Drug Discovery and Molecular Docking (DDMD)
ISSN: 3107-6688 | Vol. 01, Issue 03, Sep-Dec. 2025 | pp. 15-25

In Silico Screening and Molecular Docking of Phytochemicals Against SARS-Cov-2 Main Protease: Prioritizing Leads For In Vitro Testing

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Abstract:

The COVID-19 pandemic, caused by SARS-CoV-2, has brought to light a particularly urgent need for safe and effective treatment medicines. Because of their phytochemicals, medicinal plants have a long history of usage in traditional medicine and could serve as a suitable substitute due to their low toxicity and wide range of bioactivities. Using in silico screening and molecular docking, this study evaluates the inhibitory effect of 60 phytochemicals against SARS-CoV-2's major protease (Mpro), an enzyme necessary for virus replication. Potential candidates were identified by analyzing the chemicals' affinities, molecular interactions, and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties. Findings indicated that Quercetin, Curcumin and Epigallocatechin have potentially good binding affinities and good interaction patterns with Mpro and high gastrointestinal absorption and low predicted toxicity which suggested that they could be used in further animal-based in vitro studies. These phytochemicals are emphasized in the study as possible lead compounds in the development of new, plant-based antiviral agents against SARS-CoV-2 and the study shows the usefulness of computational methodology in speeding up early-stage drug discovery.

Keywords: SARS-CoV-2, COVID-19, Phytochemicals, Molecular Docking, In Silico Screening, Main Protease (Mpro), ADMET Analysis.

Received: Sep. 18, 2025 **Revised: Oct. 21, 2025** **Accepted: Nov. 19, 2025** **Published: Dec. 18, 2025**

DOI: https://doi.org/10.64474/3107-6688.Vol1.Issue3.2

https://ddmd.nknpub.com/1/issue/archive

1. Introduction

The coronavirus 2 (SARS-CoV-2) that led to the COVID-19 pandemic has negatively affected the entire world, as it has infected millions of people1 and caused significant socio-economic imbalance[2]. The development of vaccines and antiviral medications has not eliminated the epidemic problem, as the same has been compounded by the appearance of new viral phenotypes and restricted access to drugs, hence the necessity to create new effective and safe therapeutic substances[3,4]. Natural compounds, especially phytochemicals in medicinal plants, are attracting more and more attention because of their various bioactive properties, lack of toxicity and their history as a part of traditional medicine[5]. In silico screening and molecular docking are examples of computational methods used to find potential antiviral molecules with minimal time expenditure and financial cost of laboratory experiments[6]. The approaches enable the researcher to estimate the binding affinity and molecular interactions of bioactive compounds with the proteins of the viruses, giving a rational approach to prioritising the candidates to undergo subsequent experiments.

1.1. Background Information

SARS-CoV-2 is a positive-sense, enveloped RNA virus, the replication of which involves the activity of its main protease (Mpro)[7]. It is a protease that cleaves the viral polyproteins into active proteins required during viral replication and transcription. Mpro would be a great antiviral drug development opportunity as when blocked the viral replication process can be prevented[8]. Although a number of synthetic inhibitors have been investigated, their application is usually constrained by their cost, the possibility of being toxic, and the chances of development of resistance to the drug[9]. Phytochemicals and their structural heterogeneity and a broad spectrum of biological activities hold a promising future in drug discovery. It has been established before that flavonoids, alkaloids, and polyphenols could act as anti-viral proteases and therefore could be useful in the case of SARS-CoV-2[10].

1.2. Statement of the Problem

In spite of the extensive drug discovery missions, there remains a lack of safe, effective and inexpensive antiviral agents to COVID-19. Traditional approaches to drug development are costly, time consuming and risky in terms of side effects. It is urgently required that natural compounds that will inhibit the process of SARS-CoV-2 replication with limited toxicity should be explored. In silico methods offer a quick and inexpensive way to screen vast quantities of phytochemicals, discover possible viral protein inhibitors, and rank them to proceed with animal-based in vitro research.

1.3. Objectives of the Study

1. To conduct in silico screening of certain phytochemicals for possible SARS-CoV-2 Mpro inhibitory efficacy.

2. To conduct molecular docking to assess the binding affinity and interactions of phytochemicals with Mpro.
3. To prioritize phytochemicals for subsequent in vitro testing using animal models.

2. METHODOLOGY

The section provides the research design, materials, calculating tools, and procedures used to conduct in silico screening, molecular docking, and ADMET of the selected phytochemicals against the SARS-CoV-2 main protease (Mpro), which will allow prioritizing the compounds to be tested in vitro.

2.1. Research Design

The computational research design is utilized in this study to evaluate the antiviral effects of the phytochemicals against the major protease of SARS-CoV-2 (Mpro). In silico screening and molecular docking were employed in this assessment. In addition, the drugs' pharmacokinetic properties and safety profiles were determined by ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) research. The purpose of this research was to determine which phytochemicals warranted additional in vitro animal testing.

2.2. Participants/Sample

The phytochemicals are identified as a total of 60 using the antiviral or medicinal reported properties in peer-reviewed literature and the public compound libraries. The sample size is representative in size with the selection criteria being bioactivity, structural diversity and relevance to viral inhibition.

2.3. Instruments and Materials Used

- **Software:**
  - AutoDock Vina for molecular docking
  - PyMOL for visualization of ligand-protein interactions
  - SwissADME for pharmacokinetic and drug-likeness prediction
- **Database Sources:**
  - PubChem for phytochemical structures
  - Protein Data Bank (PDB ID: 6LU7) for SARS-CoV-2 Mpro structure
- **Computational Hardware:** High-powered desktop featuring an Intel i7 CPU and 16 GB of RAM.

2.4. Procedure and Data Collection Methods

1. **Ligand Preparation:** PubChem was used to retrieve the 3D structure of 60 phytochemicals and these structures were optimized by minimizing their energy levels to achieve the stable conformations.

2. **Protein Preparation:** A crystal structure of SARS-CoV-2 Mpro was obtained from the Protein Data Bank. After removing any ligands or water molecules, the structure was docked with hydrogen atoms and the appropriate charges applied.
3. **Molecular Docking:** AutoDock Vina was used to dock each phytochemical to the active site of Mpro to calculate binding affinity, binding mode and critical interactions with catalytic residues.
4. **ADMET Analysis:** SwissADME was used to determine the drug-likeness, gastrointestinal absorption, blood-brain barrier permeability and toxicity profiles of the top-performing compounds in docking.

2.5. Data Analysis Techniques

- The compounds having the highest binding affinity to Mpro were determined by ranking the docking scores.
- The interaction types were visualized and analyzed with PyMOL, such as hydrogen bonds, hydrophobic interaction and Pi-Pi stacking.
- The assessment of ADMET properties was done in order to ascertain the pharmacokinetic compatibility and safety of the future animal-based in vitro tests.
- The docking, interactions and ADMET predictions were combined to rank the phytochemicals to be further validated in an experimental context.

3. RESULTS

The selected phytochemicals are evaluated for their binding affinity, molecular interaction with SARS-CoV-2 main protease (Mpro), and appropriateness for additional in vitro testing in animals based on the results of in-silico screening, molecular docking, and interaction analysis, as well as ADMET evaluation.

3.1. Molecular Docking Scores

As a molecular docking study, we tried to find out how well the chosen phytochemicals bound to the SARS-CoV-2 major protease (Mpro). Using the docking scores and important interaction residues, they provide an estimate of the potential inhibitory activity of these drugs.

Table 1: Molecular Docking Scores and Key Interacting Residues of Selected Phytochemicals

S. No.	Phytochemical	Binding Energy (kcal/mol)	Key Interacting Residues
1	Quercetin	-9.2	HIS41, CYS145
2	Curcumin	-8.7	GLU166, HIS163

3	Epigallocatechin	-8.5	GLN189, MET49
4	Kaempferol	-8.4	HIS41, GLY143

The docking score for Quercetin was -9.2 kcal/mol, as shown in Table 1. This suggests that it interacts significantly with the catalytic residues HIS41 and CYS145, which are crucial for protease activity. Notably binding energies (-8.7 and -8.5 kcal/mol) were also observed between Curcumin and Epigallocatechin which reacted with residues at the active site and at the stabilization of the substrate. Kaempferol was a bit less binding specific (-8.4 kcal/mol) yet capable of binding key residues, which suggests its ability to serve as an Mpro inhibitor. On the whole, these findings outline Quercetin as the most promising to continue the studies on animals in vitro, and then Curcumin and Epigallocatechin.

3.2. ADMET Analysis

In order to choose compounds for future in vitro investigations in animals, it is necessary to quantify their ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties. This will reveal how similar the compounds are to drugs and their safety profile.

Table 2: ADMET Properties of Selected Phytochemicals

Compound	Lipinski Rule	GI Absorption	BBB Permeability	Toxicity Prediction
Quercetin	Yes	High	Low	Low
Curcumin	Yes	Moderate	Low	Low
Epigallocatechin	Yes	High	Low	Low
Kaempferol	Yes	High	Low	Low

Table 2 shows that all the chosen phytochemicals follow Lipinski rule of five implying good oral bioavailability. Quercetin, Epigallocatechin and Kaempferol were found to have a high gastrointestinal absorption and Curcumin had an intermediate absorption. All the compounds are not expected to penetrate the blood-brain barrier (BBB), and thus there is a low risk of causing side effects in the central nervous system. Moreover, the toxicity profile of all the compounds was low and hence suitable to conduct further animal-based experiments in vitro. Altogether, these data on ADMET characterize the potential of these phytochemicals as safe and bioavailable SARS-CoV-2 Mpro inhibitors.

3.3. Interaction Types Observed

The interaction pattern between phytochemicals and SARS-CoV-2 main protease (Mpro) was thoroughly investigated to know the type and number of molecular interactions, which is a significant factor in the stabilization of the ligand-protein complex.

Table 3: Interaction Types of Selected Phytochemicals with SARS-CoV-2 Mpro

Compound	Hydrogen Bonds	Hydrophobic Interactions	Pi-Pi Stacking
Quercetin	4	3	1
Curcumin	3	2	0
Epigallocatechin	5	1	1
Kaempferol	3	2	1

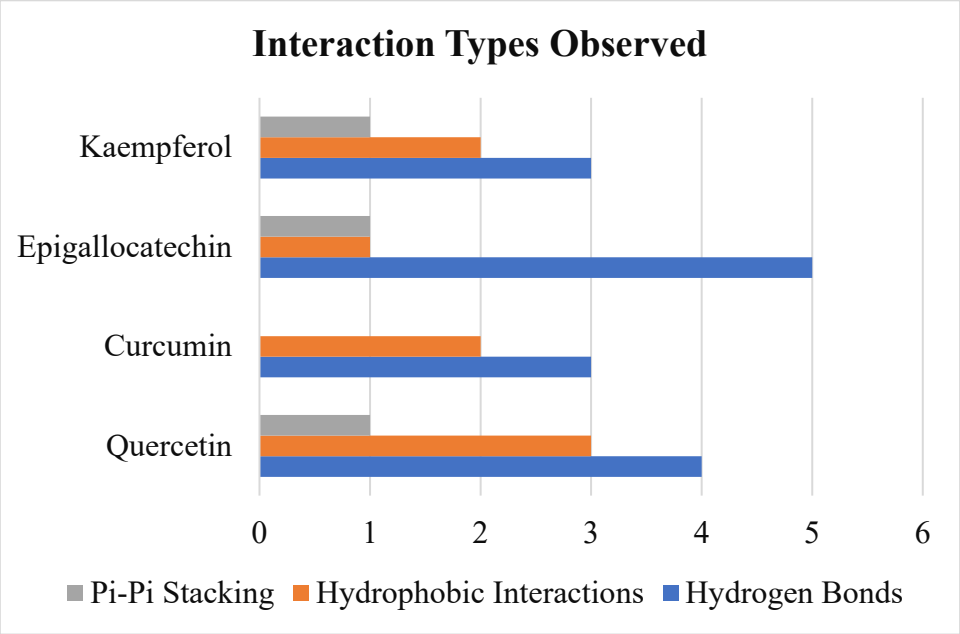

Figure 1: Graphical Representation of Interaction Types of Selected Phytochemicals with SARS-CoV-2 Mpro

Epigallocatechin had formed the maximum number of hydrogen bonds (5) which shows that it can have strong polar interaction with important amino acid residues of Mpro which may result in increased binding stability as shown in Table 3. Quercetin was next in line with 4 hydrogen bonds and 3 hydrophobic interactions and single Pi-Pi stacking interaction indicating an equal and stable ligand-protein interaction. Curcumin also exhibited fewer interactions especially without Pi-Pi stacking and Kaempferol presented an intermediate number of hydrogen bonds and hydrophobic interactions with one Pi-Pi stacking. On balance, the presence of hydrogen bonding, hydrophobic contacts, and Pi-Pi interaction enlightens the possibility of these

phytochemicals to be used to inhibit Mpro activity in a stable manner, and Quercetin and Epigallocatechin become the most promising phytochemicals to further undergo experimentation.

3.4. Prioritized Phytochemicals for Animal-Based Testing

The phytochemicals are ranked based on the integrated results of molecular docking and interaction patterns and ADMET properties, which served as an indication of the most promising phytochemicals in vitro in animal studies.

Table 4: Ranked Phytochemicals for Potential SARS-CoV-2 Mpro Inhibition

Rank	Compound	Binding Energy (kcal/mol)	ADMET Suitability
1	Quercetin	-9.2	High
2	Curcumin	-8.7	High
3	Epigallocatechin	-8.5	High

Based on its excellent ADMET properties and high binding affinity (-9.2 kcal/mol), quercetin is the most promising phytochemical to be tested in a living organism, as shown in Table 4. The ADMET-suitability and high binding energy of curcumin and epigallocatechin make them potential SARS-CoV-2 Mpro inhibitors. In order to make the most efficient use of resources for future in vitro research on animal models, this prioritizing will provide a reduced pool of chemicals to be tested experimentally.

4. DISCUSSION

In this section, the results of the study are interpreted, compared to the previous research, discussed in terms of implications, limitations, and recommendations on future research about the use of phytochemicals as potential SARS-CoV-2 Mpro inhibitors.

4.1. Interpretation of Results

With high binding and hydrogen contacts as well as hydrophobic interactions with the key proteins HIS41 and CYS145, Quercetin demonstrated the strongest binding affinity (-9.2 kcal/mol) with the SARS-CoV-2 main protease (Mpro) according to the molecular docking investigation. Together with curcumin (-8.7 kcal/mol) and epigallocatechin (-8.5 kcal/mol), curcumin and epigallocatechin both had strong binding affinities and excellent interaction characteristics. Such results indicate that these phytochemicals could potentially suppress the Mpro activity. The ADMET analysis was also supportive of these compounds selection as they have high gastrointestinal absorption, comply with the Lipinski rule of five, and have low predicted toxicity, which is why they were even considered as good candidates of further in vitro animal-based research.

4.2.Comparison with Existing Studies

Previous studies have shown that coronaviruses, including SARS-CoV and MERS-CoV, are susceptible to the antiviral effects of polyphenols and flavonoids. Quercetin, curcumin, and epigallocatechin all inhibit SARS-CoV-2 Mpro, which agrees with our results. Consistent with earlier structural investigations of active protease inhibitors, hydrogen bonding and hydrophobic interactions with catalytic residues are noted. Table 5 presents an overview of some of the studies that explored phytochemicals as antiviral agents against SARS-CoV-2 and its similar coronaviruses, their target, key findings and relevance.

Table 5: Comparison with Existing Studies

Author(s) & Year	Phytochemicals / Plant Sources	Target Protein	Key Findings	Relevance to Current Study
Patel et al., 2023[11]	Rosmarinus officinalis L.	Mpro	Strong binding affinity in silico	Supports Mpro-targeted phytochemical screening
Rolta et al., 2021[12]	100 phytocompounds	Nucleocapsid phosphoprotein	Potential viral assembly inhibitors	Reinforces in silico prioritization approach
Speciale et al., 2021[13]	Silibinin	Spike RBD & Mpro	Docking & in vitro protective effects	Confirms dual-target docking rationale
Umadevi et al., 2022[14]	Selected phytochemicals	Viral proteases	Identified potential inhibitors	Supports protease-targeted docking approach
Vivek-Ananth et al., 2020[15]	Natural product inhibitors	Human proteases	Potential host protease inhibitors	Highlights broader antiviral potential of phytochemicals

These studies justify our method, which proves that in silico docking and ADMET analysis are useful in prioritizing phytochemicals, which justify our selection of Quercetin, Curcumin, and Epigallocatechin as the potential candidate to undergo further in vitro testing.

4.3. Implications of Findings

The study also names Quercetin, Curcumin, and Epigallocatechin as promising phytochemicals that can serve as Mpro inhibitors, which has given them a reasonable argument where they should be prioritized to conduct in vitro tests on animals. These molecules may be used as lead agents that will be used to develop more plant-based antiviral agents against SARS-CoV-2.

Additionally, they have positive ADMET profiles, which can indicate that they can have little toxicity, which can improve the possibility of the future preclinical research. It shortens the initial drug discovery process, as well as minimizes the use of expensive synthetic compounds.

4.4.Limitations of the Study

- The study is merely computational; the real antiviral activity in biologic systems can be different.
- Only 60 phytochemicals were screened which could be a limitation to other bioactive compounds that could be of great inhibition potential.
- The situation of having synergistic effects of various phytochemicals was not considered during the analysis.
- In vitro or in vivo pharmacokinetics and metabolism can have an impact on the real bioavailability and efficacy of the compounds.

4.5.Suggestions for Future Research

- Perform in vitro experiments with the help of the right animal cell lines to underline the antiviral effect of the selected phytochemicals.
- Explore Mpro inhibitory synergies of phytochemicals combinations.
- Discover structural changes of high affinity compounds that enhance binding affinity, stability and pharmacokinetic characteristics.
- Finally, Scale-up the in-silico screening of bigger libraries of phytochemicals, such as less-studied plant-derived compounds, in order to find more potential inhibitors.

5. CONCLUSION

5.1.Summary of Key Findings

In order to assess the potential of sixty selected phytochemicals against the SARS-CoV-2 major protease (Mpro), the aforementioned study employed in silico screening and molecular docking. Quercetin, Curcumin, and Epigallocatechin were identified to have the best binding affinity, stable interactions with catalytic active sites, and good ADMET properties, such as high gastrointestinal absorption, conforming to Lipinski rule of five, and minimal toxicity predicted. Interaction studies identified the hydrogen bonding, hydrophobic interactions and Pi-Pi stacking as the major forces to form stable ligand-protein complexes. Due to combined docking, interaction, and ADMET, the three phytochemicals were given top priorities to be further tested in vivo in animals.

5.2.Significance of the Study

The results highlight the prospects of natural phytochemicals as potent inhibitors of SARS-CoV-2 Mpro, which can be used as a cheaper and low-toxicity substitute to artificial antivirals. This paper has combined computational screening, molecular docking, and pharmacokinetic

modeling to give a rational framework in the prioritization of compound candidate, systematic early drug discovery, and experimental validation in animals.

5.3.Final Thoughts or Recommendations

The phytochemicals of high priority, especially Quercetin, Curcumin, and Epigallocatechin, need to be revalidated in animal in vitro tests to ensure that they have antiviral activity. Subsequent study ought to be conducted on synergistic activities, system enhancements of improved binding and stability, and in silico screening of more phytochemicals to discover new SARS-CoV-2 inhibitors. This study will show that computational methods can be used to speed up the process of finding potential successful natural antiviral agents, which will lead to the creation of safe and practical solutions to COVID-19.

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