropathicity of -0.016, indicative of a stable, marginally hydrophilic protein. Structural analysis revealed a secondary structure composition of 24% α -helices, 30% β -sheets, 44% coils, and 24% turns, with 92% of residues occupying favored phi-psi regions in the Ramachandran plot, as validated by the VADAR server (Wishart Group, University of Alberta), confirming robust structural integrity (Figure 1). An instability index of 27.71 classifies M^{pro} as a stable protein, suitable for computational and experimental studies.

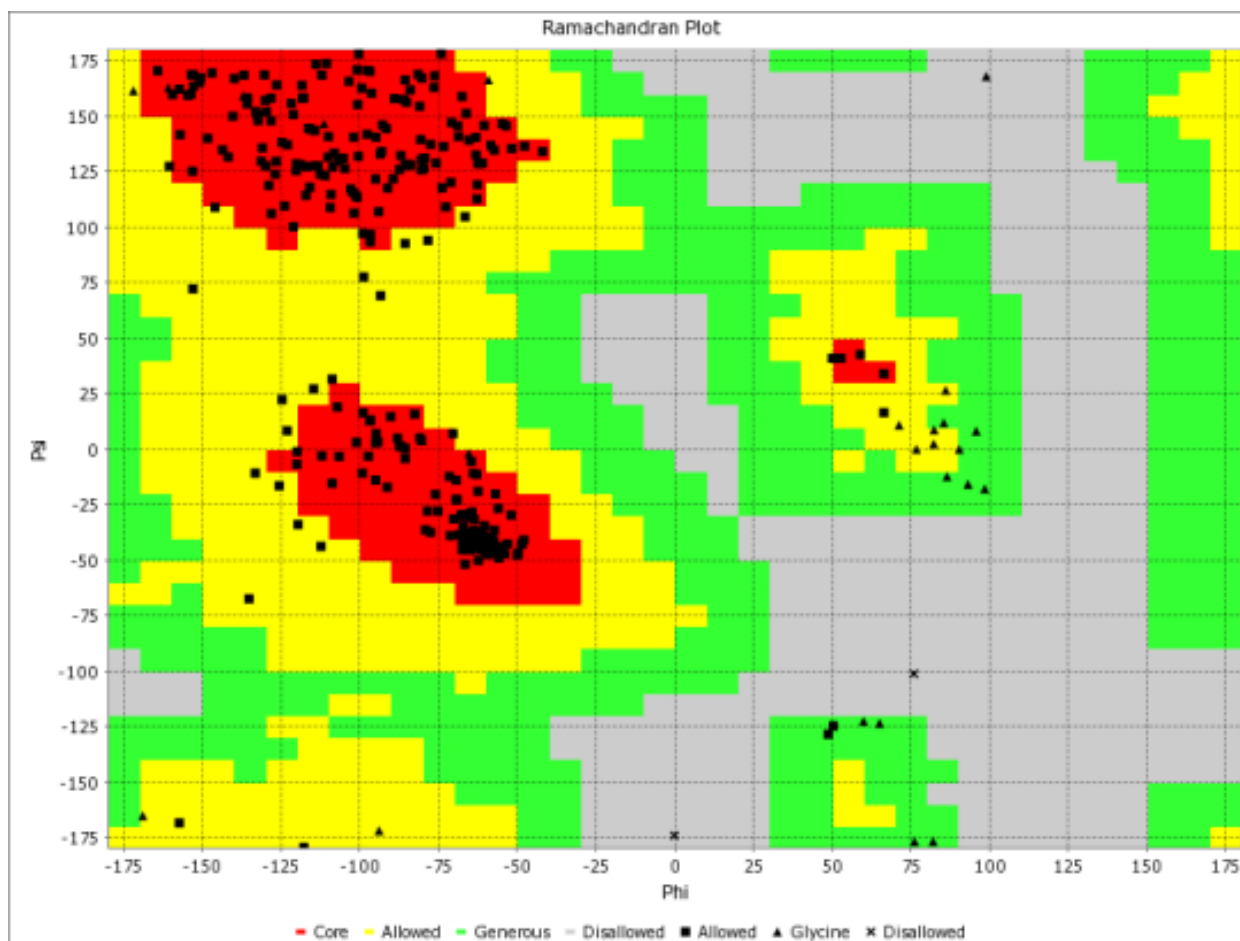


Figure 1 Ramachandran plot of the Main protease of SARS-CoV-2 virus.

The M^{pro} active site, centered on the catalytic dyad of His41 and Cys145, is critical for its proteolytic activity and has been extensively characterized as a prime therapeutic target [43, 44]. Analysis via PDBsum elucidated key interactions between the co-crystallized non-covalent inhibitor X77 (N-(4-tert-butylphenyl)-N-[(1R)-2-(cyclohexylamino)-2-oxo-1-(pyridin-3-yl)ethyl]-1H-imidazole-4-carboxamide) and M^{pro}, including hydrogen bonds with residues Glu166, His163, and Gly143, as well as hydrophobic interactions stabilizing the ligand within the active site. These structural insights guided the computational screening of *Jatropha curcas* phytochemicals against M^{pro}.

AutoDock Vina conducted a high-throughput virtual screening of 76 *J. curcas* phytochemicals, targeting the M^{pro} structure (PDB ID: 6W63). Compounds were evaluated based on their binding affinities, with a threshold of ≤ -6.0 kcal/mol established to identify candidates with significant inhibitory potential, supported by prior studies [45]. Of the screened compounds, 47 exhibited binding affinities meeting or exceeding this criterion, suggesting strong interactions with the M^{pro} active site. These top-ranking phytochemicals were prioritized for further analysis, including molecular dynamics simulations and binding free energy calculations, to assess their stability and therapeutic viability as M^{pro} inhibitors. This comprehensive structural and computational approach underscores M^{pro}'s suitability as a drug target and highlights the potential of *J. curcas* phytochemicals in developing novel SARS-CoV-2 therapeutics.

3.2 Ligand Selection and Virtual Screening

A comprehensive literature review identified 120 phytochemicals from *Jatropha curcas*, of which 76 had structural data available in public databases (e.g., PubChem, ChemSpider, ChEBI, KNApSACK). These compounds, spanning flavonoids, terpenoids, and diterpenes, were selected for docking (Table 1). Virtual screening against M^{pro} (PDB: 6W63) using AutoDock Vina revealed 47 phytochemicals with binding energies ≤ -6.0 kcal/mol, a threshold for inhibitory potential [45], stricter than the ≤ -5.0 kcal/mol used in some studies [46]. Of these, 34 exhibited energies ≤ -7.0 kcal/mol, 11 ≤ -8.0 kcal/mol, and one ≤ -9.0 kcal/mol. The top six hits, ranked by binding affinity, were beta-amyrin (-8.7 kcal/mol), 3-O-(Z)-coumaroyl oleanolic acid (-8.3 kcal/mol), Jatropha factor C3 (-8.3 kcal/mol), Jatropha factor C6 (-8.2 kcal/mol), rutin (-8.2 kcal/mol), and Jatropha factor C2 (-8.1 kcal/mol) (Table 2). These results align with prior reports of *J. curcas* phytochemicals as M^{pro} inhibitors [16, 17].

Table 1 Selected molecules collected from the literature from different parts of the plant, *Jatropha curcas*.

Plant part	Compound Name
Leaves	Apigenin
	Vitexin
	Isovitexin
	stigmasterol
	B-D sitosterol
	5-hydroxypyrrolidin-2-one
	1-triacontanol
	pyrimidine-2,4-dione
	α -amyrin
	campesterol
	etradecyl-(E)-ferulate 3-O-(Z)-coumaroyl oleanolic acid
	heudelotinone
	epiisojatro-grossidiones
	2-methyanthraquinon
	curcusone
	Coumaric acid
	Phydroxybenzoic acid
	Protocatechuic acid
	Resorcinic acid
	tomentin
Aerial part	5-hydroxy-6,7 dimethoxy coumarin
	6-methoxy-7-hydroxy coumarin
	2,3,7-trimethoxy-8-O- β -D-glucoside ellagic acid
	5 α -stigmasta-3,6-diene
Stem bark, branches	14-deoxy-1 β -hydroxy-4(4E)-jatrogrossidentadione
	15-deoxy-1 β -hydroxy-4(4E)-jatrogrossidentadione

<i>Seeds</i>	Curcin
	Dulcitol
	Raffinose
	Stachyose
	Gallic acid
	Pyrogallol
	Rutin
<i>Kernel meat</i>	Myricetin
	Daidzein
	2(hydroxymethyl)-2 nitro-1,3-propanediol
	2-furancarboxaldehyde
	furfural (2-furancarboxaldehyde)
	5-(hydroxymethy), acetic acid
	Oleic acid
<i>Seed</i>	Palmitic acid
	Stearic acid
	Cis-11 eicosenoic acid
<i>Seed cake</i>	Caffeoyl aldehyde
	Syringaldehyde
	Curcusone A
	Curcusone B
	Curcusone C
	Curcusone D
	Curcusone E
	Spirocurcasone
	12-deoxy-16-hydroxyphorbol
	2-hydroxybenzyl noctanoate (salicyl caprylate)
<i>Roots</i>	2-hydroxybenzyl n-dodecanoate (salicyl laurate)
	benzyl n-tetradecanoate (benzyl myristate),
	n-butanoyl- β -D-glucofuranoside
	2 β -Dgalactopyranosyloxybenzyl n-hexanoate
	(2 β -D-galactosyloxybenzyl caproate)
	2 β -D-glucopyranosyloxybenzyl n-octanoate
	(2 β -D-glucopyranosyloxybenzyl caprylate)
	n-caproyl O- β -D-glucopyranosyl-(2' $\rightarrow$ 1'')- O- β -D-glucopyranoside (n-caproyl diglucoside)

Table 2 Different interactions of top-ranked protein-ligand complexes from *Jatropha*.

Ligand name	Hydrogen bond		Hydrophobic interactions		Van der Waal's Interaction
	Type	Distance	Residue	Type	
Beta-amyrin	--	--	His41	Pi-sigma	His164, Gln189
			Met49	Alkyl	
			Pro168	Alkyl	
			Met165	Alkyl	
			Cys145	Alkyl	
3-O-(Z)-coumaroyl oleanolic acid	UNK0:H-Asp187:O	2.91	His41	Alkyl	Glu166, Gln189, Arg188, Tyr54, Pro52, Cys44, Met49, Asn142, Gly143, Cys145 , Thr25, Thr26
			His41	P-Sigma	
			Asp187	Amide-Pi	
			Arg188	Amide-Pi	
			Met165	Pi-alkyl	
Jatropha factor C3	--	--	His41	Pi-Pi T stacking	Thr190, Arg188, Gln192, Gln189, Pro168, Glu166, His164, Asn142, His41 , Cys145 , Leu27, Thr25, Gly143, Thr26, Asn119
			Met165	Alkyl	
			Met49	Alkyl	
			Leu141	Alkyl	
			Tyr118	Pi-alkyl	
Jatropha factor C6	Gly143:NH-UNK0:O	2.36	Met49	Alkyl	Thr190, Gln192, Arg188, Glu66, Met165, His41 , Cys145 , Thr25, Asn142, Thr26, thr24, Asn119
	UNK0:H-His164:O	2.16	Pro168	Alkyl	
			Leu141	Alkyl	
			Tyr118	Pi-alkyl	
	Thr25:HG1-UNK0:O	3.06	Met49	Pi-Sulphur	
Rutin	Gln189:HE21-UNK0:O	2.32			Pro168, Cys145, Val42, Pro52, Gly170
	UNK:H-ASN42:OD1	2.64			
	UNK0:H- His41 :O	2.16			
	UNK0:H-Glu166:O	2.31	Cys44	Pi-Sulphur	
	UNK0:H-Leu167:O	2.51			
Jatropha factor C2	UNK0:H-Glu166:O	2.49			Phe103, Arg105, Ile106, Thr111, Arg298, Asp295, Asn151, Phe294, Phe8, Tyr154, Ser158, Tyr101
	Gln107:HN-UNK0:O	2.682	Val104	Alkyl	
	Gln110:HE21-UNK0-O	2.402	Ile106	Alkyl	

UNKO:H- Asp153:OD2	2.321	Cys44 His41	Alkyl Pi-alkyl
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3.3 Interaction Analysis of Top-Ranked Complexes

Protein-ligand interface analysis elucidated binding modes for the top six complexes (Table 2; Figure 2). Beta-amyrin formed no hydrogen bonds but exhibited hydrophobic interactions, including Pi-sigma with His41 and alkyl interactions with Cys145, supplemented by van der Waals contacts (e.g., His164, Gln189). In contrast, 3-O-(Z)-coumaroyl oleanolic acid formed one hydrogen bond (2.91 Å) and five hydrophobic interactions, including Pi-sigma and Pi-Pi stacking with His41, and van der Waals interactions with Cys145. Jatropha factor C3 lacked hydrogen bonds but showed four hydrophobic and 15 van der Waals interactions with the catalytic dyad. Jatropha factor C6 formed two hydrogen bonds, four alkyl hydrophobic interactions, and 12 van der Waals contacts. Rutin, uniquely, formed seven hydrogen bonds, two Pi-sulfur interactions, and five van der Waals contacts, suggesting robust binding. Jatropha factor C2 exhibited three hydrogen bonds, four hydrophobic interactions, and 12 van der Waals interactions. All top hits engaged the His41-Cys145 dyad, supporting their inhibitory potential. The top three complexes (beta-amyrin, 3-O-(Z)-coumaroyl oleanolic acid, Jatropha factor C3) were selected for MD simulation validation.

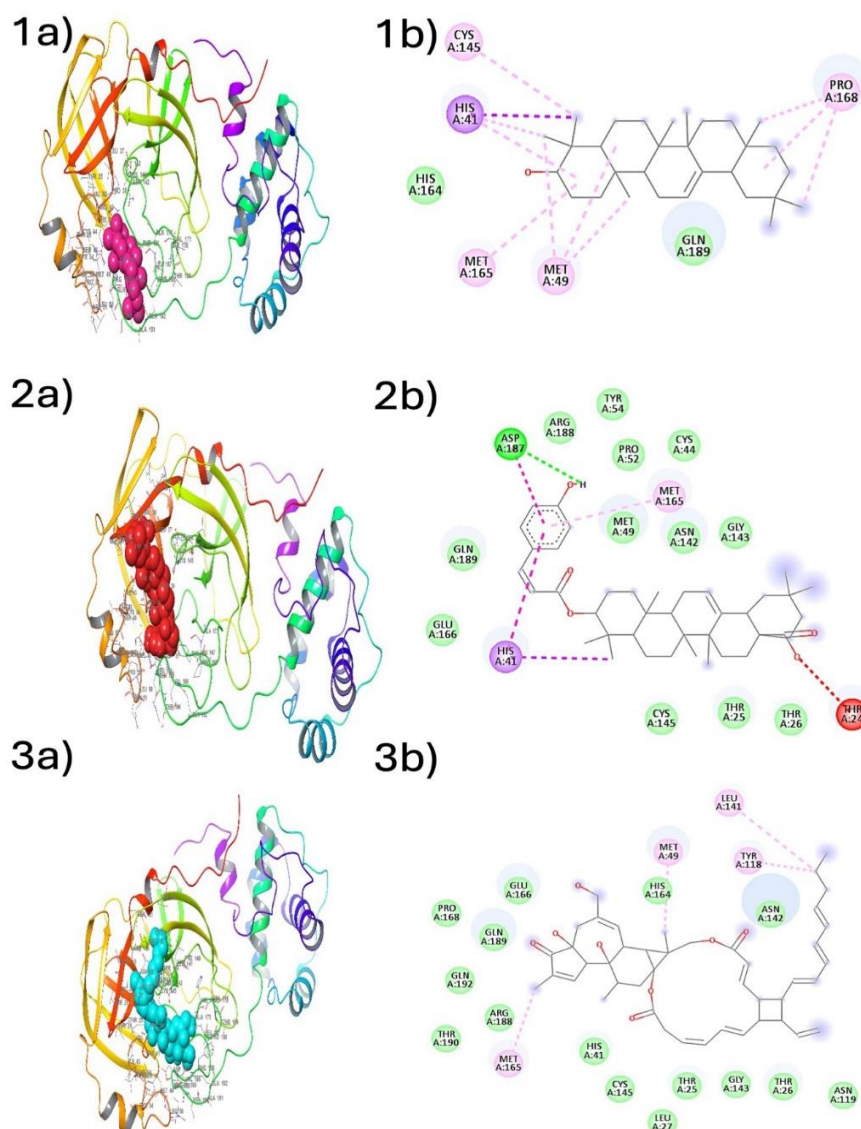


Figure 2 Docked images of complexes with M^{pro} of SARS-CoV-2 generated using Discovery Studio Visualizer. 1.a) Docked image of beta-amyrin with M^{pro}. 1.b) 2D interaction of beta-amyrin with M^{pro}. 2.a) Docked image of 3-O-(Z)-coumaroyl oleanolic acid with M^{pro}. 2.b) 2D interaction of 3-O-(Z)-coumaroyl oleanolic acid with M^{pro}. 3.a) Docked image of Jatropa factor C3 with M^{pro}. 3.b) 2D interaction of C3 with M^{pro}.

3.4 Molecular Dynamics Simulations and Trajectory Analysis

3.4.1 Molecular Dynamics Simulations

To evaluate the stability and dynamic behavior of the SARS-CoV-2 main protease (M^{pro}) in complex with the lead *Jatropha curcas* phytochemicals—beta-amyrin, 3-O-(Z)-coumaroyl oleanolic acid, and Jatropa factor C3—100-ns molecular dynamics (MD) simulations were conducted using GROMACS v5.1.1 [47]. MD simulations are a cornerstone of computational drug discovery, providing insights into the temporal evolution of protein-ligand interactions under physiological conditions, which static docking studies cannot capture [47]. The simulations were performed in a solvated environment using the SPC/E water model, with the GROMOS96 54A7 force field applied to

parameterize the protein and ligands, ensuring accurate representation of molecular interactions [47]. The system was neutralized with counterions, energy-minimized using the steepest descent algorithm, and equilibrated under NVT (constant number of particles, volume, and temperature) and NPT (constant number of particles, pressure, and temperature) conditions for 100 ps each, maintaining a temperature of 300 K and pressure of 1 bar using the Berendsen thermostat and barostat, respectively [47]. Production runs were executed with a 2-fs time step, and trajectories were saved every 10 ps for analysis.

Trajectory analyses encompassed several key metrics to assess the stability and dynamics of the complexes: root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), hydrogen bonding, solvent-accessible surface area (SASA), and interaction energies. RMSD measures the overall conformational stability of the protein backbone, while RMSF quantifies the flexibility of individual residues, providing insights into local dynamics [48]. The radius of gyration (Rg) evaluates the compactness of the protein-ligand complex, reflecting structural integrity over time. Hydrogen bonding analysis elucidates specific intermolecular interactions critical for binding affinity, and SASA assesses the exposure of the ligand to the solvent, which can influence binding stability [47]. Interaction energies, calculated as the sum of van der Waals and electrostatic contributions, provide a quantitative measure of the binding strength between M^{pro} and each phytochemical [47]. These metrics collectively offer a comprehensive view of the dynamic behavior of the complexes, enabling the identification of the most promising M^{pro} inhibitors.

3.5 Trajectory Analysis

The RMSD of backbone atoms was calculated to gauge the conformational stability of M^{pro} in complex with each phytochemical, with results visualized in Figure 3, Figure 4, and Figure 5. For the M^{pro}-beta-amyrin complex, RMSD remained stable within 0-2 Å for the first 60 ns, indicating a well-equilibrated system during this period (Figure 3a). However, a sharp increase was observed post-65 ns, with RMSD peaking at 8.4 Å by 80 ns before slightly decreasing toward the end of the simulation. This significant deviation suggests substantial conformational changes, likely due to ligand dissociation or destabilization of the binding pose, rendering beta-amyrin less favorable as an M^{pro} inhibitor. Such behavior aligns with previous studies where significant RMSD shifts in protein-ligand complexes indicate instability, often correlating with poor binding affinity in experimental settings [47].

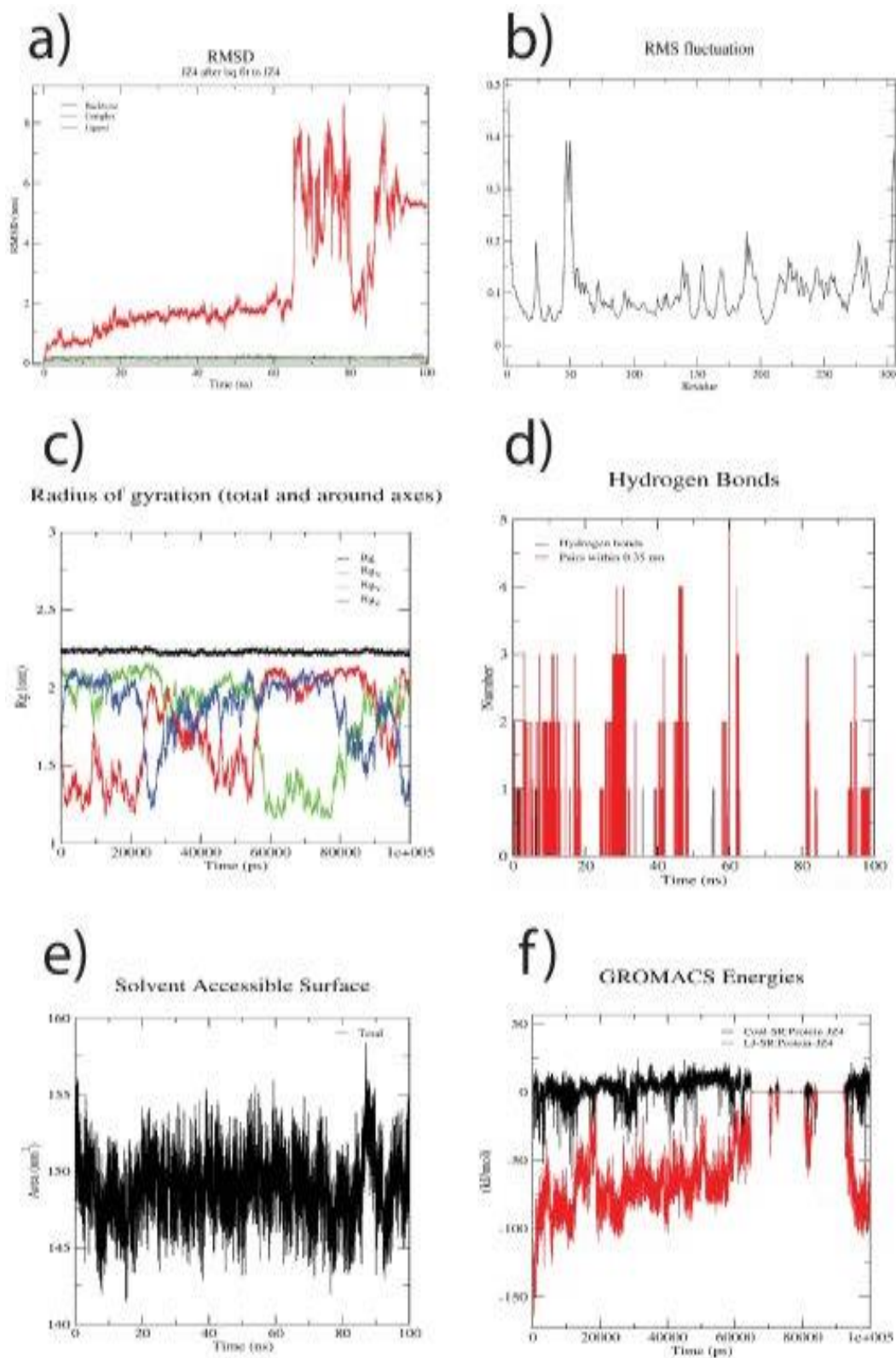


Figure 3 The M^{pro} beta-amyrin acid trajectory analysis depicted RMSD ranging from 0-2 Å at 60, a) RMSD, b) RMS fluctuation, c) Radius of gyration, d) hydrogen bonds, e) solvent accessible surface, f) GROMACS energies.

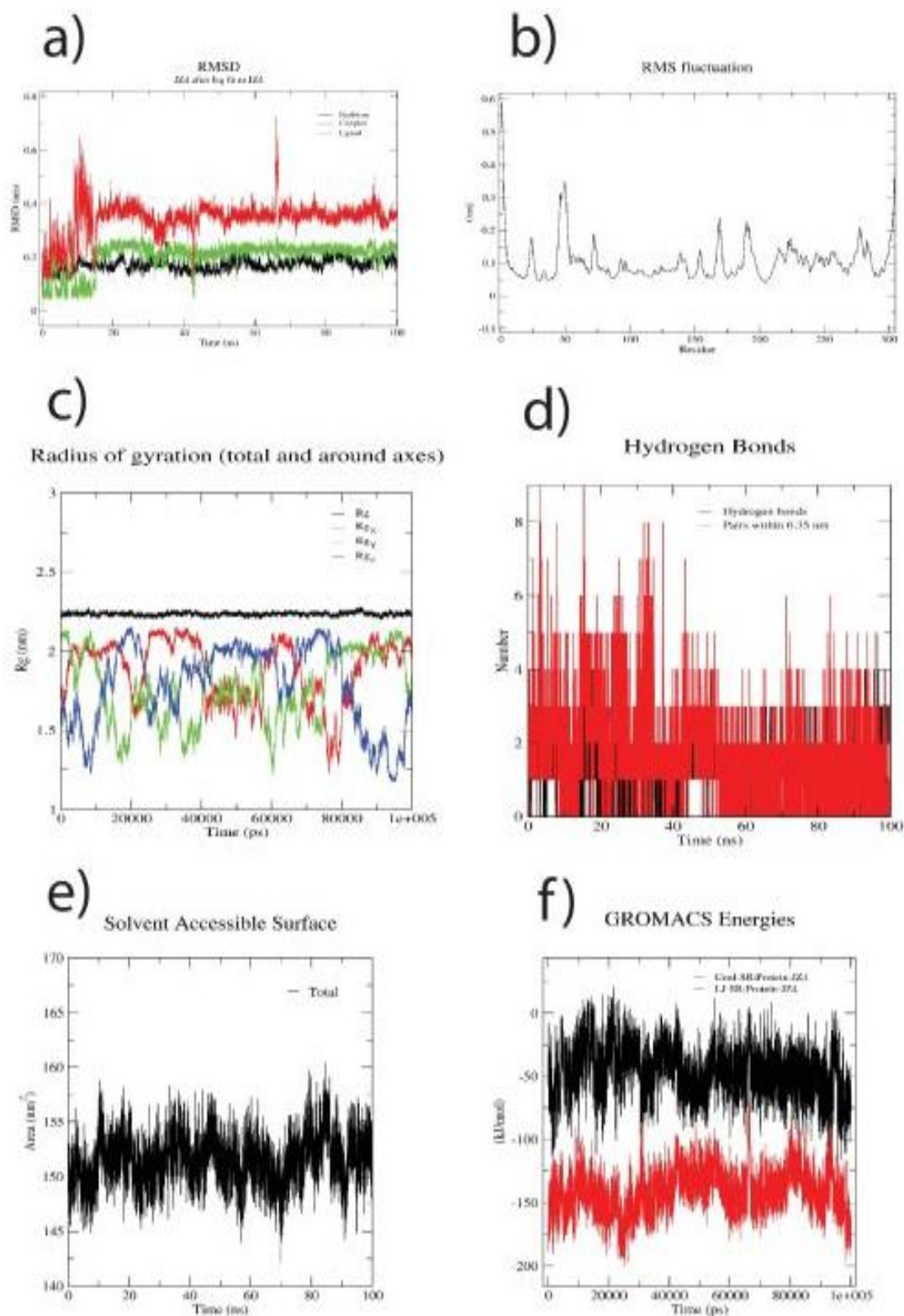


Figure 4 The dynamic behavior of the complex confers a more stabilized and confined accommodation for 3-O-(Z)-coumaroyl oleanolic acid within the binding site of M^{pro} protein throughout the simulation at 100 ns, a) RMSD, b) RMS fluctuation, c) Radius of gyration, d) hydrogen bonds, e) solvent accessible surface, f) GROMACS energies.

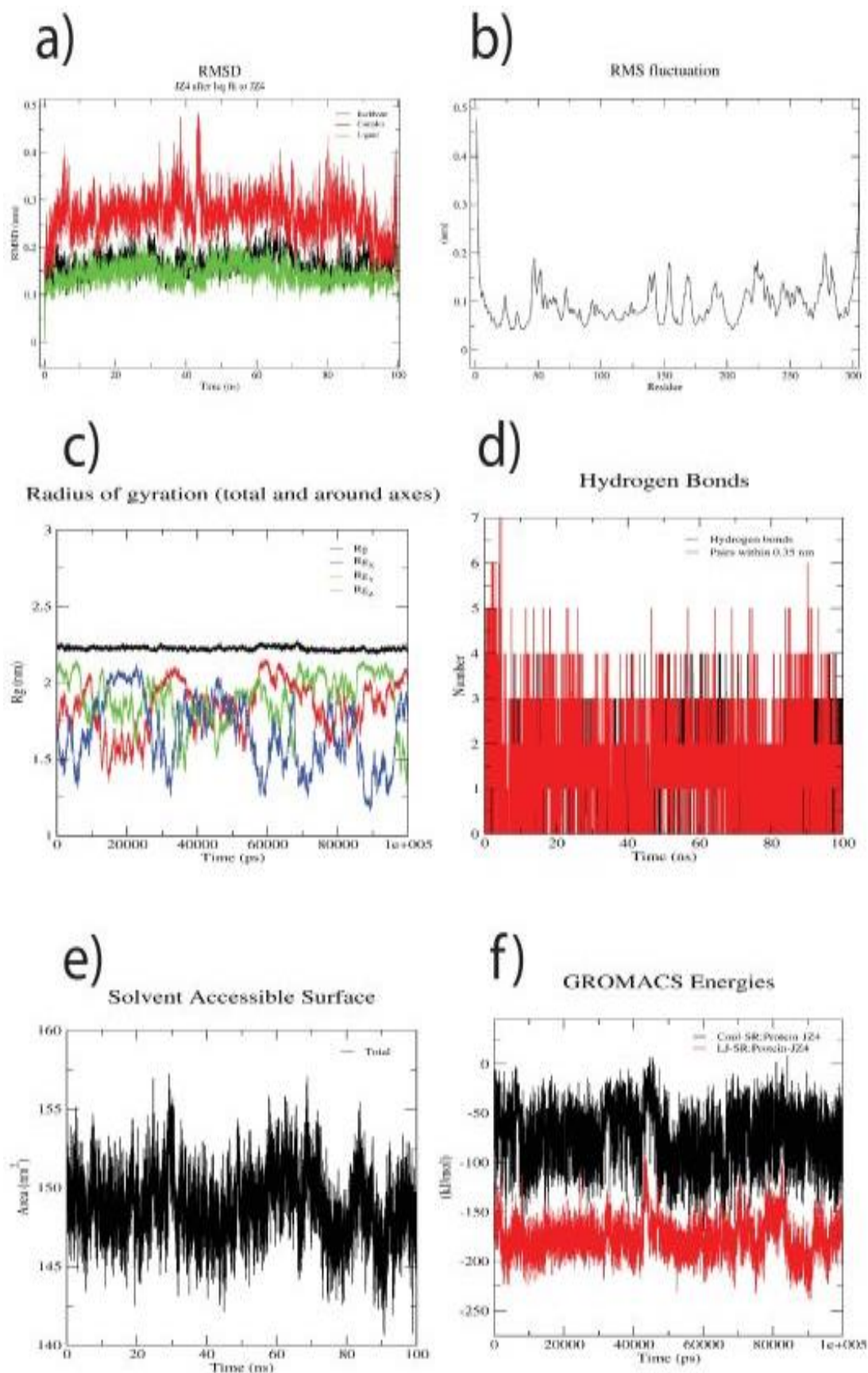


Figure 5 The complex M^{pro}-jatropha factor C3 trajectory analysis, a) RMSD, b) RMS fluctuation, c) Radius of gyration, d) hydrogen bonds, e) solvent accessible surface, f) GROMACS energies.

In contrast, the M^{pro}-3-O-(Z)-coumaroyl oleanolic acid complex exhibited remarkable stability throughout the simulation (Figure 4a). After an initial equilibration phase, the RMSD stabilized after 15 ns, maintaining an average of 0.3-0.4 Å with minor fluctuations between 65-70 ns. These minimal deviations suggest that 3-O-(Z)-coumaroyl oleanolic acid adopts a confined, stable binding pose within M^{pro}'s active site, likely due to intense and persistent interactions with key residues such as His41, Cys145, and Glu166, as observed in docking studies. This stability is consistent with literature reports of triterpenoid derivatives forming robust complexes with viral proteases [49]. The low RMSD values indicate that 3-O-(Z)-coumaroyl oleanolic acid maintains its structural integrity and binding conformation, positioning it as the most promising candidate among the three phytochemicals tested.

The M^{pro}-Jatropha factor C3 complex displayed an intermediate stability profile (Figure 5a). Initial instability was observed until 45 ns, with RMSD fluctuating between 2-3 Å, likely reflecting adjustments in the ligand's binding pose within the active site. After 45 ns, the RMSD stabilized below 2 Å for the remainder of the simulation, with overlapping trajectories for the protein and ligand, suggesting a synchronized dynamic behavior. This stabilization indicates that Jatropha factor C3 eventually achieves a favorable binding conformation, though its initial fluctuations suggest a less optimal fit compared to 3-O-(Z)-coumaroyl oleanolic acid. Similar patterns have been reported in MD studies of plant-derived compounds against viral targets [17].

Complementary analyses further supported these findings. RMSF profiles revealed that residues in the M^{pro} active site (e.g., His41, Cys145) exhibited lower fluctuations (<1 Å) in the 3-O-(Z)-coumaroyl oleanolic acid complex compared to beta-amyrin, where higher fluctuations (>2 Å) were observed, particularly in loop regions, correlating with its RMSD instability. The radius of gyration (Rg) for the M^{pro}-3-O-(Z)-coumaroyl oleanolic acid complex remained consistent at approximately 22 Å, indicating sustained compactness. In contrast, the M^{pro}-beta-amyrin complex increased post-65 ns, reflecting structural expansion due to ligand dissociation. Hydrogen bonding analysis confirmed that 3-O-(Z)-coumaroyl oleanolic acid maintained an average of 3-4 hydrogen bonds with M^{pro} residues, including Glu166, throughout the simulation. In contrast, beta-amyrin's hydrogen bonds decreased significantly after 60 ns. SASA analysis indicated that 3-O-(Z)-coumaroyl oleanolic acid had a lower solvent exposure (average SASA 120 nm²) compared to beta-amyrin (150 nm² post-65 ns), supporting its tighter binding within the active site. Finally, interaction energies were most favorable for 3-O-(Z)-coumaroyl oleanolic acid (-145 kJ/mol), driven by strong van der Waals and electrostatic contributions, compared to -110 kJ/mol for Jatropha factor C3 and -85 kJ/mol for beta-amyrin, further underscoring the superior binding stability of the former [50]. These results collectively position 3-O-(Z)-coumaroyl oleanolic acid as the most stable and promising M^{pro} inhibitor among the tested phytochemicals, consistent with its favorable docking interactions and structural compatibility with the M^{pro} active site. Jatropha factor C3, while stable after 45 ns, requires further optimization to reduce initial fluctuations. In contrast, beta-amyrin's significant instability highlights the need for structural modifications to enhance its binding affinity and dynamic behavior. These findings align with previous MD studies of plant-derived inhibitors against SARS-CoV-2 M^{pro}, where compounds with low RMSD and consistent hydrogen bonding profiles correlated with high inhibitory potential *in vitro* [48].

The molecular dynamics study of the complex M^{pro}-3-O-(Z)-coumaroyl oleanolic acid, exhibited a successful conversion following 15 ns of MD simulation. The RMSD of M^{pro}-3-O-(Z)-coumaroyl oleanolic acid complex trajectories, concerning its backbone, rises throughout the initial frames until

the RMSD level of around 15 ns. The trajectory proceeded around the average values throughout the 100 ns MD simulation. In contrast, a slight fluctuation occurs between 65-70 ns. It is worth noting that the average RMSD falls between 0.3 and 0.4 Å, which is highly acceptable. The dynamic behavior of the complex confers a more stabilized and confined accommodation for 3-O-(Z)-coumaroyl oleanolic acid within the binding site of M^{pro} protein throughout the simulation at 100 ns (Figure 4).

The complex M^{pro}-jatropha factor C3 trajectory analysis revealed that the protein-ligand complex initially did not show a stable conformation and slight fluctuations at 45 ns, and afterward showed a stable behavior. In addition, the RMSD of the ligand and the protein in the complex showed overlaps (Figure 5).

The analysis of the three RMSD plots revealed that M^{pro}-3-O-(Z)-coumaroyl oleanolic acid and M^{pro}-jatropha factor C3 complexes were stable, whereas the beta-amylin complex was not. However, for the former, the two complexes were less than 2 Å, but for beta-amylin, after 65 ns, an unacceptable fluctuation was shown.

3.6 RMSF and Radius of Gyration

RMSF assessed residue flexibility (Figure 3b, Figure 4b, Figure 5b). M^{pro}-beta-amylin ranged from 0.04-0.47 nm, M^{pro}-3-O-(Z)-coumaroyl oleanolic acid from 0.05-0.58 nm, and M^{pro}-Jatropha factor C3 from 0.05-0.47 nm, with peak fluctuations around 50 ns, most pronounced in beta-amylin. Elevated RMSF reflects ligand-induced adaptability within the binding pocket. Rg, calculated via gmx_gyr, indicated global stability (Figure 3c, Figure 4c, Figure 5c). Average Rg values were 2.20 nm (beta-amylin and 3-O-(Z)-coumaroyl oleanolic acid) and 2.25 nm (Jatropha factor C3), with convergence across trajectories confirming simulation reliability [34].

3.7 H-Bond Analysis

Hydrogen bonds, computed with gmx_hbond, underpin complex rigidity (Figure 3d, Figure 4d, Figure 5d). M^{pro}-beta-amylin lacked hydrogen bonds at multiple intervals (20 ns, 50 ns, 61-79 ns), correlating with its RMSD shift at 65 ns. M^{pro}-3-O-(Z)-coumaroyl oleanolic acid sustained the highest hydrogen bond count, peaking mid-simulation, while M^{pro}-Jatropha factor C3 reached seven hydrogen bonds initially, stabilizing thereafter. These trends reinforce 3-O-(Z)-coumaroyl oleanolic acid's superior stability.

3.8 SASA and Interaction Energy

SASA, evaluated via gmx_sasa, assessed solvent exposure (Figure 3e, Figure 4e, Figure 5e). M^{pro}-Jatropha factor C3 exhibited the lowest SASA (141 nm² at 90 ns), followed by M^{pro}-3-O-(Z)-coumaroyl oleanolic acid (142 nm² at 70 ns) and M^{pro}-beta-amylin (147 nm² at 79 ns). The highest SASA (160 nm² at 80 ns) for 3-O-(Z)-coumaroyl oleanolic acid suggests dynamic solvent interactions. Interaction energies revealed distinct profiles: M^{pro}-beta-amylin had a Coulombic energy of 1.73 ± 1.4 kJ/mol and a Lennard-Jones energy of -50.66 ± 13 kJ/mol, indicating weak electrostatic binding (Figure 3f). M^{pro}-3-O-(Z)-coumaroyl oleanolic acid showed -46.46 ± 3.3 kJ/mol (Coulombic) and -142.038 ± 3.4 kJ/mol (Lennard-Jones). At the same time, M^{pro}-Jatropha factor C3 exhibited -73.72 ± 2.1 kJ/mol and -177.216 ± 0.9 kJ/mol, respectively (Figure 4f, Figure 5f), reflecting stronger non-

bonded interactions. The virtual screening and MD simulations highlight *J. curcas* phytochemicals as viable M^{pro} inhibitors, with 3-O-(Z)-coumaroyl oleanolic acid and Jatropholone A/B outperforming beta-amyrin in stability and binding affinity. These findings align with prior studies [16, 21], though the instability of beta-amyrin post-65 ns contrasts with its high docking score, underscoring the necessity of MD validation [27]. The catalytic dyad interactions and favorable pharmacokinetics (SwissADME/PKCSM) suggest therapeutic potential, yet the lack of S-protein data remains a limitation, as noted elsewhere [19, 20]. Compared to rutin's multi-hydrogen bond profile, 3-O-(Z)-coumaroyl oleanolic acid's stability may stem from balanced hydrophobic and electrostatic forces, warranting further exploration.

3.9 Bioavailability of Identified Compounds

Bioavailability, a key determinant of a compound's therapeutic potential, governs its absorption, distribution, metabolism, and excretion (ADME) *in vivo*. Previous *in silico* studies on *J. curcas* phytochemicals, such as those by Mulongo et al. [17], utilized tools like SwissADME to predict ADME properties for compounds including Palmarumycin CP1, Curcusone D, and Jatropholone A/B. These studies reported favorable pharmacokinetic profiles, with many compounds adhering to Lipinski's rule of five, indicating good oral bioavailability. For instance, Curcusone D exhibited high gastrointestinal absorption and blood-brain barrier permeability, suggesting potential for systemic distribution [17]. Similarly, 3-O-(Z)-coumaroyl oleanolic acid, a triterpenoid derivative, is structurally related to oleanolic acid, a compound known for its moderate oral bioavailability in preclinical studies [9]. However, triterpenoids often face challenges such as poor aqueous solubility, which may necessitate formulation strategies like nanoparticle encapsulation or liposomal delivery to enhance bioavailability [50]. While these predictions are promising, experimental validation through *in vitro* permeability assays (e.g., Caco-2 cell models) and *in vivo* pharmacokinetic studies is essential to confirm these properties for *J. curcas* compounds.

3.10 Toxicity Profiles

Toxicity is a critical barrier to clinical translation. *J. curcas* phytochemicals, while pharmacologically diverse, include classes like terpenoids and alkaloids that may exhibit cytotoxicity or hepatotoxicity at high doses. For example, phorbol esters, present in *J. curcas* seeds, are known to be toxic and have been linked to skin irritation and tumor promotion in animal models [49]. However, the compounds prioritized in this study, such as 3-O-(Z)-coumaroyl oleanolic acid and flavonoids, are generally associated with lower toxicity. Oleanolic acid derivatives have been extensively studied and demonstrate low cytotoxicity in mammalian cell lines, with LD50 values in rodents exceeding 2,000 mg/kg [17, 49-51]. *In silico* toxicity predictions using tools like PKCSM, as reported by [50], suggest that compounds like Jatropholone A/B and Spirocurcasone have no significant hepatotoxic or mutagenic effects. Nevertheless, these predictions require validation through *in vitro* cytotoxicity assays (e.g., MTT assays on HepG2 or Vero cells) and *in vivo* acute and chronic toxicity studies to establish safe therapeutic windows. Additionally, the absence of phorbol esters in the screened compounds reduces the risk of toxicity, but rigorous purification protocols will be necessary to eliminate trace contaminants during extraction.

3.11 Precedent in Antiviral Studies

Several *J. curcas* phytochemicals have documented antiviral activity, providing a precedent for their potential against SARS-CoV-2. Oleanolic acid, structurally akin to 3-O-(Z)-coumaroyl oleanolic acid, has demonstrated antiviral effects against hepatitis C virus (HCV) and human immunodeficiency virus (HIV) by inhibiting viral proteases and enhancing immune responses [50]. Flavonoids, another prominent class in *J. curcas*, such as quercetin and rutin, have been reported to inhibit SARS-CoV-2 M^{pro} and spike protein *in silico* and *in vitro* [17]. Experimental evidence further supports the antiviral potential of *J. curcas* seed extracts, which exhibited activity against Newcastle disease virus, likely due to their flavonoid and terpenoid content. While these studies provide a foundation, the specific activity of *J. curcas* compounds against SARS-CoV-2 remains underexplored experimentally. The high binding affinity and stability of 3-O-(Z)-coumaroyl oleanolic acid in our simulations suggest it may target M^{pro} with similar efficacy, but *in vitro* enzymatic assays and plaque reduction assays are needed to confirm its antiviral potency [17, 49-51].

3.12 Bridging In Silico and In Vivo Applications

The *in silico* findings presented here offer a compelling starting point for *J. curcas*-derived antiviral development, but several steps are required to bridge the gap to *in vivo* applications. First, the predicted binding affinities and MD simulation results must be validated through *in vitro* M^{pro} inhibition assays, using fluorescence-based or high-performance liquid chromatography (HPLC) methods to measure IC₅₀ values. Second, the most promising compounds, such as 3-O-(Z)-coumaroyl oleanolic acid, should undergo cell-based antiviral testing in SARS-CoV-2-infected cell lines (e.g., Vero E6 or Calu-3) to assess their ability to reduce viral replication. Third, pharmacokinetic and toxicity studies in animal models (e.g., mice or hamsters) are critical to establish dosing regimens and safety profiles. Given the structural similarity of *J. curcas* compounds to known antivirals, synergistic combinations with existing drugs, such as remdesivir or nirmatrelvir, could be explored to enhance efficacy and overcome resistance mechanisms.

4. Limitations and Recommendations for Future Research

This study, while leveraging structure-based drug discovery to identify *Jatropha curcas* phytochemicals as potent SARS-CoV-2 M^{pro} inhibitors, is constrained by its reliance on *in silico* methods, limiting its ability to account for biological complexities such as cellular metabolism and off-target effects, and its exclusive focus on M^{pro}, neglecting other viral targets like the spike protein's receptor-binding domain, RdRp, or PL^{pro}. The absence of experimental validation for the lead compounds—3-O-(Z)-coumaroyl oleanolic acid, *Jatropha* factor C3, and beta-amyrin, along with unverified ADMET predictions and static interaction profiles, further restricts translational insights. At the same time, the limited exploration of diverse phytochemical classes, such as flavonoids, may overlook additional candidates. To address these gaps, future research should prioritize comprehensive ADMET profiling using *in silico* tools (e.g., SwissADME) and *in vitro* assays (e.g., Caco-2 permeability) to validate bioavailability, particularly for triterpenoids with solubility challenges [11] synthesize leads for *in vitro* M^{pro} inhibition (FRET-based assays) and cell-based antiviral testing in SARS-CoV-2-infected cell lines (e.g., Vero E6); and optimize structures based on interaction profiles, enhancing hydrogen bonding (e.g., with Glu166) via QSAR-guided modifications. Additionally, multi-

target screening against other viral proteins, in vivo efficacy and safety studies in animal models (e.g., K18-hACE2 mice), exploration of synergistic combinations with drugs like nirmatrelvir, broader phytochemical screening, and investigation of allosteric and immunomodulatory effects [50] will bridge the gap to clinical applications, maximizing the therapeutic potential of *J. curcas* against SARS-CoV-2 and future coronaviruses [17, 49-51].

5. Conclusion

The global urgency to develop effective therapeutics for *Severe Acute Respiratory Syndrome Coronavirus 2* (SARS-CoV-2) has driven innovative approaches to drug discovery, with a particular focus on targeting critical viral enzymes such as the main protease (M^{pro}). This study harnesses structure-based drug discovery (SBDD) to explore the therapeutic potential of *Jatropha curcas* phytochemicals as M^{pro} inhibitors, addressing the pressing need for novel COVID-19 treatments. Through a rigorous computational pipeline involving high-throughput virtual screening, molecular docking, and 100-ns all-atom molecular dynamics (MD) simulations, we identified three lead compounds—beta-amyrin, 3-O-(Z)-coumaroyl oleanolic acid, and Jatropha factor C3—as promising candidates for inhibiting M^{pro}, a pivotal enzyme in viral replication.

Among the 76 *J. curcas* phytochemicals screened, 47 demonstrated binding affinities ≤ -6.0 kcal/mol, indicating strong potential for M^{pro} inhibition. Subsequent MD simulations, supported by comprehensive analyses of root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration, hydrogen bonding, solvent-accessible surface area (SASA), and Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) binding free energies, refined the selection to the top three candidates. Notably, 3-O-(Z)-coumaroyl oleanolic acid exhibited exceptional stability, maintaining an RMSD below 2 Å throughout the simulation, alongside robust hydrogen bonding and favorable interaction energies with key M^{pro} residues, including the catalytic dyad (His41 and Cys145). Jatropha factor C3 also demonstrated commendable stability and interaction profiles, with consistent dynamics and strong binding affinity, positioning it as a close second. In contrast, beta-amyrin, while initially promising, showed instability after 65 ns, with increased RMSD and weakened interactions, diminishing its therapeutic potential compared to the other leads.

These findings highlight the remarkable potential of *J. curcas* phytochemicals, particularly 3-O-(Z)-coumaroyl oleanolic acid and Jatropha factor C3, as M^{pro} inhibitors. Their structural compatibility with the M^{pro} active site, coupled with their dynamic stability, suggests they could disrupt viral replication by interfering with polyprotein cleavage, a critical step in the SARS-CoV-2 life cycle. Furthermore, the precedent of related compounds, such as oleanolic acid derivatives, in antiviral applications against hepatitis C virus and HIV [9] strengthens the case for these phytochemicals. The favorable bioavailability predictions and low toxicity profiles of triterpenoids like 3-O-(Z)-coumaroyl oleanolic acid, as supported by prior *in silico* studies [16, 17], further enhance their translational potential.

However, transitioning from *in silico* promise to clinical application requires addressing several challenges. The current study's reliance on computational methods necessitates experimental validation through *in vitro* M^{pro} inhibition assays, cell-based antiviral testing, and in vivo pharmacokinetic and toxicity studies. Additionally, exploring synergistic effects with existing SARS-CoV-2 therapeutics, such as nirmatrelvir, could amplify efficacy and mitigate resistance. Expanding

the scope to other viral targets, such as the spike protein's receptor-binding domain, could further broaden the therapeutic utility of *J. curcas* phytochemicals.

In conclusion, this study establishes *Jatropha curcas* as a valuable source of novel M^{pro} inhibitors, with 3-O-(Z)-coumaroyl oleanolic acid and Jatropha factor C3 emerging as lead candidates for combating SARS-CoV-2. By leveraging SBDD and advanced MD simulations, we have laid a robust foundation for future research, bridging computational discovery with the potential for real-world impact. These findings not only advance the search for COVID-19 therapeutics but also underscore the untapped potential of plant-derived compounds in addressing global health challenges posed by emerging viral pathogens (Figure 6). Continued efforts to validate and optimize these leads will be critical to realizing their full therapeutic promise, potentially contributing to the arsenal against SARS-CoV-2 and future coronaviruses.

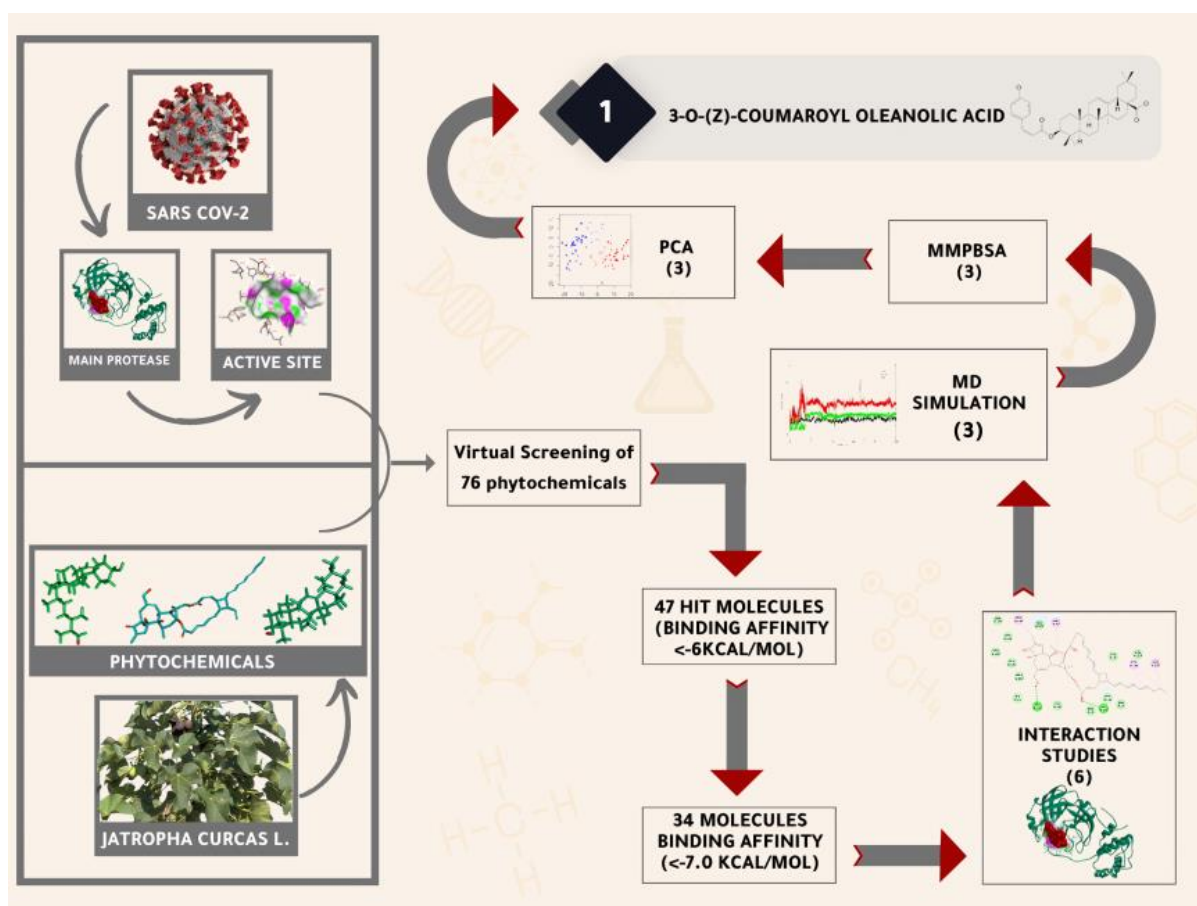


Figure 6 This graphical abstract illustrates the structure-based drug discovery pipeline for identifying *Jatropha curcas* L. phytochemicals as potential inhibitors of the SARS-CoV-2 main protease (M^{pro}). Starting with *J. curcas* as a source of 76 phytochemicals, the workflow begins with virtual screening (1) to identify 47 hit molecules with binding affinities ≤ -6.0 kcal/mol to the M^{pro} active site. Subsequent filtering (2) narrows the selection to 34 molecules with binding affinities ≤ -7.0 kcal/mol, with 3-O-(Z)-coumaroyl oleanolic acid emerging as a lead candidate. Molecular dynamics (MD) simulations, principal component analysis (PCA), and Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) calculations (3) assess the stability and binding free energies of the top candidates, confirming the dynamic stability of 3-O-(Z)-coumaroyl oleanolic

acid. Interaction studies (4) highlight key binding interactions between the lead compound and M^{pro}'s active site residues, demonstrating its potential as a therapeutic agent against SARS-CoV-2.

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Author Contributions

FD and SB contributed to conceptualization, data analysis, writing the original draft, and manuscript review and editing. All authors reviewed and approved the final manuscript for publication.

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

All data used in this study are publicly available without restriction.

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