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# Computational Screening of Phytochemicals as Potential Inhibitors of SARS-CoV-2 Main Protease using Molecular Docking and Dynamics-Based Approaches

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## ABSTRACT

The main protease (Mpro) of SARS-CoV-2 is a crucial enzyme needed for viral replication and has gathered significant interest in COVID-19 therapeutic research as a drug target. In the current study, we used an integrative approach that included computational methods to assess a curated collection of phytochemicals from plants as potential inhibitors of the Mpro. A library of 50 phytochemicals were screened through a docking program to identify the ones with the best binding affinity to the Mpro active site. The leading candidates were put through molecular dynamics (MD) simulations to examine the stabilities of protein-ligand complexes, and made use of MM-PBSA binding free energy calculations to quantify the strengths of bindings of the proteins and ligands. We also evaluated each phytochemical with ADMET profiling to detect whether they had drug-likeness and pharmacokinetic characteristics. Withanoside V and Kaempferol-3-O-rutinoside exhibited the best profiles of the lipophilicness, reversible bind of 3 for the catalytic residues (His41, Cys145), low predicted toxicity, and high GI absorptions. Overall, the data suggests that some phytochemicals may be effective leads for developing novel anti-SARS-CoV-2 antiviral agents. Furthermore, this work supports the ability of in silico methods to accelerate drug discovery based on natural products.

***KEYWORDS:*** *SARS-CoV-2, Main protease (Mpro), Phytochemicals, Molecular docking, Molecular dynamics simulation, MM-PBSA, ADMET, COVID-19, Natural product drug discovery*

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## INTRODUCTION

Discovered in 2019, SARS-CoV-2 is causing a global pandemic of COVID-19. The pandemic has resulted in a global effort to identify effective drug therapies for COVID-19. One of the major drug targets in the SARS-CoV-2 lifecycle is the main protease, Mpro, also referred to as 3CLpro. The function of Mpro is critical in the cleavage of viral polyproteins into functional elements necessary for the replication and transcription of the virus (Zhang et al., 2020). As Mpro has a highly conserved structure with no close human homolog, it is an optimal candidate for drug designing (Jin et al., 2020).

Phytochemicals, or plant-derived, natural compounds, have been an important source of drug therapy (e.g., perfuming acid; chemotheraputic agent paclitaxel). Many phytochemicals have demonstrated antiviral, anti-inflammatory, and immunomodulatory properties in various preclinical models (Srivastava et al, 2021). The scope of phytochemical structures are biologically relevant candidates for drug discovery pipelines (Atanasov et al, 2021). Given the increasing abundance of phytochemical databases and the knowledge residing in traditional medicine, natural compounds may be valuable alternatives or adjuncts to synthetic antiviral therapies.

Computational methods, such as molecular docking and molecular dynamics (MD) simulations allow researchers to appraise numerous libraries of nutraceutical compounds (Hollingsworth & Dror, 2018). These methods reliably, quickly, and accurately evaluate binding agreement, predict the conformity of a compound, and reject undesirable compounds prior to initiating any costly laboratory studies. In global health emergency contexts,

computational methods provide rapid, in silico drug discovery pathways.

## Materials and Methods:

### Software and Tools Implemented

Computational workflow was accomplished utilizing a series of open-source and academic tools. The molecular docking was done through the use of AutoDock Vina (v1.1.2) and GROMACS (v2022.2) for molecular dynamics simulations. The visualization and preparation of proteins were conducted in PyMOL and AutoDockTools. The structures of the phytochemicals were downloaded from PubChem and minimized using Open Babel. The ADMET properties and drug-likeness characteristics of the compounds were determined using the SwissADME and pkCSM web servers. The MM-PBSA plugin in GROMACS was utilized to perform the calculations for binding free energy estimation.

### Preparing the protein

We downloaded the crystal structure of SARS-CoV-2 main protease (Mpro) in complex with an inhibitor from RCSB Protein Data Bank (PDB ID: 6LU7). We further cleaned the protein structure by deleting the inhibitor, water molecules, and other heteroatoms in PyMOL. Hydrogen atoms were added, and Kollman charges applied using AutoDockTools, before exporting the protein structure in PDBQT format for docking.

### Phytochemical Library Creation

A collection of 50 phytochemicals with antiviral, anti-inflammatory, or immunomodulatory activity was created from PubChem, the IMPPAT database, and new literature on plant-based SARS-CoV-2 inhibitors. The 2D structures were downloaded in SDF format and converted to 3D structures in Open Babel, geometry optimized, and energy minimized using the MMFF94 force field, and then saved as PDBQT format for docking. Molecular Docking

The docking studies were conducted using AutoDock Vina, maintaining the docking grid focused on the active site of Mpro incorporating catalytic residues His41 and Cys145. The size of the docking grid box was about 22 × 24 × 24 Å and the exhaustiveness option was set to 8. Docking was performed on each compound and the top binding pose of each compound was selected based on the binding affinity (kcal/mol) to analyze further. Ligand–protein interactions were representatively visualized using PyMOL and Discovery Studio Visualizer.

### Molecular Dynamics Simulations

The 5 docked complexes selected were subjected to 100 ns of molecular dynamics simulations using GROMACS. The protein–ligand complex was placed in a cubic box containing TIP3P water molecules and neutralized with $Na^+$/$Cl^-$ ions, using the CHARMM36 force field for the protein and ligand topology created with CGenFF. The system was energy minimized using the steepest descent algorithm, equilibrated under NVT (100 ps) and NPT (100 ps) ensembles, and run for 100 ns at 300 K. Trajectories were analyzed for RMSD, RMSF, radius of gyration, and hydrogen bonding.

### Binding Free Energy calculations

The MM-PBSA method was used to compute the binding free energy of the ligand-protein complexes over the last 10 ns of the MD trajectories. Energies, which were broken down independent of protein and ligand, were recorded for the van der Waals, electrostatic, polar solvation, and SASA terms from the g_mmpbsa tool. Lastly, compounds with the most favorable ΔG_bind values were chosen for the second analysis.

### ADMET and Drug-Likeness Prediction

The top 10 docked compounds were evaluated for pharmacokinetic properties using SwissADME and pkCSM. Parameters that were assessed included : oral bioavailability, lipophilicity (LogP), gastrointestinal absorption, blood-brain barrier penetration and cytotoxicity. Compounds violated more than one of Lipinski's Rule of Five or indicated potential hepatotoxicity or mutagenicity were excluded from final selection.

### Review of Computational Methodologies for Screening Phytochemicals Against SARS-CoV-2 Mpro

Due to the rapid and destructive nature of the COVID-19 pandemic-related shifts and resultant urgency, there have been a surge of research exploring the next generation of antiviral approaches including some agents that target SARS-CoV-2 proteins like Mpro (a protease enzyme critical to viral replication and transcription) (Zhang et al., 2020; Jin et al., 2020). Some of these studies have implemented computational methodologies to determine whether phytochemicals could act as natural competitive inhibitors of Mpro. Researchers have employed in silico methods to harness the discovery potential of biological activity of compounds found in plants as potential antiviral agents. Articles published to date have leveraged in silico methods, including molecular docking, molecular dynamics (MD) simulations, binding free energy estimation, and ADMET (absorption distribution metabolism excretion toxicity) assessments (Srivastava et al., 2021; Patel et al., 2021; Salamat et al., 2023). In this section we review the computational steps that have been

published, and that have been used to screen phytochemicals against SARS-CoV-2 Mpro in the literature. We will describe a number of standardized workflows and pipelines, indicate which software researchers have used and how they/plants/ligands were prepared, provide the parameters used, describe the common methods of analysis of the results, and mention some similarities in conclusions from analyses performed.

In summary, these computational perspectives throughout the pandemic have revealed an actual blend of bioinformatics, structural biology with natural products chemistry. For example, after performing clustering and docking and ultimately predicting the fit or affinity of the promising elements obtained, researchers report experimental validation of lead compounds with strong individual ligand binding affinity and stable structures of protein-ligand interaction contortions to validate in vitro (Ullah et al., 2021; Jaiswal et al., 2022). Most of the published papers are in line with a standard pipeline that contains some steps in the following strong order: protein and ligand preparation, molecular docking, perhaps molecular dynamics, followed by assessment of the ADMET. The pipeline may be linear, however the authors vary on how much of their proposed common pipeline is implemented. For example, an author(s) may report high-throughput docking of hundreds or thousands of phytochemicals, while another author(s) may only dock a couple of peptides as comparison (Mahmud et al., 2021; Kulkarni et al., 2022).

**Results:**

**Molecular Docking Results**

Molecular docking was executed for a curated library of 50 phytochemicals against the active site of SARS-CoV-2 Mpro (PDB ID: 6LU7) with binding affinities ranging from -5.2 to -9.4 kcal/mol. The top five compounds based on docking score were Withanoside V, Kaempferol-3-O-rutinoside, Racemoside A, Shatavarin IX, Apigenin-7-O-glucoside. These compounds all exhibited favorable interactions with the essential catalytic and surrounding residues like His41, Cys145, Glu166, and Met165. Table 1 outlines the top ranking compounds and their docking score.

**Table 1. Top 5 phytochemicals docked against SARS-CoV-2 Mpro**

| Compound | Binding Affinity (kcal/mol) | Key Interactions |
|---|---|---|
| Withanoside V | -9.4 | His41, Cys145, Glu166 |
| Kaempferol-3-O-rutinoside | -9.1 | Cys145, Met165, Gln189 |
| Racemoside A | -8.8 | His41, Glu166, Thr190 |
| Shatavarin IX | -8.6 | Cys145, Glu166, Gln189 |
| Apigenin-7-O-glucoside | -8.5 | His41, Met49, Gln192 |

Figure 1.

Root Mean Square Deviation (RMSD) and hydrogen bond analysis of Mpro–ligand complexes during 100ns of molecular dynamics simulation.

A. RMSD plots showing the backbone stability of SARS-CoV-2 Mpro in complex with Withanoside V, Kaempferol-3-O-rutinoside, and Racemoside A. All complexes retained backbone fluctuation within approximately 1.5–2.5 Å suggesting a conformationally stable protein.
B. Hydrogen bond analysis showing that Withanoside V maintained 4–6 potential hydrogen bonds consistently, while Kaempferol-3-O-rutinoside and Racemoside A sustained 3-5 bonds on average. Overall, the association with the three ligands in response to 100ns of MD supports the notion of strong, stable binding over extended timescales.

**Molecular Dynamics Simulation**

To determine the stability of the top three protein-ligand complexes, we conducted 100 ns MD simulations with GROMACS. Plotting the RMSD, we found the Mpro backbone stabilized (fluctuations of 1.5–2.3 Å), while the ligand RMSDs indicated very strong binding stability. The Withanoside V–Mpro complex had the least deviation (mean RMSD of ~1.9 Å) and formed 4-6 persistent hydrogen bonds completely through the trajectory. The RMSF for the active site residues (His41, Cys145) indicated limited fluctuations, which demonstrates that structural integrity was maintained. The radius of gyration (Rg) values showed a stable value and suggested that the protein remained compact.

Figure 1. RMSD and hydrogen bond plots for top ligand–Mpro complexes.

**Binding Free Energy Calculations**

The MM-PBSA analysis was calculated for the last 20 ns of each MD trajectory of the three best scoring complexes. The most favorable binding free energy was with Withanoside V (ΔG_bind = -55.8 kcal/mol),

followed by Kaempferol-3-O-rutinoside (-50.2 kcal/mol) and Racemoside A (-47.5 kcal/mol). Van der Waals and electrostatic interactions were the primary contributors to the binding energy.

**Table 2. MM-PBSA Binding Free Energy Values**

| Compound | ΔG_bind (kcal/mol) |
|---|---|
| Withanoside V | -55.8 |
| Kaempferol-3-O-rutinoside | -50.2 |
| Racemoside A | -47.5 |

Figure 2.

This table summarizes the estimated binding free energies (ΔG_bind) of the best three phytochemicals docked to SARS-CoV-2 Mpro, calculated using the MM-PBSA method from the last 20 ns of each molecular dynamics trajectory. The most favorable binding was obtained for

Withanoside V, which showed a ΔG_bind of -55.8 kcal/mol. The other phytochemicals are Kaempferol-3-O-rutinoside (-50.2 kcal/mol) and Racemoside A (-47.5 kcal/mol). The negative energy values were beyond sufficient for indicating the binding process, which is favorable and spontaneous. It seems the differentiating energy contributions can be attributed mostly to van der Waals and electrostatic interactions which would suggest close and stable binding of the ligand in the active site of Mpro.

**Evaluation of ADMET & Drug-Likeness**

The top 10 compounds from the docking studies were evaluated with the SwissADME and pkCSM web servers. All the compounds were compliant with Lipinski's Rule of Five with the exception of Racemoside A and Shatavarin IX, which both exceeded the molecular weight limit. Two compounds, Withanoside V and Kaempferol-3-O-rutinoside, had the highest predicted oral absorption, both displayed a low predicted toxicity, no AMES mutagenicity, and acceptable solubility parameters. Apigenin-7-O-glucoside was only flagged for potentially inhibiting CYP450 metabolism. Additionally, any compounds that failed ADMET filters were deprioritized for further analysis.

**Table 3. ADMET Summary of Top Compounds**

| Compound | Rule of 5 | GI Absorption | Toxicity Risk | Notes |
|---|---|---|---|---|
| Withanoside V | Pass | High | Low | Favorable profile |
| Kaempferol-3-O-rutinoside | Pass | High | Low | Good bioavailability |
| Racemoside A | Fail | Moderate | Low | High MW |
| Shatavarin IX | Fail | Low | Moderate | Exceeds Ro5 |
| Apigenin-7-O-glucoside | Pass | High | Medium | Possible CYP450 inhibition |

Figure 3.

Presents ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiles for the top five phytochemicals based on the results of docking and MD. Withanoside V and Kaempferol-3-O-rutinoside demonstrated a high pharmacokinetic potential showing from the lipinski's rule of 5. With a high prediction for gastrointestinal absorption and low findings for toxicity. Because of their greater than ideal molecular weights, Racemoside A and Shatavarin IX were identified as having poor risk for a good ADMET profile. Apigenin-7-O-glucoside is mildly likely than others to demonstrate drug-like action, but it does present moderate likelihood of toxicity risk and possible CYP450 inhibition, which would require further safety profiling.

These findings indicate that Withanoside V and Kaempferol-3-O-rutinoside are viable options for further experimental testing as SARS-CoV-2 Mpro inhibitor candidates.

**Discussion:**

This research involved investigating the potential usage of phytochemicals as inhibitors for SARS-CoV-2 Mpro (main protease) using an integrated in silico investigation that combined molecular docking, molecular dynamics (MD) simulations, binding free energy estimation using MM-PBSA, and ADMET profiling. The computational workflow was aimed to simulate the early-stage of drug discovery process in which natural products with high binding affinity, stable protein-ligand interactions, and favourable pharmacokinetic profiles were investigated.

The docking results offered various high-affinity candidate compounds to proceed with, including Withanoside V, Kaempferol-3-O-rutinoside, and Racemoside A (binding energies were ranging from -8.5 to -9.4 kcal/mol). The selected compounds had reproducible interactions with the important Mpro catalytic residues His41, Cys145 and Glu166, and these binding sites were consistent with prior studies

showing Mpro-targeting compounds. Based on molecular dynamics simulations, the promotional ranking of all top-ranked protein-ligand complexes were stable for 100 ns, with all RMSDs remaining less than 2.5 Å and each of the trajectories retaining hydrogen bonding. Withanoside V had the most stable dynamics (RMSD and hydrogen bond interactions) in conjunction to the others.

Binding free energy calculation utilizing the MM-PBSA protocol confirmed the above docking and MD results, with Withanoside V providing the most favorable ΔG_bind value (-55.8 kcal/mol) followed closely by Kaempferol-3-O-rutinoside and Racemoside A. These results are indicative of strong thermodynamic favourability and tight binding at the Mpro active site, likely due to van der Waals and electrostatic factor contributions.

The additional ADMET predictions provided a well refined list of candidates. Withanoside V and Kaempferol-3-O-rutinoside turned out to be the best candidates in terms of high gastrointestinal absorption, low toxicity, as well as being Lipinski's Rule of Five compliant. Although Racemoside A and Shatavarin IX performed well during the docking exercise, their high molecular weights and poor oral bioavailability lead to demotion of both candidates.

Apigenin-7-O-glucoside passed just about all the pharmacokinetic filters prior to the process, but carried moderate toxicity and the potential to inhibit CY450 activity.

The results of this study are in good agreement with earlier studies of the antiviral activity of Withania somnifera derivatives and flavonoids many of which were able to inhibit viral proteases that included SARS-CoV-2 protease. However, this study contributes to the literature by encompassing docking, MD, and MM-PBSA studies into a useful systematic ranking of phytochemical leads and ADMET profiling.

However, despite the good results, there are several limitations. This work is strictly based on computer predictions only and has no experimental validation via biochemical assays or cell line studies. The study is also limited by the use of one protein conformation and the knowledge that scoring functions may have had limitations. While ADMET tools are helpful, per estimate, and needs to be verified with actual laboratory experiments.

Future work should include in vitro validation of the top-ranked compounds against recombinant Mpro. The in vitro validation should use relevant enzyme inhibition assays including fluorometric $IC_{50}$ determination and subsequent antiviral testing against SARS-CoV-2 in relevant cell lines. Expansion of the compound library with the use of a high-throughput screen or AI-based virtual screening approach could yield novel lead compounds. Additionally, conduct the structure–activity relationship (SAR) studies and chemical optimization in order to develop compounds with improved potency and bioavailability from the most promising hits.

In summary, this computational study provides further encouragement to view phytochemicals, and especially Withanoside V and Kaempferol-3-O-rutinoside, as potential inhibitors of SARS-CoV-2 Mpro. There is merit in continuing to combine natural product chemistry and computational biology to find the next novel antiviral agents.

**Conclusion:**

This investigation utilized a robust computational pipeline to investigate possible phytochemicals as molecules indicating SARS-CoV-2 main protease activity (Mpro). Ultimately, a variety of plant-molecules were identified to have good potential as antiviral agents, based on computational drug discovery approaches. The most promising compounds were Withanoside V and Kaempferol-3-O-rutinoside, which exhibited strong binding interaction, good stability and favorable biopharmaceutical properties. While the results of this investigation support the use of phytochemicals in COVID-19 drug discovery, additional studies are needed using biochemical assays and cell-based assays to validate these findings. This effort demonstrates the potential of utilizing and combining natural product research with computational scientific drug discovery platforms to facilitate and expedite the discovery of antiviral therapeutics against viruses such as SARS-CoV-2.

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