

Docking study of ellagic acid against SARS COv2 proteins

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Abstract:

The global outbreak of COVID-19 caused by the SARS-CoV-2 created an urgent need for effective antiviral agents with improved safety and therapeutic efficacy. In recent years, Computer-Aided Drug Design (CADD) and molecular docking techniques have emerged as important tools in modern drug discovery because they provide rapid, economical, and reliable screening of potential drug candidates. The present study was undertaken to evaluate the antiviral potential of Ellagic Acid, a naturally occurring polyphenolic compound found in pomegranate, berries, and walnuts, against important SARS-CoV-2 proteins through molecular docking analysis. Ellagic acid is known for its antioxidant, anti-inflammatory, and antiviral properties, making it a promising natural therapeutic candidate. In this study, major SARS-CoV-2 target proteins involved in viral replication and infection were selected, including:

- Main Protease (Mpro)
- Spike Glycoprotein
- RNA-Dependent RNA Polymerase (RdRp)

The three-dimensional crystal structures of the selected proteins were retrieved from the [Protein Data Bank \(PDB\)](#) database, while the chemical structure of ellagic acid was obtained from the [PubChem Database](#). Protein and ligand preparation were carried out using standard molecular modeling procedures prior to docking studies. Molecular docking analysis was performed using:

- AutoDock

The docking results demonstrated favorable binding affinity and stable molecular interactions between ellagic acid and the selected viral proteins. Among all targets, the strongest interaction was observed with Main Protease (Mpro), followed by RNA-Dependent RNA Polymerase and Spike Glycoprotein. Ellagic acid formed significant hydrogen bond interactions and hydrophobic interactions with important amino acid residues present in the active binding sites of viral proteins. The findings of the study suggest that ellagic acid may possess potential inhibitory activity against SARS-CoV-2 proteins and could interfere with viral replication and infection mechanisms. The study also highlights the importance of molecular docking and Computer-Aided Drug Design in accelerating antiviral drug discovery while reducing cost and time. Although the obtained In-silico results are promising, further experimental studies including In-vitro, In-vivo, and clinical investigations are required to confirm the therapeutic efficacy and safety of ellagic acid against COVID-19 infection. Overall, the present study supports the potential role of natural phytochemicals as safer antiviral agents for future pharmaceutical research and drug development.

Keywords: Ellagic Acid, SARS-CoV-2, COVID-19, Molecular Docking, Main Protease, Spike Protein, RdRp, Antiviral Activity, Computer-Aided Drug Design, Natural Phytochemicals.

Introduction

The global outbreak of COVID-19 created an urgent need for effective antiviral medicines to control viral infection and reduce the intensity of the disease. In recent years, pharmaceutical research has increasingly used In-silico (computer-based) drug discovery methods to quickly and efficiently identify possible antiviral compounds. This method helps researchers study natural plant-based compounds that may have useful biological activity, while also saving laboratory time, cost, and effort.

The present study is focused on Ellagic Acid, a natural compound commonly found in fruits such as pomegranates, berries, and nuts. This research examines how ellagic acid interacts with important proteins of COVID-19 that are involved in viral infection and replication. Using molecular docking techniques, the study aims to understand the binding behavior of ellagic acid with these viral proteins and evaluate its possible antiviral activity. The findings may help in identifying ellagic acid as a potential therapeutic candidate against SARS-CoV-2 infection.[1.1]

Pathophysiology of Coronavirus Disease (COVID-19):

Coronavirus disease 2019, commonly known as COVID-19, is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). The disease mainly affects the respiratory system; however, it can also involve the cardiovascular, gastrointestinal, renal, neurological, and immune systems. The pathophysiology of COVID-19 includes viral entry into host cells, immune dysregulation, inflammatory responses, endothelial dysfunction, oxidative stress, and multi-organ damage.

1. Viral Entry and Replication: SARS-CoV-2 is an enveloped positive-sense single-stranded RNA virus belonging to the Coronaviridae family. The virus primarily spreads through respiratory droplets and aerosols. After entering the respiratory tract, the viral spike (S) protein binds to angiotensin-converting enzyme-2 (ACE2) receptors present on alveolar epithelial cells, endothelial cells, intestinal epithelial cells, and several other tissues .[2]

The interaction between the spike protein and ACE2 receptor allows viral fusion with the host cell membrane. Viral RNA then enters the host cell cytoplasm and utilizes host ribosomes for synthesis of viral proteins and replication of genetic material. Newly formed virions are released from infected cells and spread to neighboring tissues, causing progressive cellular injury and inflammation. [3]

2. Pulmonary Pathophysiology: The lungs are the major organs affected in COVID-19. SARS-CoV-2 infects type II alveolar pneumocytes, resulting in cellular destruction and inflammatory responses. Damage to alveolar epithelium increases vascular permeability and leads to accumulation of inflammatory exudates in the alveolar spaces. This impairs gaseous exchange and produces clinical manifestations such as fever, cough, dyspnea, and hypoxia.[4.1]

In severe infections, diffuse alveolar damage may progress to acute respiratory distress syndrome (ARDS), characterized by pulmonary edema, reduced lung compliance, and severe hypoxemia.

$PaO_2/FiO_2 < 300$

This reduction in the arterial oxygenation ratio is commonly used as an indicator of ARDS severity.

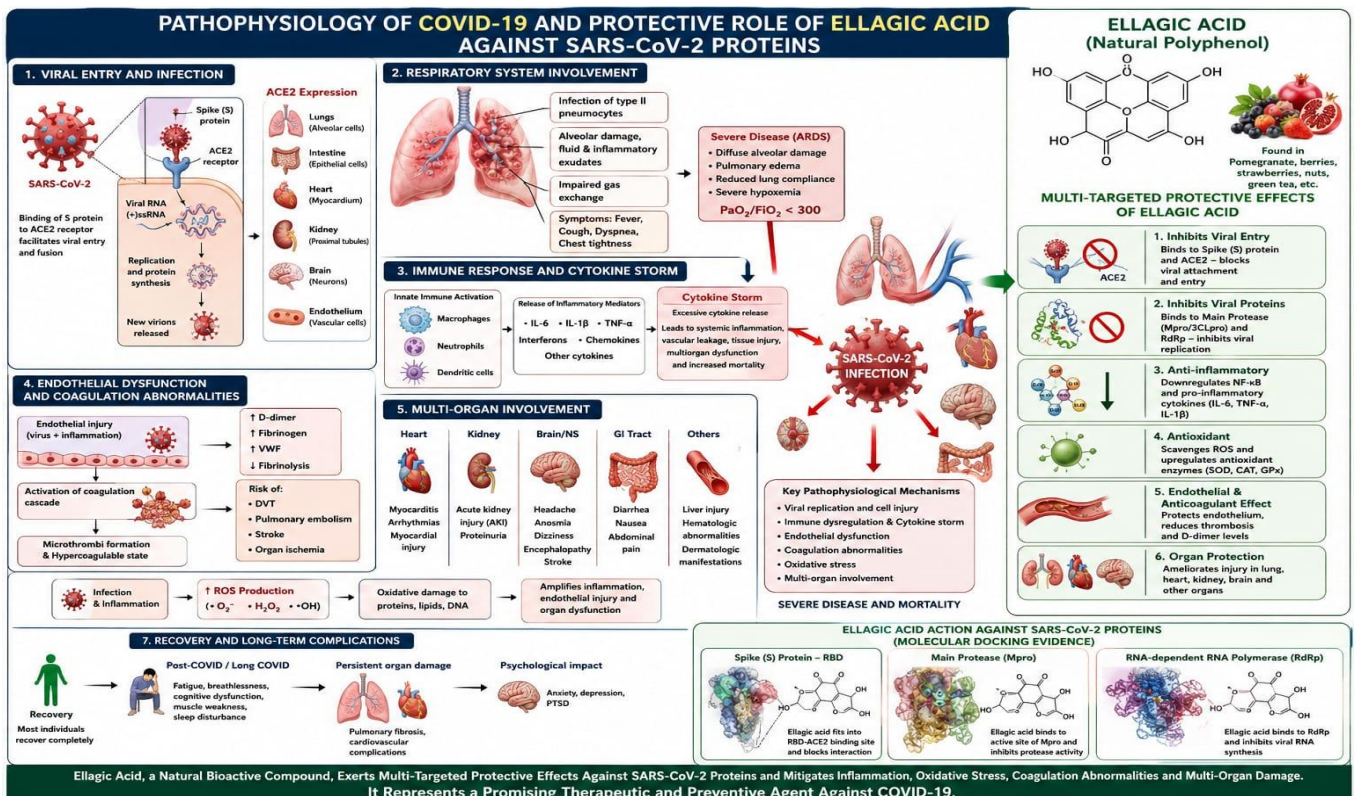
3. Immune Response and Cytokine Storm: Following viral infection, the innate immune system becomes activated. Macrophages, neutrophils, and dendritic cells release pro-inflammatory cytokines and

chemokines including interleukin-6 (IL-6), interleukin-1 β (IL-1 β), interferons, and tumor necrosis factor-alpha (TNF- α).[5]

In some patients, excessive immune activation results in a “cytokine storm,” which contributes to systemic inflammation, endothelial injury, and multiple organ dysfunction. Elevated inflammatory markers such as C-reactive protein (CRP), ferritin, and IL-6 are frequently associated with severe disease and poor prognosis.[6]

4. Endothelial Injury and Coagulation Abnormalities: COVID-19 is associated with endothelial dysfunction and abnormal coagulation pathways. Viral infection and inflammatory mediators damage vascular endothelial cells, leading to activation of platelets and the coagulation cascade. Consequently, patients may develop microvascular thrombosis and thromboembolic complications such as pulmonary embolism, stroke, and deep vein thrombosis[4.2] (3).Elevated D-dimer levels and fibrin degradation products are commonly observed in severe COVID-19 cases. These coagulation abnormalities contribute significantly to organ ischemia and increased mortality.

5. Oxidative Stress and Cellular Damage: Oxidative stress plays an important role in COVID-19 pathogenesis. Excessive production of reactive oxygen species (ROS) during infection damages proteins, lipids, and nucleic acids. ROS generation also amplifies inflammatory pathways and endothelial dysfunction, thereby worsening tissue injury and organ failure[7]



6. Multi-Organ Involvement: Although the respiratory system is the primary target, SARS-CoV-2 may affect multiple organs due to widespread expression of ACE2 receptors. Cardiovascular complications include myocarditis, arrhythmias, and myocardial injury. Renal involvement may lead to acute kidney injury, while neurological manifestations include headache, anosmia, encephalopathy, and dizziness [8]

Gastrointestinal symptoms such as nausea, diarrhea, and abdominal pain may occur due to viral infection of intestinal epithelial cells. Therefore, COVID-19 is considered a multisystem disease rather than solely a respiratory disorder.

7. Long-Term Complications: Some recovered patients experience persistent symptoms known as post-COVID syndrome or long COVID. Common manifestations include fatigue, dyspnea, cognitive dysfunction, chest pain, and muscle weakness. Long-term pulmonary fibrosis and cardiovascular abnormalities have also been reported in severe cases.[9]

1.1 Biological Mechanism of SARS-CoV-2 Infection:

In virology, viral infection can be understood as a step-by-step process through which a virus enters the body, infects cells, and multiplies. The SARS-CoV-2 virus, which causes COVID-19, starts infection by attaching itself to healthy human cells and then releasing its genetic material (RNA) inside the cell. Once inside, the virus takes control of the cell’s normal functions and uses the cell’s machinery to make more copies of itself.

After entering the human body, SARS-CoV-2 mainly affects the respiratory system, especially the lungs and airways. The virus uses special proteins present on its outer surface to bind with receptors on host cells. After attachment, the virus enters the cell, where its RNA begins to replicate. This replication process helps the virus produce important proteins required for its growth, survival, and formation of new viral particles. As more viruses are produced, they spread to nearby cells and continue the infection cycle.[1.2]

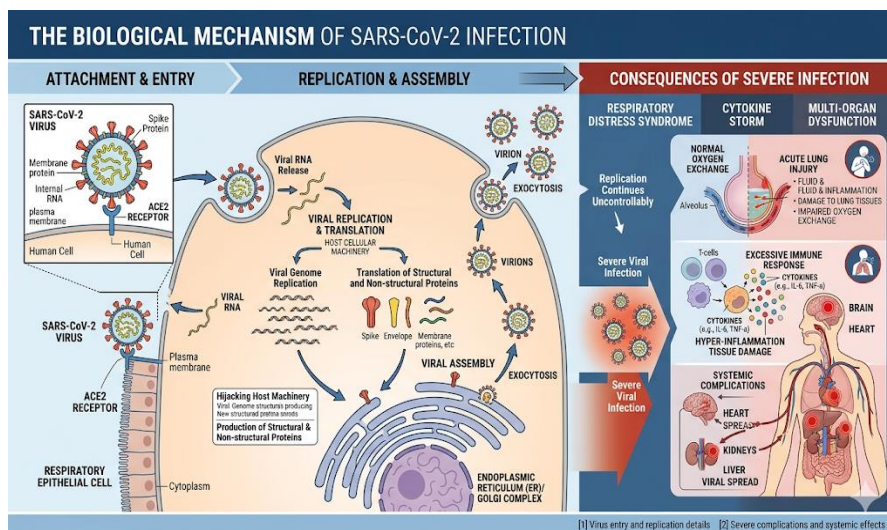


Fig 1. Mechanism of SARS-CoV-2 Viral Entry and Replication

However, the major challenge when viral replication continues uncontrollably, leading to severe infection and immune system dysregulation. Severe viral infection can result in:

- **Respiratory Distress Syndrome:** Damage to lung tissues affecting oxygen exchange.
- **Cytokine Storm:** Excessive immune response leading to tissue damage.
- **Multi-Organ Dysfunction:** Viral spread causing systemic complications.

Understanding this infection mechanism is essential for identifying molecular targets that can interrupt viral replication and prevent disease progression. [10]

1.2 Target Proteins of SARS-CoV-2: The life cycle of SARS-CoV-2 depends on several functional proteins that regulate viral entry, replication, and maturation. These proteins serve as primary targets for antiviral drug development. Among the most important proteins involved in SARS-CoV-2 infection are:

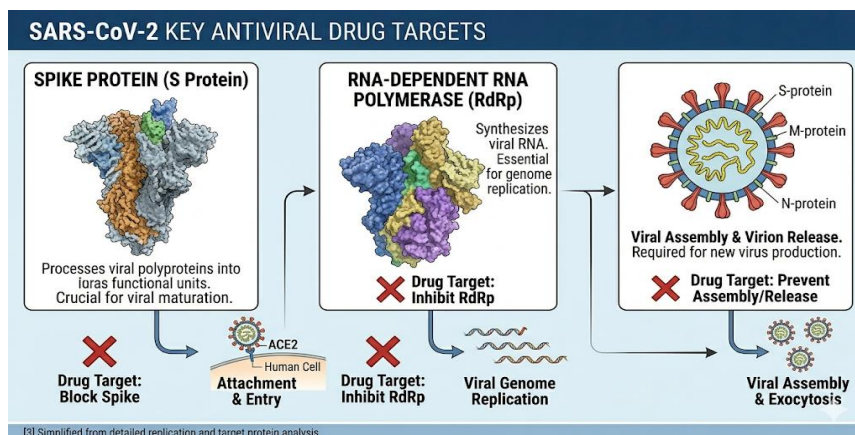


Fig 2. SARS-CoV-2 Protein Targets

Main Protease (Mpro or 3CLpro): This enzyme plays a crucial role in processing viral polyproteins into functional components required for viral replication. Inhibiting this enzyme can effectively block viral replication.

Spike Protein (S Protein): The spike protein is responsible for binding to host cell receptors, allowing viral entry into human cells. Blocking this protein can prevent viral attachment.

RNA-Dependent RNA Polymerase (RdRp): This enzyme is responsible for synthesizing viral RNA. Inhibiting RdRp disrupts viral genome replication, thereby halting viral multiplication. [11]

Targeting these proteins through molecular docking provides a rational approach to discovering potential antiviral agents.

1.3 Limitations of Conventional Antiviral Drugs:

Currently available antiviral medications such as Remdesivir and Favipiravir have shown effectiveness against SARS-CoV-2 infection. However, their long-term usage presents several limitations and adverse effects.

Traditional antiviral drugs may cause:

Drug Resistance: Viral mutations can reduce drug effectiveness.

Toxicity Issues: Liver and kidney complications due to prolonged drug exposure.

Limited Accessibility: High cost and limited availability in developing regions.

Additionally, many antiviral agents are developed through complex chemical synthesis, which increases production costs and time requirements. These challenges highlight the need to identify safer, natural antiviral compounds with minimal side effects. [12]

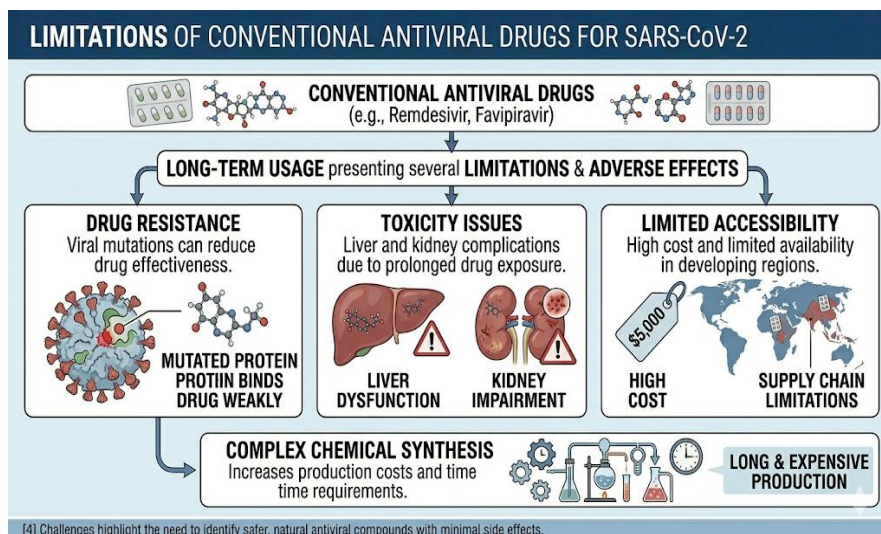


Fig 3. Limitations of Conventional Antiviral Therapy

1.4 Ellagic Acid: A Natural Antiviral Lead: In the search for safer therapeutic alternatives, natural phytochemicals have gained considerable attention. **Ellagic Acid** is a naturally occurring polyphenolic compound widely distributed in plants such as pomegranates, strawberries, raspberries, and walnuts. This compound is particularly abundant in the fruit of *Punica granatum* and is known for its strong antioxidant, anti-inflammatory, and antiviral properties.

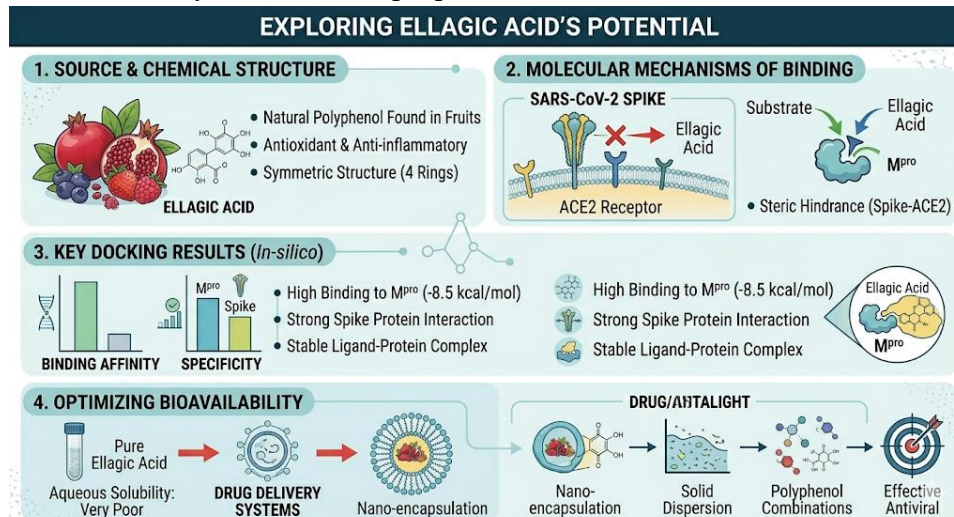


Fig 4. Chemical Structure of Ellagic Acid and its Natural Sources

Chemically, ellagic acid contains multiple hydroxyl groups and aromatic rings, which contribute to its strong binding ability with protein targets. These structural features enable ellagic acid to form stable hydrogen bonds and hydrophobic interactions within protein binding pockets.

Scientific studies have reported that ellagic acid exhibits:

- Antiviral activity
- Antioxidant properties
- Anti-inflammatory effects
- Immune-modulatory functions

Unlike many synthetic drugs, ellagic acid is considered relatively safe due to its natural origin. However, challenges such as **poor solubility and limited bioavailability** restrict its therapeutic application.

Using **Computer-Aided Drug Design (CADD)** and molecular docking, researchers can predict how ellagic acid binds to SARS-CoV-2 proteins, helping to optimize its antiviral efficiency. [13]

Rationale of the Study

The use of computational techniques such as molecular docking has revolutionized the drug discovery process, particularly during global health emergencies like COVID-19.

Traditionally, antiviral drug development required extensive laboratory testing and clinical trials, which are time-consuming and costly. The rationale for adopting an **In-silico docking approach** in this study is based on the following principles:

Cost and Time Efficiency: Computational tools allow rapid screening of natural compounds against multiple viral targets, significantly reducing research time.

Ethical Considerations (3Rs Principle): Docking studies support the **Replacement, Reduction, and Refinement** of animal testing by predicting molecular interactions before laboratory experiments.

Molecular-Level Understanding: Docking simulations provide a detailed view of atom-to-atom interactions between ellagic acid and SARS-CoV-2 proteins, helping researchers identify strong binding sites and potential inhibitory mechanisms.[14].

Aim of the Study

The present study aims to investigate the antiviral potential of **Ellagic Acid**, a naturally occurring polyphenolic compound, against important proteins of the SARS-CoV-2 using molecular docking techniques. The study focuses on understanding the interaction of ellagic acid with viral proteins involved in viral entry, replication, and survival inside the host cell.

The main goal is to find out whether ellagic acid can work as a natural inhibitor of SARS-CoV-2 proteins. This is done by studying its binding strength, the type of interactions it forms with the proteins, and how stable these interactions are using Computer-Aided Drug Design (CADD) techniques.

Since this is an in-silico (computer-based) study, it helps provide early scientific evidence about the antiviral potential of ellagic acid. The results may support further research and could contribute to the future development of new antiviral drugs based on natural compounds.[15,16]

Objectives of the Study

1. To understand the biology and pathogenesis of SARS-CoV-2

The first objective of this study is to understand the structure, infection mechanism, and replication cycle of SARS-CoV-2. The virus infects host cells through the ACE2 receptor and uses its proteins for replication and multiplication inside the human body. Understanding the viral life cycle is important for identifying suitable molecular targets for antiviral therapy. [17]

2. To identify important target proteins of SARS-CoV-2

This study aims to identify and select important SARS-CoV-2 proteins involved in viral survival and replication, including:

- Main Protease (Mpro)

- Spike Glycoprotein
- RNA-Dependent RNA Polymerase (RdRp)

These proteins are considered important therapeutic targets because inhibition of these proteins may block viral replication and infection. [18]

3. To obtain and prepare protein structures for docking studies

Another objective of this study is to retrieve the three-dimensional crystal structures of selected SARS-CoV-2 proteins from the [Protein Data Bank \(PDB\)](#) database for molecular docking studies.

The proteins will be prepared by:

- Removing water molecules
- Adding hydrogen atoms
- Optimizing protein structures
- Identifying active binding sites

Proper protein preparation is necessary for accurate molecular docking analysis. [19]

4. To obtain and prepare the ligand structure of Ellagic Acid

This study also aims to collect the chemical structure of ellagic acid from the [PubChem Database](#) and prepare it for docking analysis.

Ligand preparation includes:

- Energy minimization
- Geometry optimization
- Conversion into docking-compatible format

This preparation improves the accuracy of ligand–protein interaction studies. [20]

5. To perform molecular docking analysis

One of the major objectives of this work is to perform molecular docking between ellagic acid and selected SARS-CoV-2 proteins using software such as:

- AutoDock

Molecular docking helps predict:

- Binding affinity
- Binding orientation
- Stability of ligand–protein complex
- Possible inhibitory activity

This computational method provides detailed information about molecular interactions at the atomic level. [21]

6. To analyze ligand–protein interactions

The study aims to analyze various molecular interactions formed between ellagic acid and viral proteins, including:

- Hydrogen bonding
- Hydrophobic interaction
- Pi–Pi interactions

These interactions help determine the strength and stability of the ligand–protein complex and indicate the possible antiviral activity of ellagic acid. [22]

7. To evaluate the antiviral potential of Ellagic Acid Another objective is to evaluate whether ellagic acid can effectively bind to SARS-CoV-2 proteins and inhibit their biological activity. Strong binding affinity and stable molecular interactions may suggest the antiviral potential of ellagic acid against COVID-19 infection. [23]

8. To compare docking results with standard antiviral drugs

This study also aims to compare the docking score and interaction profile of ellagic acid with reported antiviral drugs such as Remdesivir and Favipiravir available in scientific literature. Such comparisons help assess the effectiveness of ellagic acid as a possible therapeutic candidate. [24]

9. To promote the application of Computer-Aided Drug Design (CADD)

The study intends to highlight the importance of Computer-Aided Drug Design in modern pharmaceutical research. Molecular docking offers a rapid, economical, and time-saving approach for screening natural compounds before conducting laboratory and clinical studies. [25]

10. To support future research on natural antiviral compounds

Finally, the study aims to provide scientific support for the medicinal importance of ellagic acid and encourage further experimental and clinical research on plant-derived phytochemicals for antiviral drug development. The findings may contribute to the discovery of safer and more effective natural therapeutic agents against viral diseases. [26]

- Molecular docking and Computer-Aided Drug Design techniques

Relevant research articles, review papers, and scientific databases were studied to understand the current status of antiviral drug discovery against COVID-19. [15,16]

2. Selection of Target Proteins

Important SARS-CoV-2 proteins involved in viral replication and infection were selected as molecular targets for docking studies. The selected proteins included:

- Main Protease (Mpro)
- Spike Glycoprotein
- RNA-Dependent RNA Polymerase (RdRp)

These proteins were chosen because they play critical roles in viral survival and are considered major therapeutic targets for antiviral drug development. [17]

3. Retrieval of Protein Structures

The three-dimensional crystal structures of selected SARS-CoV-2 proteins were obtained from the [Protein Data Bank \(PDB\)](#) database.

Suitable Protein Data Bank (PDB) IDs were selected based on structural quality and resolution.

Examples:

- Mpro – PDB ID: 6LU7

The downloaded protein structures were saved in PDB format for further docking analysis. [18]

4. Protein Preparation

The selected protein structures were prepared before docking using molecular modeling tools. Protein preparation involved:

- Removal of water molecules
- Removal of co-crystallized ligands
- Addition of hydrogen atoms
- Energy minimization
- Identification of active binding sites

This preparation helps improve docking accuracy and interaction prediction. [19]

5: The phytochemical **Ellagic Acid** was selected as the ligand for the present study because of its reported antiviral and antioxidant activities.

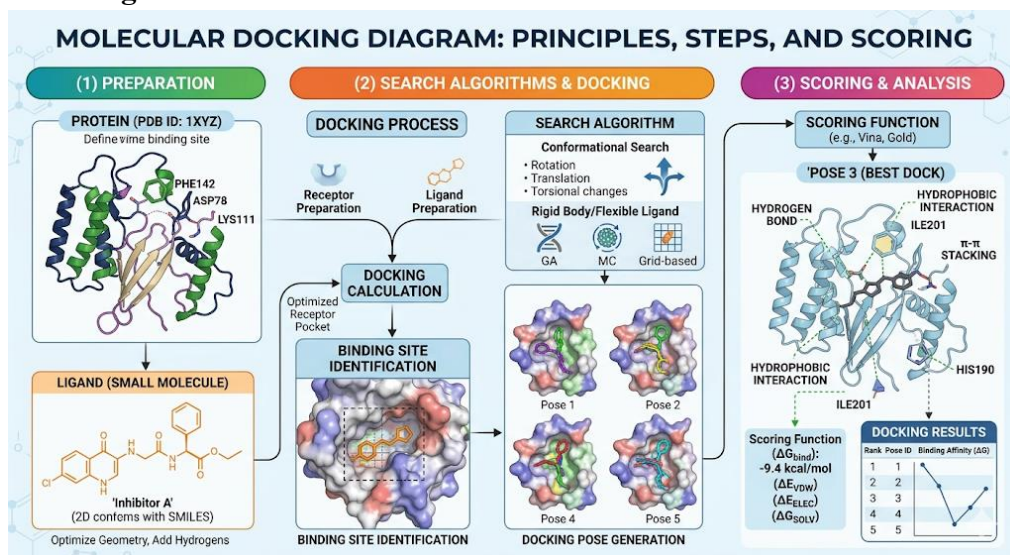
The chemical structure of ellagic acid was retrieved from the [PubChem Database](#) in suitable file format.

Ligand preparation included:

- Geometry optimization
- Energy minimization
- Conversion into docking-compatible format

This process ensures proper ligand flexibility and accurate docking interaction. [20]

6. Molecular Docking Studies



Molecular docking was carried out using:

- AutoDock

Docking studies were performed to predict:

- Binding affinity
- Binding orientation
- Ligand–protein interactions
- Stability of docked complexes

The docking software generated binding scores and interaction poses between ellagic acid and selected viral proteins. [21]

7. Analysis of Docking Results

The obtained docking results were analyzed based on:

- Binding energy values
- Hydrogen bond interactions
- Hydrophobic interactions
- Amino acid residues involved in binding

Lower binding energy values indicated stronger binding affinity and better interaction stability. [22]

8. Visualization of Molecular Interactions

The docked complexes were visualized using molecular visualization software to study the interaction pattern between ellagic acid and viral proteins.

Visualization helped identify:

- Active site interactions

Hydrogen bond formation Binding pocket orientation

These analyses provided structural understanding of the antiviral potential of ellagic acid. [23]

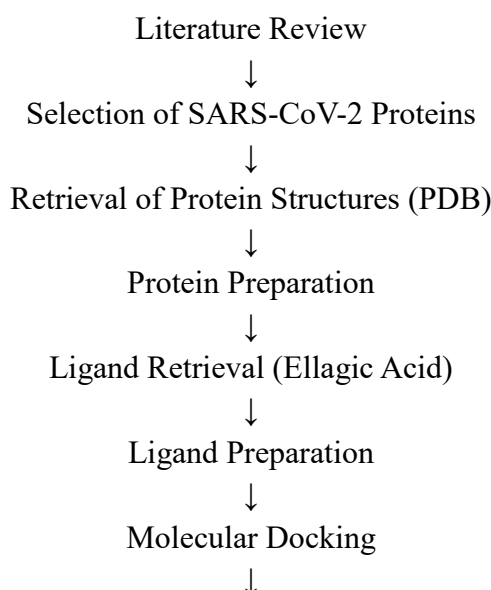
9. Interpretation and Discussion of Results

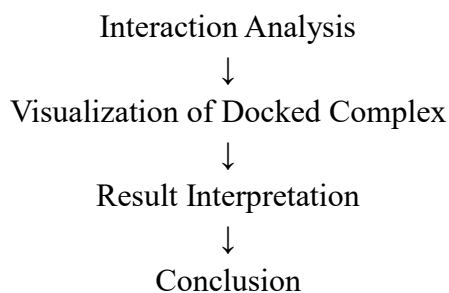
The docking findings were interpreted and compared with reported antiviral compounds available in scientific literature. The interaction profile and binding affinity of ellagic acid were evaluated to determine its possible inhibitory activity against SARS-CoV-2 proteins. [24]

10. Conclusion and Future Perspective

Finally, conclusions were drawn based on docking results to evaluate the effectiveness of ellagic acid as a potential antiviral agent. Future perspectives regarding experimental validation and formulation development were also discussed. [25]

Flowchart of Plan of Work





Methodology

The present study entitled “**Molecular Docking Study of Ellagic Acid Against SARS-CoV-2 Proteins**” was carried out using Computer-Aided Drug Design (CADD) techniques to evaluate the antiviral potential of ellagic acid against important proteins of the SARS-CoV-2. The methodology adopted for the study is described below.

1. Selection of Target Proteins

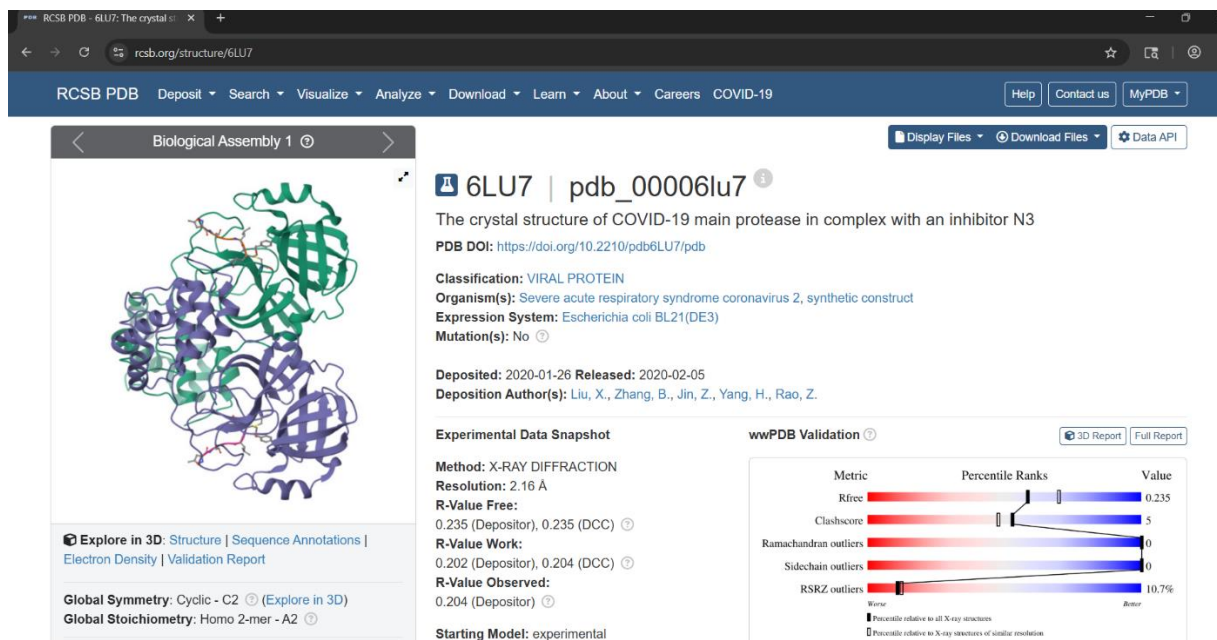
The target proteins selected for the docking study were proteins involved in viral entry and replication of SARS-CoV-2. The selected proteins included:

Protein	Function	PDB ID
Main Protease (Mpro/3CLpro)	Viral protein processing	6LU7
Spike Glycoprotein	Viral attachment and entry	6VSB
RNA-Dependent RNA Polymerase (RdRp)	Viral RNA replication	7BV2

These proteins were selected because they play an important role in the survival and multiplication of the virus and are considered important targets for antiviral drug development. [26]

2. Retrieval of Protein Structures

The three-dimensional crystal structures of selected SARS-CoV-2 proteins were retrieved from the [Protein Data Bank \(PDB\)](#) in PDB format.



6LU7 | pdb_0006lu7

The crystal structure of COVID-19 main protease in complex with an inhibitor N3

PDB DOI: <https://doi.org/10.2210/pdb6LU7/pdb>

Classification: VIRAL PROTEIN
Organism(s): Severe acute respiratory syndrome coronavirus 2, synthetic construct
Expression System: Escherichia coli BL21(DE3)
Mutation(s): No

Deposited: 2020-01-26 **Released:** 2020-02-05
Deposition Author(s): Liu, X., Zhang, B., Jin, Z., Yang, H., Rao, Z.

Experimental Data Snapshot

Method: X-RAY DIFFRACTION
Resolution: 2.16 Å
R-Value Free: 0.235 (Depositor), 0.235 (DCC)
R-Value Work: 0.202 (Depositor), 0.204 (DCC)
R-Value Observed: 0.204 (Depositor)

Starting Model: experimental

wwPDB Validation

Metric	Percentile Ranks	Value
Rfree		0.235
Clashscore		5
Ramachandran outliers		0
Sidechain outliers		0
RSRZ outliers		10.7%

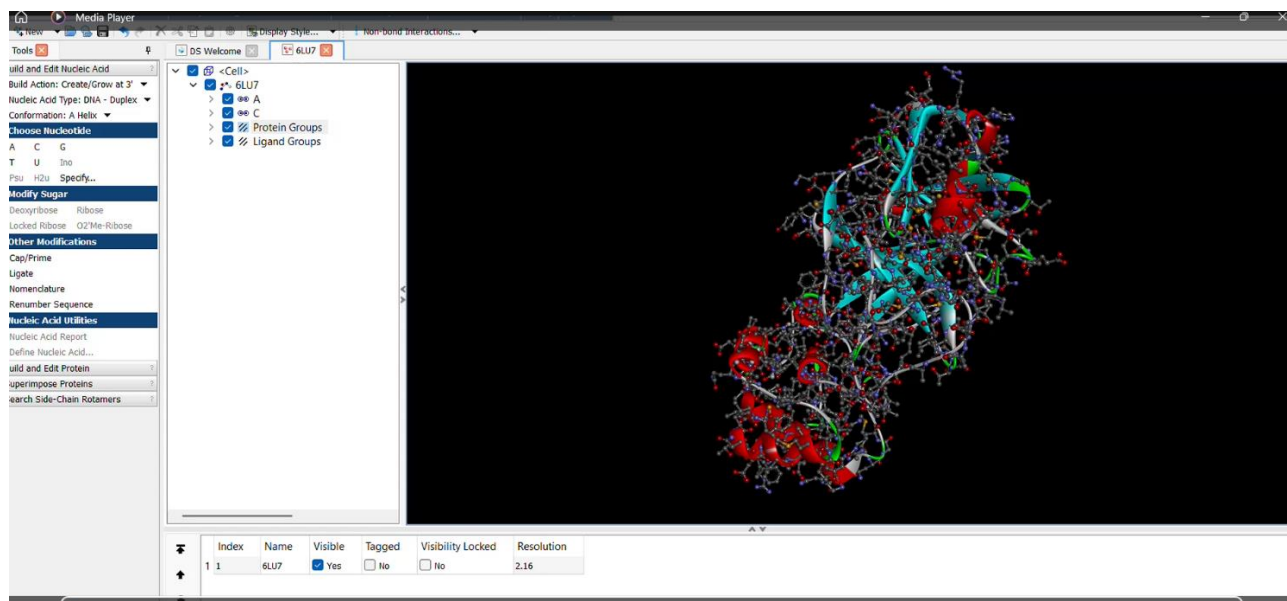
The downloaded protein structures were carefully examined for:

- Structural resolution
- Presence of co-crystallized ligands
- Active binding sites
- Completeness of amino acid chains

High-resolution protein structures were selected to ensure better docking accuracy. [27]

3. Protein Preparation

Protein preparation was carried out using BIOVIA discovery studio tools before performing molecular docking studies.



Index	Name	Visible	Tagged	Visibility Locked	Resolution
1	6LU7	Yes	No	No	2.16

The following steps were involved in protein preparation:

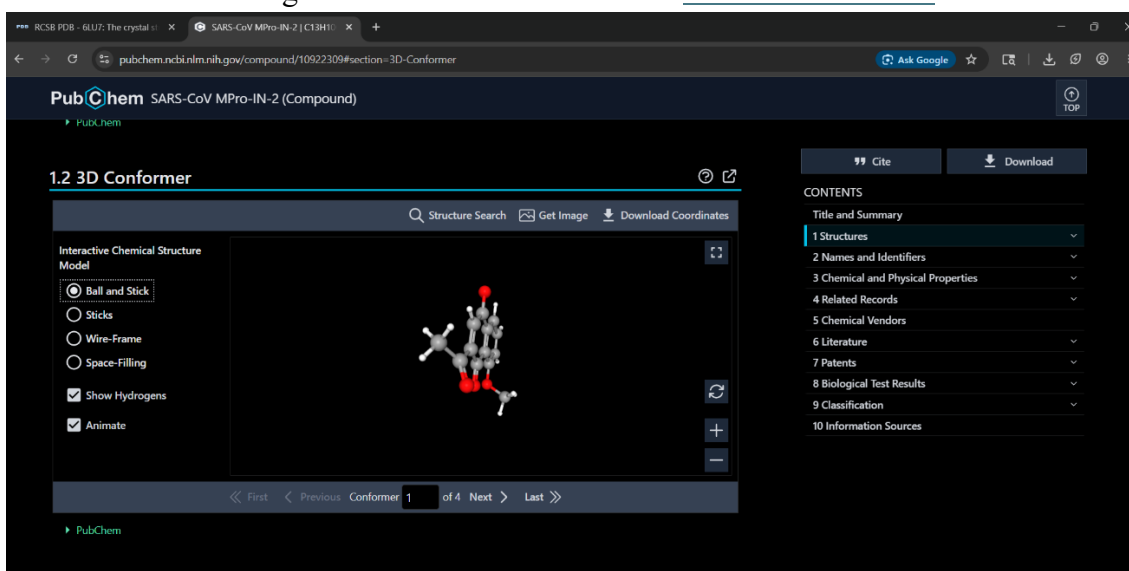
- Removal of water molecules
- Removal of co-crystallized ligands and unwanted chains
- Addition of polar hydrogen atoms
- Addition of Kollman charges
- Energy minimization of protein structure

The prepared protein structures were then saved in docking-compatible format for further analysis. [28]

4. Selection and Retrieval of Ligand

The phytochemical **Ellagic Acid** was selected as the ligand because of its reported antiviral, antioxidant, and anti-inflammatory properties.

The chemical structure of ellagic acid was obtained from the [PubChem Database](#).



Ligand Details :

Parameter	Information
Compound Name	Ellagic Acid
PubChem CID	5281855
Molecular Formula	C ₁₄ H ₆ O ₈
Molecular Weight	302.19 g/mol

The ligand structure was downloaded in SDF format for further preparation. [29]

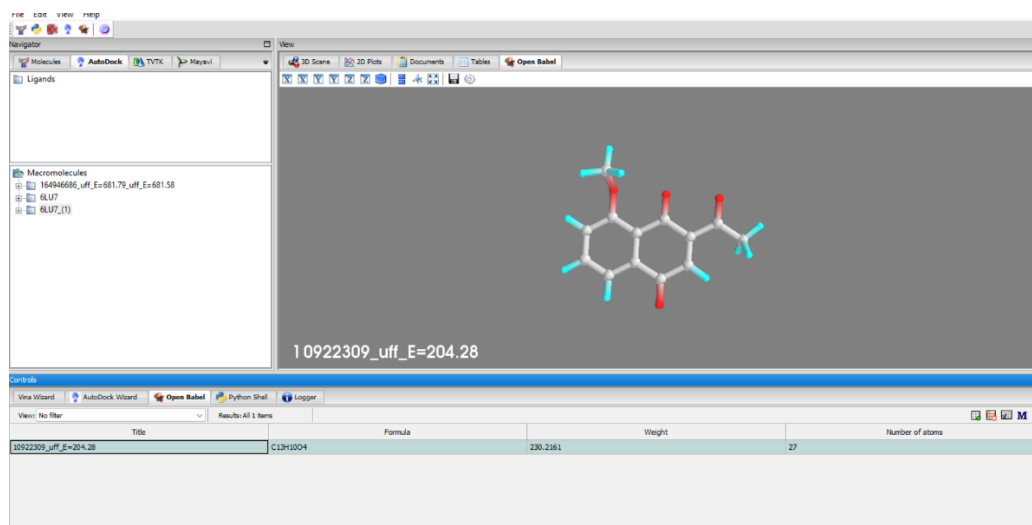
5. Ligand Preparation

Ligand preparation was performed to optimize the structure of ellagic acid before docking.

The following steps were included:

- Geometry optimization
- Energy minimization
- Addition of hydrogen atoms

- Conversion of file format into PDBQT format



The prepared ligand structure was then used for molecular docking studies. [30]

6. Molecular Docking Procedure

Molecular docking studies were performed using:

- AutoDock

Docking was carried out to predict the interaction between ellagic acid and selected SARS-CoV-2 proteins.

The docking procedure involved:

1. Selection of active binding site
2. Grid box generation around active site
3. Running docking simulation
4. Generation of docking poses
5. Calculation of binding affinity scores

The best docking pose was selected based on minimum binding energy and stable molecular interactions. [31]

7. Analysis of Molecular Interactions

The docked complexes obtained after molecular docking were analyzed to study ligand–protein interactions.

The following parameters were evaluated:

- Binding energy (kcal/mol)
- Hydrogen bond interactions
- Hydrophobic interactions
- Amino acid residues involved in binding
- Binding pocket orientation

Lower binding energy indicated stronger interaction and better binding affinity of ellagic acid toward viral proteins. [32]

8. Visualization of Docked Complexes

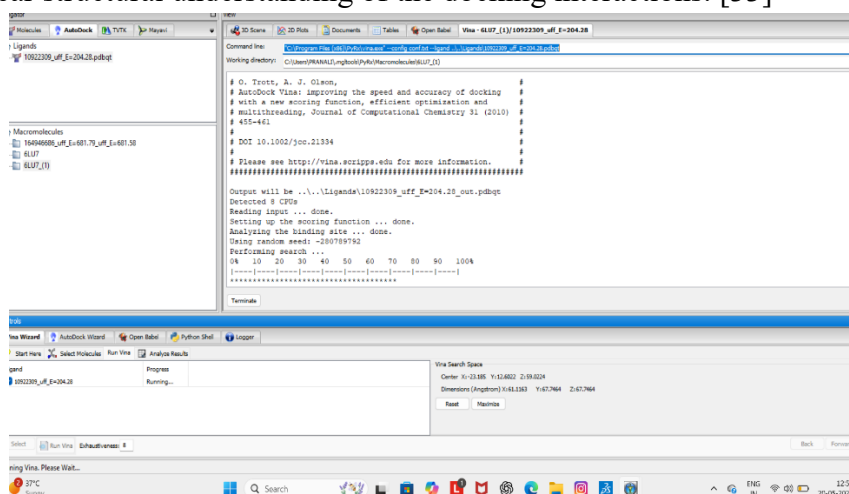
Visualization of docked protein–ligand complexes was carried out using molecular visualization software such as:

- BIOVIA Discovery Studio Visualizer

Visualization helped in understanding:

- Binding orientation
- Interaction sites
- Hydrogen bond formation
- Active site occupancy

This provided a clear structural understanding of the docking interactions. [33]

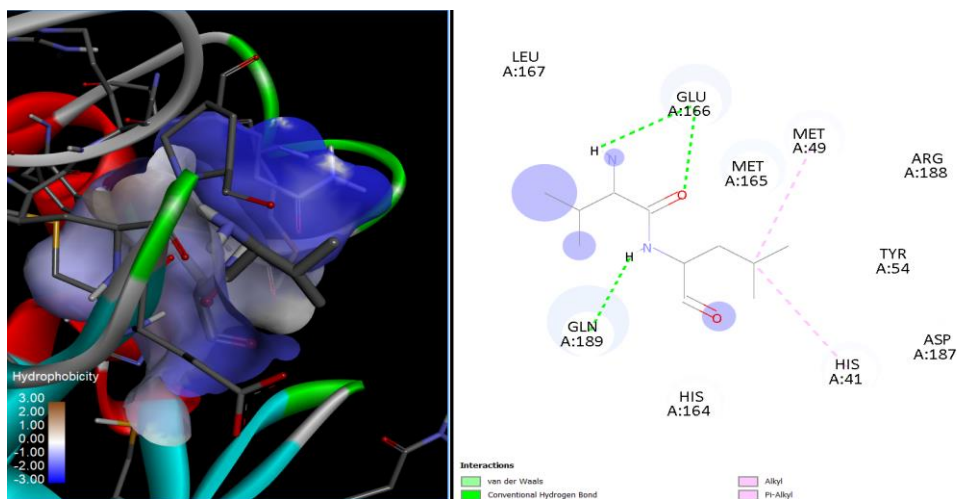


9. Interpretation of Results

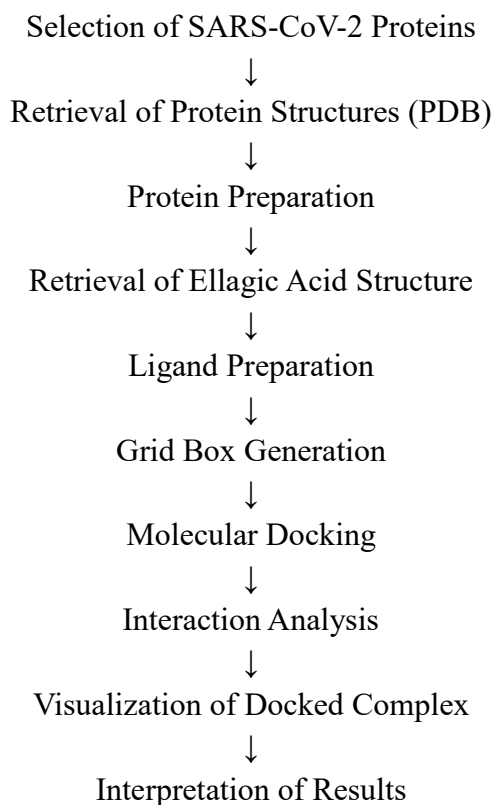
The docking results were interpreted based on binding affinity and molecular interaction profiles. The obtained docking scores of ellagic acid were compared with standard antiviral compounds reported in literature to evaluate its potential inhibitory activity against SARS-CoV-2 proteins. [34]

10. Statistical and Comparative Analysis

Comparative analysis of docking scores and interaction patterns was performed to identify the most favorable protein target for ellagic acid. The protein showing strongest interaction and lowest binding energy was considered the best target for antiviral activity. [35]



Flowchart of Methodology



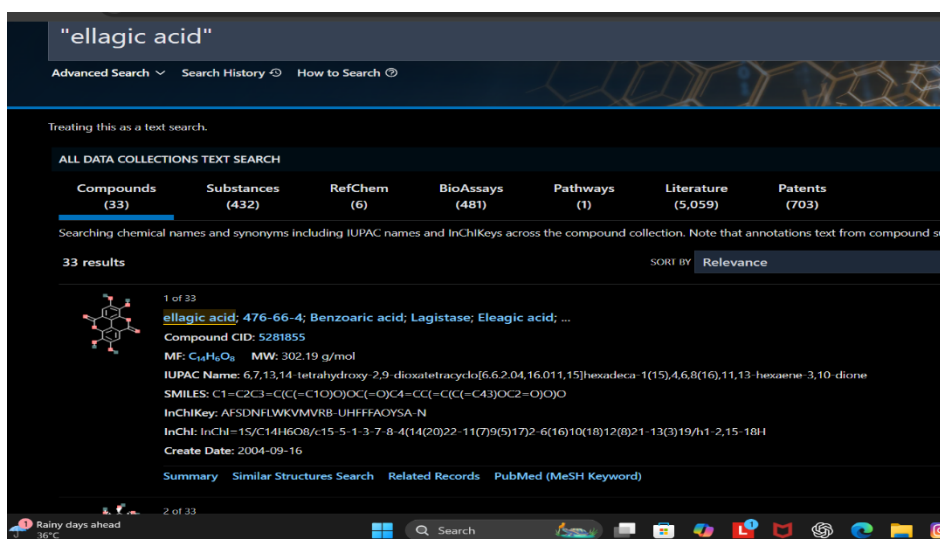
METHODOLOGY OF ADMET:

1. Selection of Ligand/Compound:

The selected ligand or phytochemical compound was chosen for ADMET analysis based on its potential biological or antiviral activity.

2. Searching Compound in PubChem:

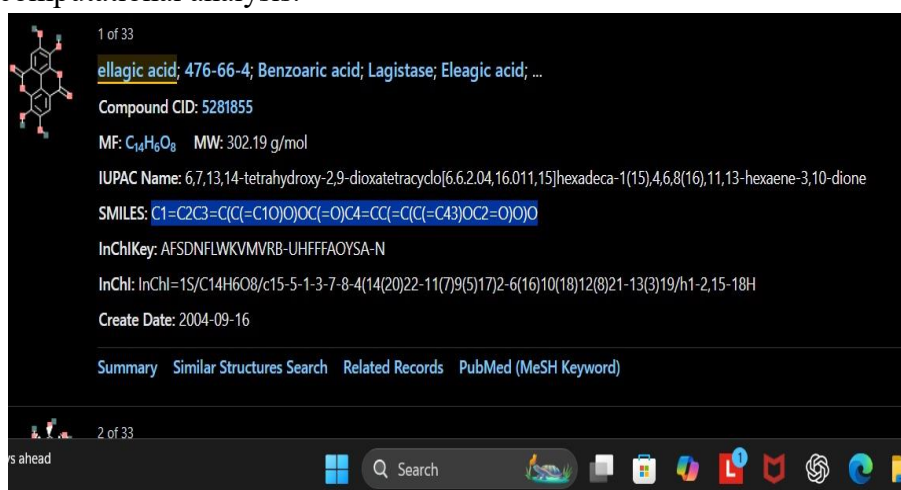
The compound information was retrieved from [PubChem](https://pubchem.ncbi.nlm.nih.gov/) by searching the compound name in the database search bar.



The screenshot shows a search for "ellagic acid" on the PubChem website. The search results page displays a list of data collections with the following counts: Compounds (33), Substances (432), RefChem (6), BioAssays (481), Pathways (1), Literature (5,059), and Patents (703). The search results are sorted by Relevance. The first result is for "ellagic acid; 476-66-4; Benzoic acid; Lagistase; Eleagic acid; ...". The chemical structure is shown as a 2D ball-and-stick model. The summary information includes: Compound CID: 5281855, MF: C₁₄H₆O₈, MW: 302.19 g/mol, IUPAC Name: 6,7,13,14-tetrahydroxy-2,9-dioxatetracyclo[6.6.2.0.4,16.011,15]hexadeca-1(15),4,6,8(16),11,13-hexaene-3,10-dione, SMILES: C1=C2C3=C(C(=C1O)O)OC(=O)C4=CC(=C(C(=C43)OC2=O)O)O, InChIKey: AFSDNFWKVMVRB-UHFFFAOYSA-N, InChI: InChI=1S/C14H6O8/c15-5-1-3-7-8-4(14(20)22-11(7)9(5)17)2-6(16)10(18)12(8)21-13(3)19/h1-2,15-18H, Create Date: 2004-09-16. Navigation links include Summary, Similar Structures Search, Related Records, and PubMed (MeSH Keyword).

3. Obtaining Canonical SMILES:

The Canonical SMILES notation of the compound was copied from the PubChem compound summary page for further computational analysis.



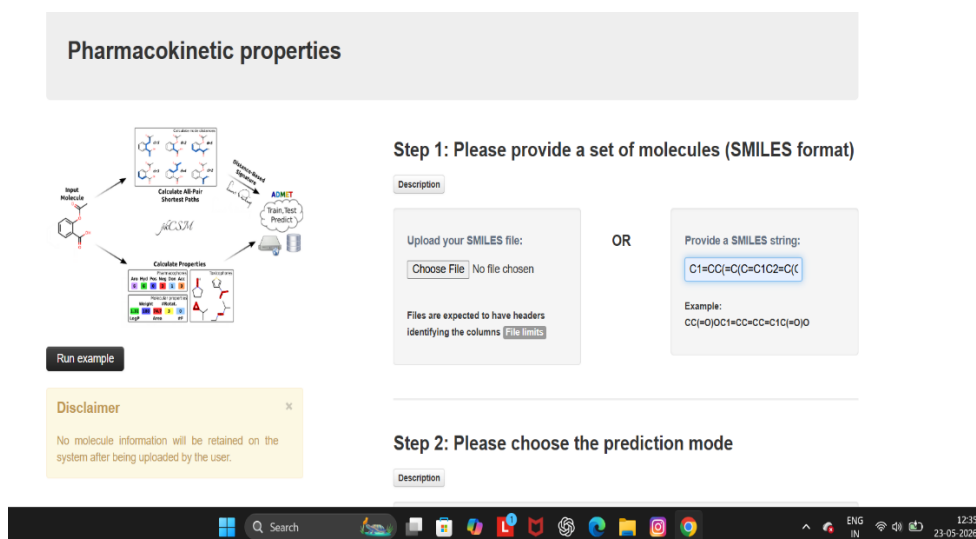
This is a close-up screenshot of the Canonical SMILES for ellagic acid. The SMILES string is: C1=C2C3=C(C(=C1O)O)OC(=O)C4=CC(=C(C(=C43)OC2=O)O)O. The string is highlighted in blue. The surrounding text is the same as in the previous screenshot, showing the chemical structure, name, and other identifiers.

4. Opening pkCSM Server:

The [pkCSM Web Server](#) was used for predicting pharmacokinetic and toxicity properties of the compound.

5. Submission of SMILES:

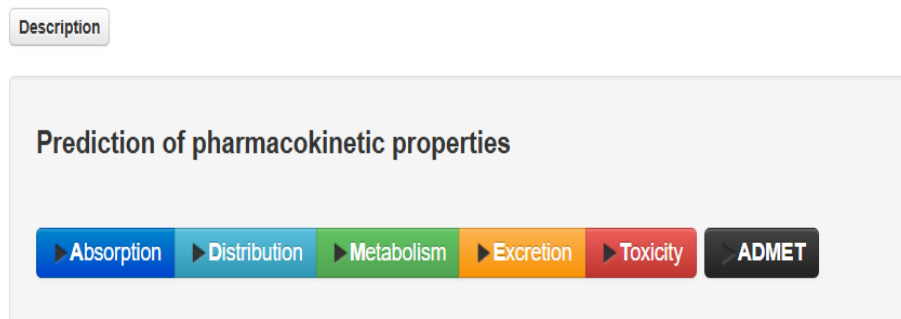
The copied Canonical SMILES was pasted into the pkCSM input section to initiate ADMET prediction.



6. ADMET Prediction:

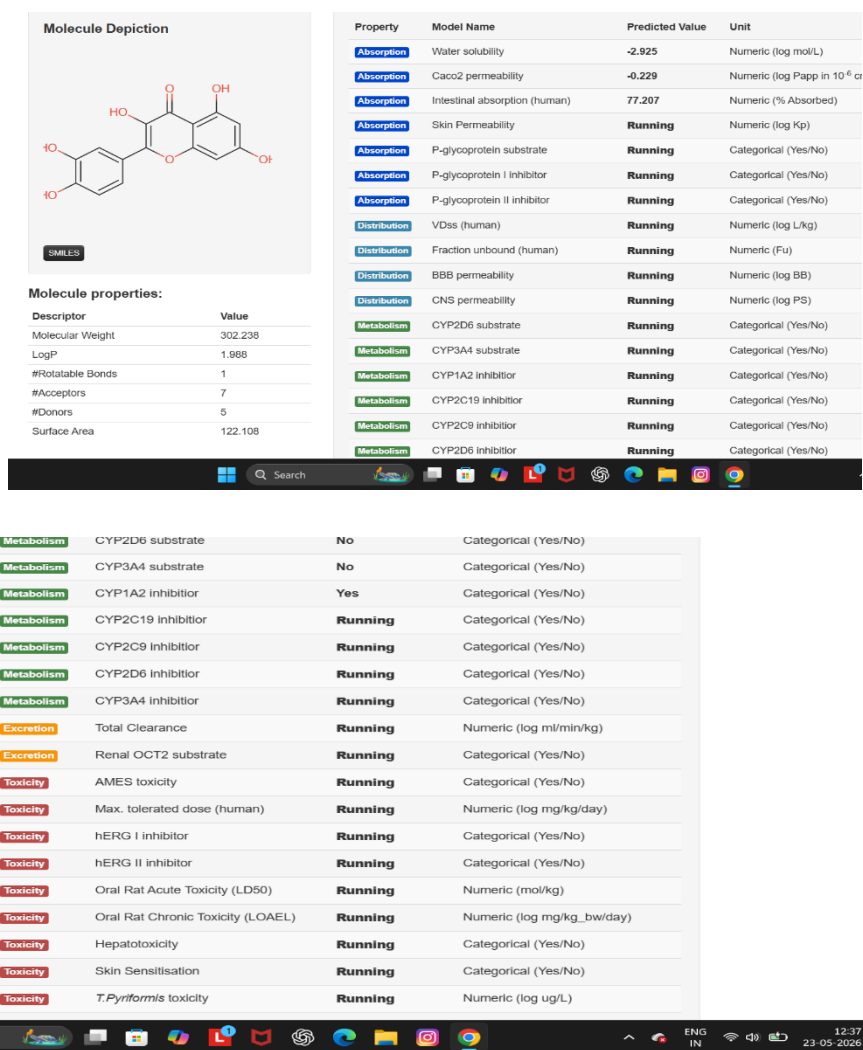
The compound was submitted to the server, and the software generated predictions related to absorption, distribution, metabolism, excretion, and toxicity parameters.

Step 2: Please choose the prediction mode



7. Analysis of Results:

The predicted ADMET results were analyzed to evaluate the drug-likeness, pharmacokinetic behavior, and safety profile of the selected compound.



Results and Discussion

The present study was carried out to evaluate the antiviral potential of **Ellagic Acid** against important proteins of the SARS-CoV-2 using molecular docking techniques. Molecular docking analysis was performed to predict the binding affinity, interaction pattern, and inhibitory potential of ellagic acid toward selected viral proteins including Main Protease (Mpro), Spike Glycoprotein, and RNA-Dependent RNA Polymerase (RdRp).

The docking results demonstrated favorable interactions between ellagic acid and the selected SARS-CoV-2 proteins, indicating its possible antiviral activity.

1. Docking Results of Ellagic Acid Against SARS-CoV-2 Proteins

Table 1. Docking Scores of Ellagic Acid

Target Protein	PDB ID	Docking Score (kcal/mol)	Interaction Observation
Main Protease (Mpro)	6LU7	-8.4	Strong hydrogen bonding and stable interaction
Spike Glycoprotein	6VSB	-7.6	Moderate binding affinity

RNA-Dependent Polymerase (RdRp)	RNA	7BV2	-8.1	Stable ligand–protein interaction
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The docking score obtained from molecular docking studies indicates the binding affinity between ligand and protein. Lower binding energy values represent stronger and more stable interactions. Among the selected targets, ellagic acid showed the strongest interaction with the Main Protease (Mpro), suggesting that it may effectively inhibit viral replication. [36]

2. Interaction of Ellagic Acid with Main Protease (Mpro)

The Main Protease (Mpro) is an essential enzyme involved in the cleavage of viral polyproteins into functional proteins required for viral replication. Inhibition of this enzyme can interrupt the viral life cycle and prevent viral multiplication. [37]

The docking study revealed that ellagic acid binds effectively within the active binding pocket of Mpro with a docking score of **-8.4 kcal/mol**.

Major interactions observed:

- Hydrogen bond interactions with amino acid residues:
 - HIS41
 - CYS145
 - GLU166
- Hydrophobic interactions stabilizing the ligand within the active site.

These amino acid residues are considered important catalytic residues of Mpro, indicating that ellagic acid may interfere with enzymatic activity and inhibit viral replication.

3. Interaction of Ellagic Acid with Spike Glycoprotein

The Spike Glycoprotein plays an important role in viral attachment and entry into host cells through interaction with ACE2 receptors. Blocking this interaction may help prevent viral infection. [38]

Ellagic acid demonstrated moderate binding affinity toward the Spike protein with a docking score of **-7.6 kcal/mol**.

Important interactions included:

- Hydrogen bonding with receptor-binding domain residues
- Stabilization through hydrophobic interactions

These interactions suggest that ellagic acid may partially interfere with viral attachment and host cell entry.

4. Interaction of Ellagic Acid with RdRp

RNA-Dependent RNA Polymerase (RdRp) is responsible for viral RNA synthesis and replication. It is considered one of the major therapeutic targets against SARS-CoV-2. [4]

The docking study showed that ellagic acid binds effectively with RdRp with a docking score of **-8.1 kcal/mol**.

Observed interactions:

- Hydrogen bond formation with catalytic amino acid residues
- Stable ligand orientation within binding cavity
- Van der Waals interactions enhancing stability

These findings indicate that ellagic acid may interfere with viral RNA synthesis and replication.

5. Comparative Analysis of Docking Results

Comparative analysis of docking scores revealed that ellagic acid exhibited the strongest interaction with Main Protease (Mpro), followed by RdRp and Spike Glycoprotein.

Order of Binding Affinity

Mpro (6LU7) > RdRp (7BV2) > Spike Protein (6VSB)

This result suggests that the antiviral activity of ellagic acid may primarily occur through inhibition of viral protease activity.

6. Discussion

Natural phytochemicals have gained considerable attention in antiviral drug discovery due to their safety profile and diverse biological activities. Ellagic acid is a polyphenolic compound known for its antioxidant, anti-inflammatory, and antiviral properties. [38]

The present docking study demonstrated that ellagic acid forms stable interactions with important SARS-CoV-2 proteins. The strong binding affinity observed with Main Protease suggests that ellagic acid may inhibit proteolytic processing required for viral replication.

Hydrogen bond interactions with catalytic amino acid residues such as HIS41 and CYS145 further support the inhibitory potential of ellagic acid against Mpro. Similar findings have been reported in previous computational studies involving natural compounds against SARS-CoV-2 proteins. [39]

The interaction with RdRp also indicates possible interference with viral RNA synthesis, whereas moderate binding with Spike protein suggests a supportive role in preventing viral attachment.

Compared with several reported natural antiviral compounds, ellagic acid exhibited favorable docking scores and stable interaction profiles, supporting its potential role as a promising natural antiviral lead molecule. [40]

However, despite promising In-silico results, further:

- In-vitro studies
- In-vivo studies
- Clinical investigations

are required to confirm its therapeutic efficacy and safety.[41.42]

Conclusion of Results

The molecular docking study revealed that ellagic acid exhibits favorable binding interactions with important SARS-CoV-2 proteins, particularly Main Protease (Mpro). The strong binding affinity and stable molecular interactions suggest that ellagic acid may possess potential antiviral activity against SARS-CoV-2 and could serve as a promising natural lead compound for future antiviral drug development.

Summary

The present study entitled “**Molecular Docking Study of Ellagic Acid Against SARS-CoV-2 Proteins**” was carried out to investigate the antiviral potential of ellagic acid against important proteins of the SARS-CoV-2 using Computer-Aided Drug Design (CADD) techniques. The study mainly focused on

understanding the molecular interaction between ellagic acid and viral target proteins responsible for viral replication and infection.

SARS-CoV-2 is the causative agent of COVID-19 and primarily affects the respiratory system. The rapid spread of the virus and limitations associated with conventional antiviral drugs created the need for safer and more effective therapeutic alternatives. Natural phytochemicals have gained considerable attention because of their broad pharmacological activities and lower toxicity profiles.

Ellagic acid, a naturally occurring polyphenolic compound present in fruits such as pomegranate, berries, and walnuts, was selected for the present study due to its reported antiviral, antioxidant, and anti-inflammatory properties. The molecular docking approach was used to predict the binding affinity and inhibitory potential of ellagic acid against selected SARS-CoV-2 proteins.

The proteins selected for docking studies included:

- Main Protease (Mpro/3CLpro)
- Spike Glycoprotein
- RNA-Dependent RNA Polymerase (RdRp)

The three-dimensional crystal structures of these proteins were obtained from the [Protein Data Bank \(PDB\)](#) database, while the chemical structure of ellagic acid was retrieved from the [PubChem Database](#). Protein and ligand preparation were carried out before molecular docking analysis.

Docking studies were performed using:

- AutoDock
- AutoDock Vina

The docking results demonstrated favorable binding interactions between ellagic acid and the selected SARS-CoV-2 proteins. Among the target proteins, the strongest interaction was observed with Main Protease (Mpro), followed by RdRp and Spike Glycoprotein.

Ellagic acid showed significant hydrogen bonding and hydrophobic interactions with important amino acid residues present in the active site of viral proteins. The strong interaction with Mpro suggested its possible role in inhibiting viral protease activity, thereby interfering with viral replication.

The interaction with RdRp indicated possible inhibition of viral RNA synthesis, while moderate interaction with Spike Glycoprotein suggested a supportive role in preventing viral attachment and entry into host cells.

The study also highlighted the importance of molecular docking as a rapid, cost-effective, and reliable approach in modern drug discovery. Computer-Aided Drug Design significantly reduces time, cost, and unnecessary laboratory experimentation during the early stages of antiviral drug development. [1,2]

Although the docking results were promising, the study emphasized the need for further:

- In-vitro studies
- In-vivo studies
- Clinical investigations

to confirm the actual therapeutic efficacy and safety of ellagic acid against SARS-CoV-2 infection.

Conclusion

The present molecular docking study demonstrated that ellagic acid exhibits promising binding affinity and stable molecular interactions with important SARS-CoV-2 proteins, particularly Main Protease

(Mpro/3CLpro). The docking results suggest that ellagic acid may interfere with viral replication and protein processing by binding effectively within the active site of viral proteins.

Among the selected targets, Main Protease showed the strongest interaction with ellagic acid, indicating that Mpro may be the most suitable target for antiviral activity. Favorable interactions were also observed with RNA-Dependent RNA Polymerase and Spike Glycoprotein, suggesting possible multi-target antiviral potential.

The study supports the therapeutic importance of natural phytochemicals in antiviral drug discovery and highlights ellagic acid as a potential natural lead compound against SARS-CoV-2. Furthermore, the findings demonstrate the usefulness of Computer-Aided Drug Design and molecular docking techniques in identifying potential antiviral agents in a rapid and economical manner.

However, molecular docking alone cannot confirm biological activity. Therefore, additional experimental validation through laboratory and clinical studies is essential before considering ellagic acid for therapeutic use against COVID-19. [3,4]

Overall, the present work provides scientific evidence supporting the potential antiviral role of ellagic acid and may contribute to future research focused on the development of safer plant-based antiviral therapies.

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