IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

In Silico Evaluation Of *Piper Longum* Bioactives Targeting SARS-Cov-2 ORF8: A Phylogeny-Guided Docking Approach

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ABSTRACT

The ongoing COVID-19 pandemic, caused by SARS-CoV-2, necessitates the identification of effective antiviral agents targeting key viral proteins. This study employed a computational approach to identify potential inhibitors from phytochemicals derived from *Piper longum* against the SARS-CoV-2 ORF8 protein, implicated in immune evasion. Phylogenetic analysis using MEGA11 was conducted on major spike protein variants to select an evolutionarily conserved viral protein, leading to the choice of ORF8 (PDB ID: 8CSA) for molecular docking. Fifty bioactive compounds from *Piper longum* were retrieved from PubChem and prepared for docking through format conversion. Drug-likeness was evaluated using Lipinski's Rule of Five via the SCFBio Drug Design Server, and toxicity profiles were predicted with pkCSM to ensure favorable pharmacokinetic properties. Molecular docking simulations were performed in ArgusLab, focusing on binding affinities and interaction profiles with the ORF8 protein. The methodology integrates phylogenetics, drug-likeness screening, and docking to systematically identify promising phytochemicals as potential antiviral agents against SARS-CoV-2. Preliminary results revealed that several plant-based compounds exhibited strong binding affinities towards critical residues of the ORF8 active site. This *in silico* investigation supports the rationale for utilizing natural compounds in drug discovery pipelines and highlights the ORF8 protein as a novel antiviral target in combating COVID-19. Future studies will involve pharmacokinetic profiling and molecular dynamics simulations to validate the stability and drug-likeness of the top-ranking ligands.

Keywords: *SARS-CoV-2, ORF8 protein, molecular docking, natural compounds, antiviral phytochemicals, drug discovery, in silico analysis.*

INTRODUCTION

In the wake of the COVID-19 pandemic, caused by the novel coronavirus SARS-CoV-2, humanity faces an unprecedented global health crisis marked by widespread suffering and significant mortality. Despite tireless global efforts, the quest for effective therapeutic strategies remains ongoing, underscoring the urgent need for novel approaches. Central to the pathogenesis of COVID-19 is the severe damage inflicted on lung tissues by the virus, yet the underlying mechanisms driving this pathology remain only partially understood.

Among the various viral components, the ORF8 protein—represented structurally by PDB ID: 8CSA—emerges as an enigmatic accessory protein whose cellular functions are still being unravelled. Together with other accessory proteins such as ORF3b, ORF6, and ORF7a, ORF8 has been implicated in modulating the host immune response, particularly by antagonizing the interferon-I (IFN-I) pathway. However, the precise roles of these proteins in immune evasion and viral pathogenesis remain poorly characterized, highlighting the need for further exploration of their molecular interactions and therapeutic potential.

Amidst the ongoing search for effective interventions, a beacon of hope lies in the ancient wisdom of Ayurveda—the world's oldest holistic medical system. Rooted in scientific principles and centuries of empirical knowledge, Ayurveda offers a rich repository of natural remedies that have historically addressed a wide array of ailments. As modern medicine grapples with rising healthcare costs and accessibility issues, revisiting Ayurveda offers a promising and sustainable approach to therapeutic discovery.

One such Ayurvedic remedy, *Piper longum* (long pepper), has been traditionally employed to treat respiratory illnesses and enhance immunity. Its relevance to COVID-19 is particularly noteworthy due to its immunomodulatory and anti-inflammatory properties. In recent years, scientific interest in *Piper longum* has surged, with researchers isolating and characterizing bioactive phytochemicals that may contribute to its therapeutic effects.

This study aims to investigate the potential of long pepper phytoconstituents as inhibitors of the SARS-CoV-2 ORF8 protein using in silico molecular docking approaches. By bridging traditional knowledge with modern computational biology, we explore the therapeutic promise of *Piper longum* as a natural, accessible, and cost-effective antiviral agent. Furthermore, this integrative approach not only contributes to the development of plant-based drug discovery pipelines but also supports the sustainable use of native botanical resources in the formulation of modern pharmaceuticals.

In essence, our work seeks to harmonize time-honoured traditions with cutting-edge science, opening new avenues for inclusive and resilient healthcare in the face of present and future pandemics.

MATERIALS AND METHODS

Protein Selection Using Phylogenetic Analysis for Docking

SARS-CoV-2 has been identified as the causative agent of COVID-19, making its viral proteins important therapeutic targets. To identify a relevant and evolutionarily conserved protein for docking studies, phylogenetic analysis was conducted using the **MEGA11 software**. Five major mutated spike protein strains of SARS-CoV-2 were analyzed to determine their evolutionary relationships.

From the phylogenetic tree generated, one major ancestral strain was selected for further study based on its evolutionary significance and structural availability. The **ORF8 protein** (PDB ID: **8CSA**), known for its role in immune evasion, was chosen for molecular docking.

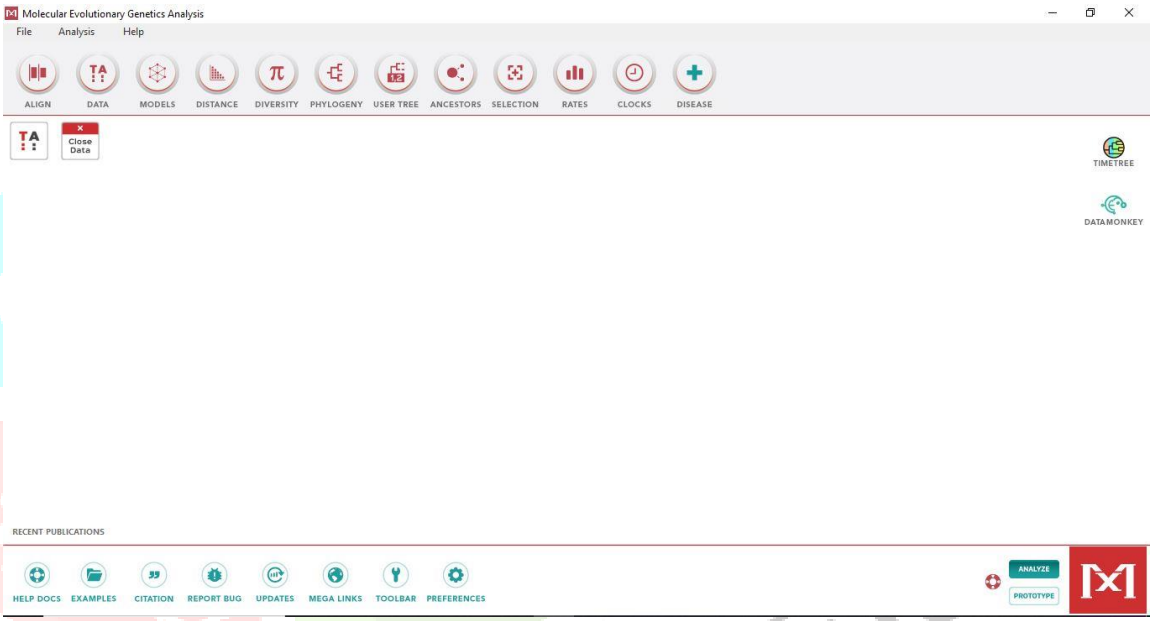


Figure: *Phylogenetic tree generated using MEGA11 software*

Protein Structure Retrieval

The **3D structure** of the SARS-CoV-2 ORF8 protein (PDB ID: 8CSA) was retrieved from the **Protein Data Bank (PDB)** [https://www.rcsb.org/]. This structure was experimentally determined and includes detailed resolution data essential for accurate docking.

Figure: *Three-dimensional structure of SARS-CoV-2 ORF8 (8CSA)*

Preparation of Ligand Molecules

Fifty bioactive phytochemicals from *Piper longum* (long pepper) with reported antiviral activity were identified through literature mining. The **2D structures** of these compounds were downloaded in **.SDF format** from the **PubChem** database [https://www.ncbi.nlm.nih.gov/pccompound].

Using **PyMOL**, the downloaded files were converted to **.MOL2** and **.PDB** formats for compatibility with docking software.

Prediction of Drug-Likeness and Toxicity

The drug-likeness of the selected compounds was evaluated using the **SCFBio Drug Design Server** [http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp], which applies **Lipinski's Rule of Five**:

- **Molecular weight (MW) ≤ 500 Dalton**
- **LogP ≤ 5**
- **Hydrogen bond acceptors (HBA) ≤ 10**
- **Hydrogen bond donors (HBD) ≤ 5**

Compounds violating more than one of these parameters are considered to have poor oral bioavailability.

Toxicity profiles of the compounds were predicted using **pkCSM** [https://biosig.lab.uq.edu.au/pkcsm/prediction], which utilizes graph-based signatures to estimate pharmacokinetic properties, including absorption, distribution, metabolism, excretion, and toxicity (ADMET).

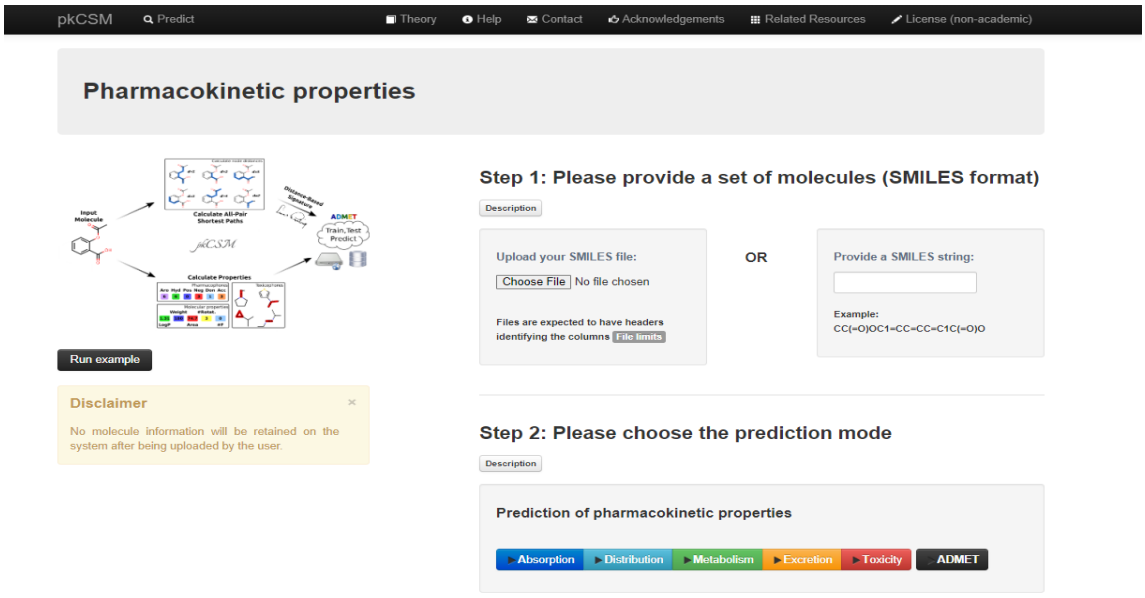


Figure: *SCFBio Drug-Likeness Server Interface*

Figure: *pkCSM Pharmacokinetic Prediction Server Interface*

Molecular Docking Using ArgusLab

Molecular docking was performed using **Arguslab**, a free molecular modeling software compatible with Windows. After preparing both the target protein (8CSA) and the ligands, docking simulations were conducted to assess binding affinity and interactions.

- The protein was loaded into Arguslab and the active site was defined using known binding residues.
- Ligands were then imported, and docking calculations were carried out using a **shape-based search algorithm** and the **AScore scoring function**.
- The **AScore** reflects the binding energy between the ligand and protein, where lower scores indicate stronger interactions.
- Compounds were ranked based on docking scores, and the best binding interactions were selected by evaluating hydrogen bonds and proximity to the substrate-binding site.

RESULTS AND DISCUSSIONS

Identifying compound from Phylogenetic analysis

Phylogenetic analysis, a fundamental aspect of evolutionary biology, seeks to understand the evolutionary relationships among organisms by studying their genetic material. MEGA (Molecular Evolutionary Genetics Analysis) is a powerful software package widely used for phylogenetic analysis. MEGA 11, the latest version at the time of my last update, offers an array of tools and features for conducting robust phylogenetic studies**.**

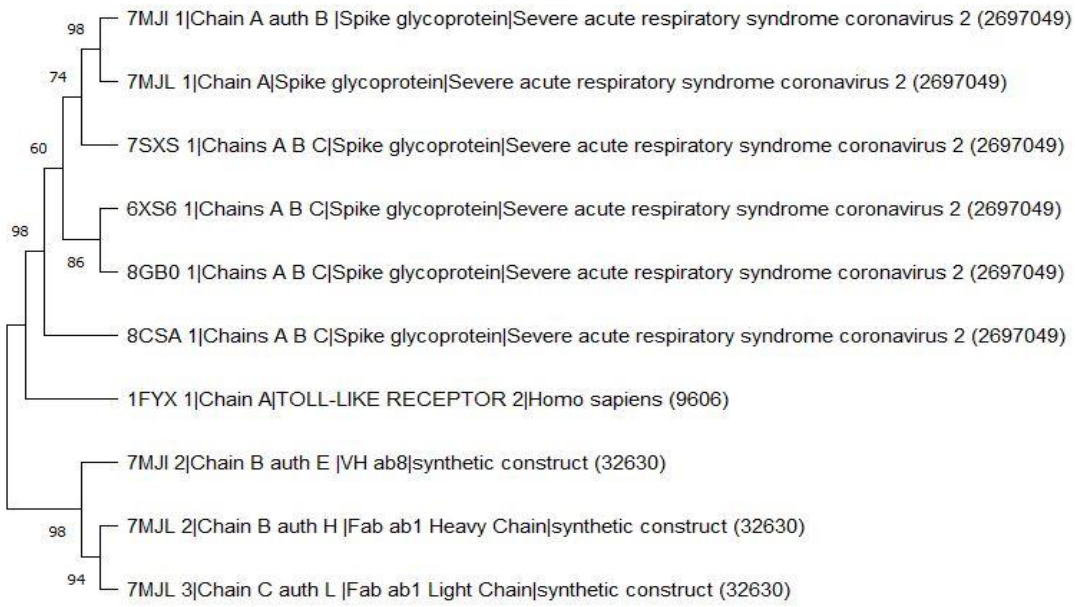


Figure: Using MEGA 11 Phylogenetic Software Minimum Neighbourhood Method and Bootstrap Format (8CSA) Protein identified, it is having Minimum Neighbourhood Sequence Similarity.

Through MEGA 11 These major Variants below Analysed by the method phylogenetic analysis constructed maximum Likely hood in Bootstrap Method with 50 Replication 1 thread

Alpha variant (B.1.1.7) N501Y(7MJL),P681H(1FYX),Del69-70()

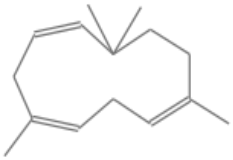
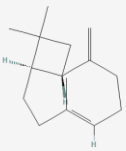
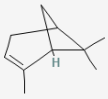
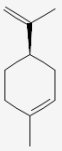
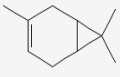
Beta variant (B.1.351)E484K(8CSA)

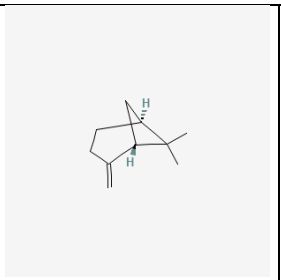
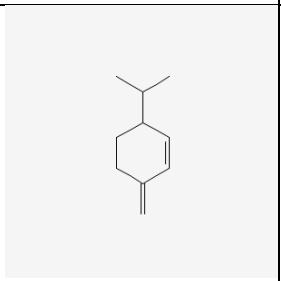
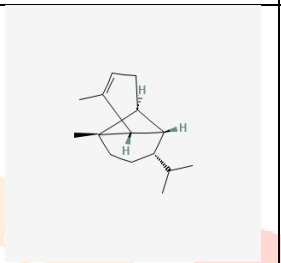
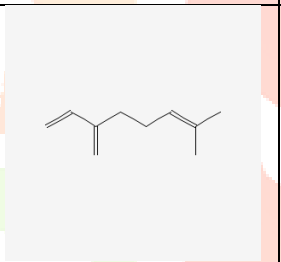
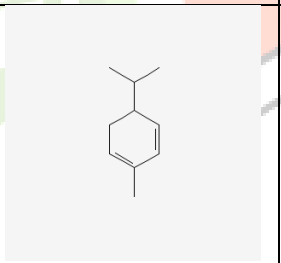
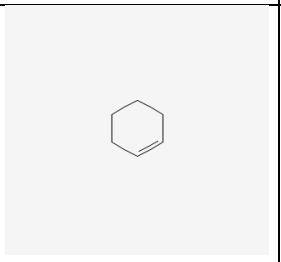
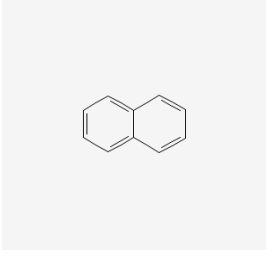
Gamma variant (P.1)

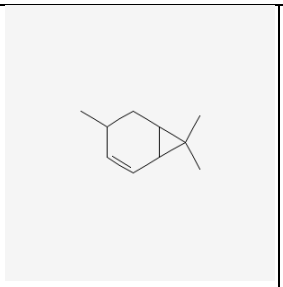
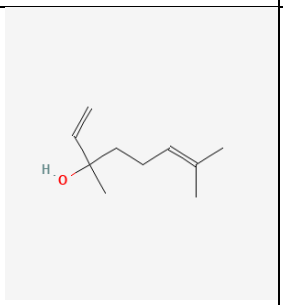
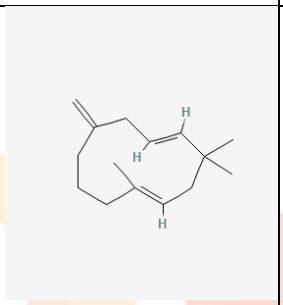
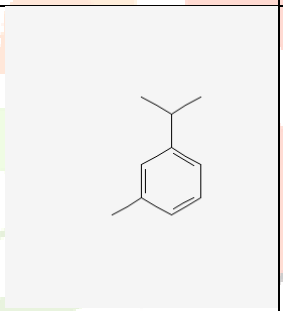
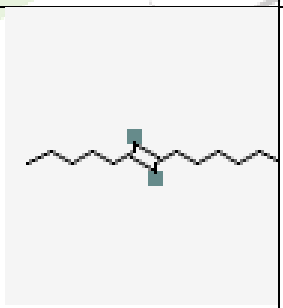
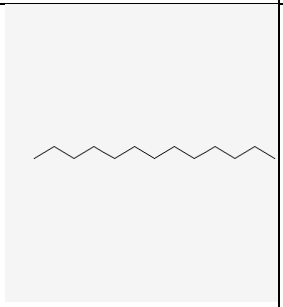
Delta variant (B.1.617.2)L452R(7SXS)

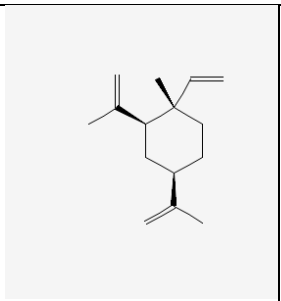
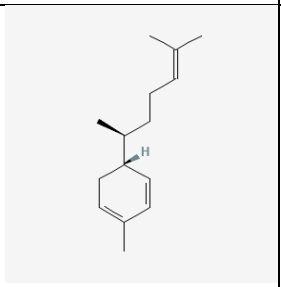
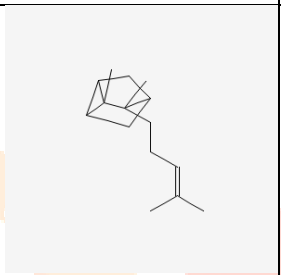
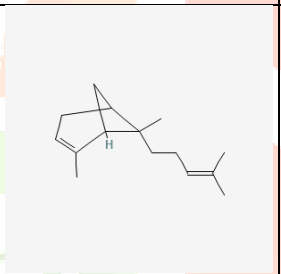
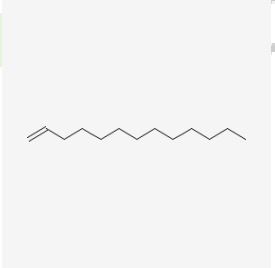
Omicronvariant(B.1.1.529)P681H(1FYX)H655Y(8GB0)D614G(6XS6)N501Y(7MJI)

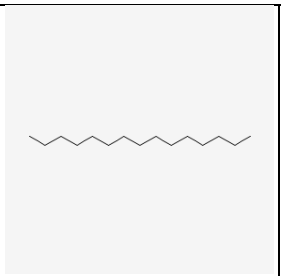
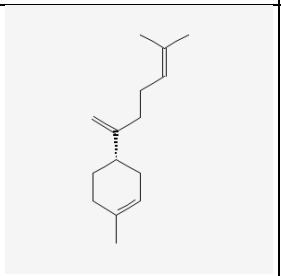
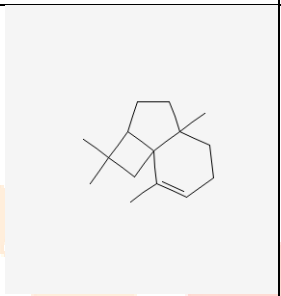
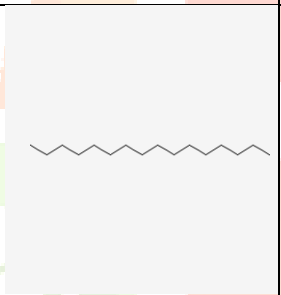
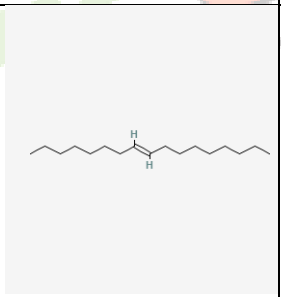
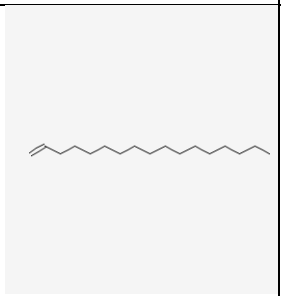
Table: 50 Compound 2D Structure Retrieved from PUBCHEM[08][14]

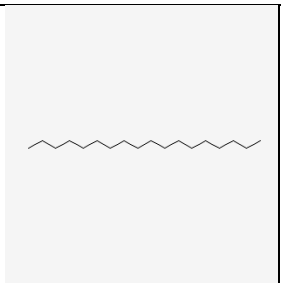
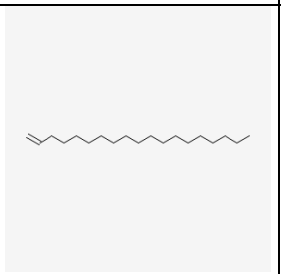
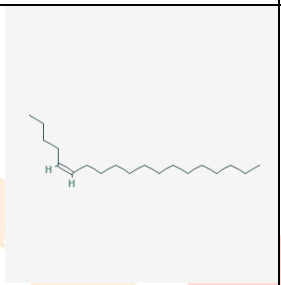
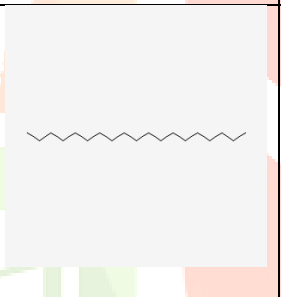
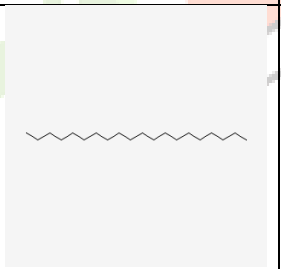
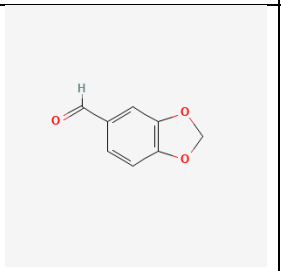
S.NO	COMPOUND NAME	PUBCHEM ID	2D STRUCTURE	CHEMICAL FORMULA	MW
1.	1,4,7-Cycloundecatrien,1,5,9,9-tetramethyl-Z,Z,Z-100			$C_{15}H_{24}$	204.3511 g/mol
2.	Caryophyllene	5281515		$C_{15}H_{24}$	204.35 g/mol
3.	Alpha-Pinene	6654		$C_{10}H_{16}$	136.26 g/mol
4.	D-Limonene	440917		$C_{10}H_{16}$	136.23 g/mol
5.	3-Carene	26049		$C_{10}H_{16}$	136.23 g/mol

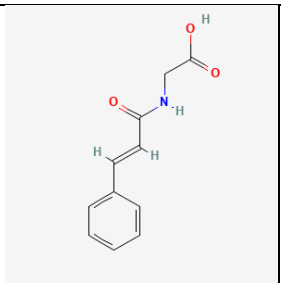
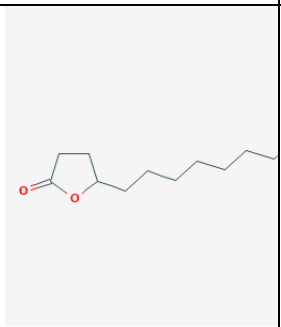
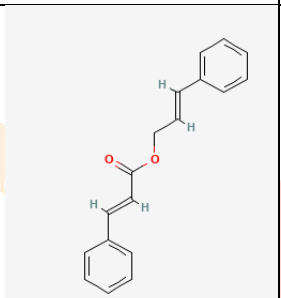
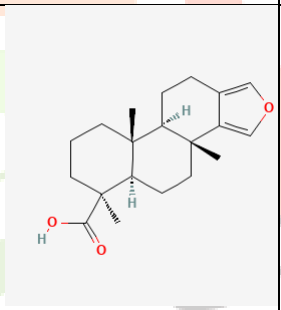
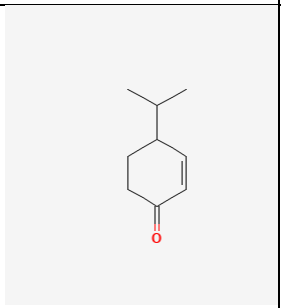
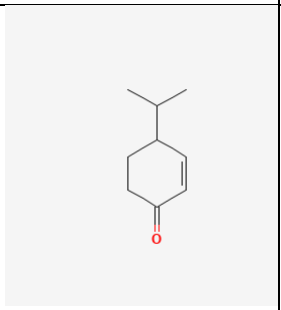
6.	(-)-beta-Pinene	440967		$C_{10}H_{16}$	136.23 g/mol
7.	Beta-phellandrene	11142		$C_{10}H_{16}$	136.23 g/mol
8.	Copaene	12303902		$C_{15}H_{24}$	204.35 g/mol
9.	Beta-Myrcene	31253		$C_{10}H_{16}$	136.23 g/mol
10.	alpha-phellandrene	7460		$C_{10}H_{16}$	136.23 g/mol
11.	Cyclohexene	8079		C_6H_{10}	82.14 g/mol
12.	Napthalene	931		$C_{10}H_8$	128.17 g/mol

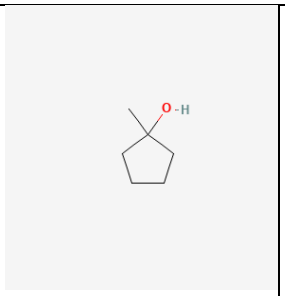
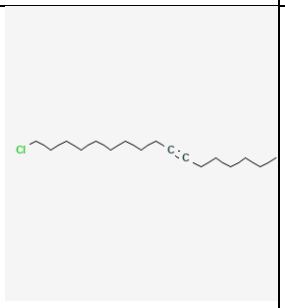
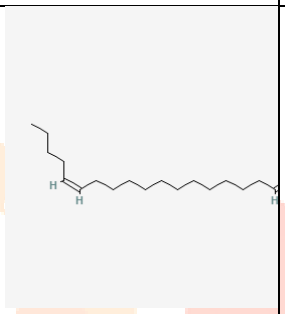
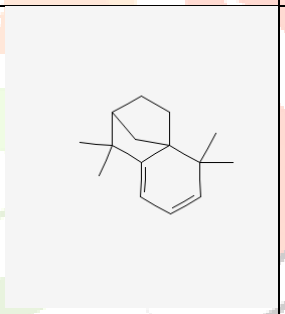
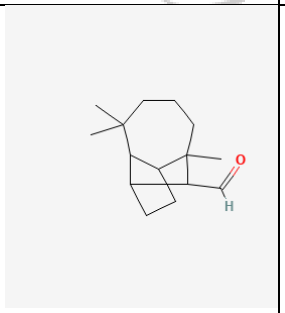
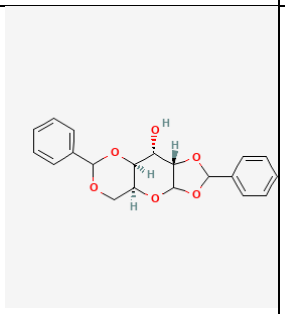
13.	(+)-4-Carene	530422		$C_{10}H_{16}$	136.23 g/mol
14.	Linalol	6529		$C_{10}H_{18}O$	154.25 g/mol
15.	Beta-Humulene	5318102		$C_{15}H_{24}$	204.35 g/mol
16.	Beta-Cymene	10812		$C_{10}H_{14}$	134.22 g/mol
17.	6-Tridecene	5364429		$C_{13}H_{26}$	182.35 g/mol
18.	Tridecane	12388		$C_{13}H_{26}$	184.36 g/mol

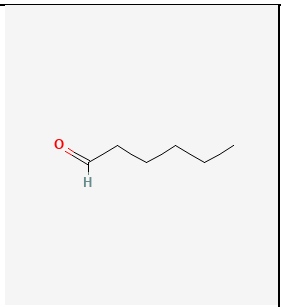
19.	β-Elemene	6918391		$C_{15}H_{24}$	204.35 g/mol
20.	Zingiberene	92776		$C_{15}H_{24}$	204.35 g/mol
21.	α-Santalene	94164		$C_{15}H_{24}$	204.35 g/mol
22.	α-Bergamotene	86608		$C_{15}H_{24}$	204.35 g/mol
23.	1-Tridecene	17095		$C_{13}H_{26}$	182.35 g/mol
24.	1 -Tetradecanol	8209		$C_{14}H_{30}O$	214.39 g/mol

25.	Pentadecane	12391		$C_{15}H_{32}$	212.41 g/mol
26.	β-Bisabolene	10104370		$C_{15}H_{24}$	204.35 g/mol
27.	(—)-α-Panasinsen	578929		$C_{15}H_{24}$	204.35 g/mol
28.	Hexadecane	11006		$C_{16}H_{34}$	226.44 g/mol
29.	8-Heptadecene	5364555		$C_{17}H_{34}$	238.5 g/mol
30.	1-Heptadecene	23217		$C_{17}H_{34}$	238.5 g/mol

31.	Octadecane	11635		$C_{18}H_{38}$	254.5 g/mol
32.	1-Nonadecene	29075		$C_{19}H_{38}$	266.5 g/mol
33.	Z-5-Nonadecene	5364560		$C_{19}H_{38}$	266.5 g/mol
34.	Nonadecane	12401		$C_{19}H_{40}$	268.5 g/mol
35.	Eicosane	8222		$C_{20}H_{42}$	282.5 g/mol
36.	Docosane	12405		$C_{22}H_{46}$	310.6 g/mol
37.	Piperonal	8438		$C_8H_6O_3$	150.13 g/mol

38.	Cinnamoylglycine	709625		$C_{11}H_{11}NO_3$	205.21 g/mol
39.	Cinnamicacid	16821		$C_{12}H_{22}O_2$	198.30 g/mol
40.	Cinnamylcinnamate	1550890		$C_{18}H_{16}O_2$	264.3 g/mol
41.	Spongia-13(16),14-dien-19-oicacid	21582644		$C_{20}H_{28}O_3$	316.4 g/mol
42.	Caryophylleneoxide	92780		$C_9H_{14}O$	138.21 g/mol
43.	2—Cyclohexen-1-one,4-(1-methylethyl)	92780		$C_9H_{14}O$	138.21 g/mol

44.	Cyclopentanol,1-methyl	73830		$C_6H_{12}O$	100.16 g/mol
45.	7—Heptadecyne,17-chloro	557030		$C_{17}H_{31}Cl$	270.9 g/mol
46.	13—Octadecenal	5364497		$C_{18}H_{34}O$	266.5 g/mol
47.	Isolongifolene,9,10—dehydro	583109		$C_{15}H_{22}$	202.33 g/mol
48.	Longifolenaldehyde	565584		$C_{15}H_{24}O$	220.35 g/mol
49.	Dibenzylidene-d-glucose	91703799		$C_{20}H_{20}O_6$	356.4 g/mol

50.	Hexanal	6184		$C_6H_{12}O$	100.16 g/mol

Predicting Gc-Ms Compounds for Molecular Docking Druglikeness

Out of 50 compounds, only 38 compounds passed the drug analysis test, which was further forwarded to the Docking studies. (Table) Refers Chemical Compounds PUBCHEM database ID, 2D Structure of the Molecule, Chemical Formula and the Chemical compound is Druglike or Non Druglike.[08][14][01][02]

Predicting Chemical Compound Druglikeness Properties by Using SCF-BIO

SCF-BIO online Web Bioinformatics web Server Predict the chemical compounds Druglikenees Properties based on LIPINSKI'S Rule of 5.

Table: Predicted Druglike Properties by Lipinski's Rule of Five

S.NO	PUBCHEM ID	COMPOUND	MASS	Log P	HBD	HBA	MOLAR REF	Drug /Non Drug
1.	5281515	Caryophyllene	204	3.961349	0	0	75.112991	Druglike
2.	6654	Alpha-Pinene	136	2.694150	0	0	45.989990	Druglike
3.	440917	D-Limonene	136	2.532510	0	0	49.191994	Druglike
4.	26049	3-Carene	136	2.654899	0	0	50.001991	Druglike
5.	440967	(-)-beta-Pinene	136	2.708449	0	0	45.965992	Druglike
6.	11142	Beta-phellandrene	136	2.543010	0	0	49.136993	Druglike
7.	12303902	Copaene	204	3.842369	0	0	73.674988	Druglike
8.	31253	Beta-Myrcene	136	2.664829	0	0	47.625992	Druglike
9.	7460	alpha-phellandrene	136	2.675900	0	0	49.891991	Druglike
10.	8079	Cyclohexene	81	1.633740	0	0	30.467995	Druglike
11.	931	Napthalene	128	1.948940	0	0	38.241997	Druglike
12.	530422	(+)-4-Carene	136	2.665399	0	0	49.946991	Druglike
13.	6529	Linalol	368		0	4		Non Druglike
14.	5318102	Beta-Humulene	204	4.020359	0	0	74.341988	Druglike
15.	10812	Beta-Cymene	134	2.499410	0	0	46.951992	Druglike
16.	5364429	6-Tridecene	182	4.023769	0	0	70.850990	Druglike
17.	12388	Tridecane	184	4.227769	0	0	73.184990	Druglike

18.	6918391	β-Elemene	204	3.849459	0	0	74.247986	Druglike
19.	92776	Zingiberene	204	4.003349	0	0	74.892990	Druglike
20.	94164	α-Santalene	204	3.999359	0	0	74.451988	Druglike
21.	86608	α-Bergamotene	204	4.021599	0	0	70.990990	Druglike
22.	17095	1-Tridecene	182	4.124589	0	0	69.449989	Druglike
23.	8209	1 -Tetradecanol	214	4.234259	1	1	79.530785	Druglike
24.	12391	Pentadecane	212	4.840350	0	0	4.840350	Druglike
25.	10104370	β-Bisabolene	204	3.859959	0	0	74.192986	Druglike
26.	578929	Caryophyllene	204	3.961349	0	0	75.112991	Druglike
27.	11006	(—)-α-Panasinsen	226	5.146641	0	0	89.585983	Non Druglike
28.	5364555	Hexadecane	238	5.248931	0	0	92.718987	Non Druglike
29.	23217	8-Heptadecene	238	5.349751	0	0	91.317986	Non Druglike
30.	11635	1-Heptadecene	254	5.759222	0	0	100.519981	Non Druglike
31.	29075	Octadecane	266	5.962332	0	0	102.251984	Non Druglike
32.	5364560	1-Nonadecene	266	5.861512	0	0	103.652985	Non Druglike
33.	12401	Z-5-Nonadecene	268	6.065513	0	0	105.986984	Non Druglike
34.	8222	Nonadecane	282	6.371803	0	0	111.453979	Non Druglike
35.	12405	Eicosane	310	6.984384	0	0	122.387970	Non Druglike
36.	8438	Docosane	150	1.137340	0	3	33.531998	Druglike
37.	709625	Piperonal	205	1.057440	2	3	48.384495	Druglike
38.	16821	Cinnamoylglycine	198	3.223379	0	2	64.697990	Druglike
39.	1550890	Cinnamicacid	264	3.222689	0	2	72.135994	Druglike
40.	21582644	Cinnamylcinnamate	316	4.297409	1	3	97.476784	Druglike
41.	92780	Spongia-13(16),14-dien-19-oicacid	138	2.229810	0	1	45.221493	Druglike
42.	92780	Caryophylleneoxide	138	2.229810	0	1	45.221493	Druglike
43.	73830	2—Cyclohexen-1-one,4-(1-methylethyl)	100	1.558940	1	1	33.570793	Druglike
44.	557030	Cyclopentanol,1-methyl	270	5.427851	0	0	93.670982	Druglike
45.	5364497	7—Heptadecyne,17-chloro	266	5.299831	0	1	96.385986	Druglike
46.	583109	13—Octadecenal	202	3.795359	0	0	72.117989	Druglike
47.	565584	Isolongifolene,9,10—	220	3.811049	0	1	75.964493	Druglike

		dehydro						
48.	91703799	Longifolenaldehyde	356	3.325558	1	6	86.840294	Druglike
49.	6184	Dibenzylidene-d-glucose	100	1.828350	0	1	33.115993	Druglike
50.	92812	Hexanal	222	3.886549	1	1	78.215790	Druglike

Toxicity Prediction by Using PKCSM

Out of 50 compounds 1 were found to be Toxic.

Table: Toxicity predicted by Using PkCSM: predicting small-molecule pharmacokinetic properties using graph-based signatures

S.NO	COMPOUND	SMILES	AMES TOXICITY
1.	1,4,7-Cycloundecatrien,1,5,9,9-tetramethyl-Z,Z,Z-100		NO
2.	Caryophyllene	CC1=CCCC(=C)C2CC(C2CC1)(C)C	NO
3.	Alpha-Pinene	CC1=CCC2CC1C2(C)C	NO
4.	D-Limonene	CC1=CCC(CC1)C(=C)C	NO
5.	3-Carene	CC1=CCC2C(C1)C2(C)C	NO
6.	(-)-beta-Pinene	CC1(C2CCC(=C)C1C2)C	NO
7.	Beta-phellandrene	CC(C)C1CCC(=C)C=C1	NO
8.	Copaene	CC1=CCC2C3C1C2(CCC3C(C)C)C	NO
9.	Beta-Myrcene	CC(=CCCC(=C)C=C)C	NO
10.	alpha-phellandrene	CC1=CCC(C=C1)C(C)C	NO
11.	Cyclohexene	C1CCC=CC1	NO
12.	Napthalene	C1=CC=C2C=CC=CC2=C1	NO
13.	(+)-4-Carene	CC1CC2C(C2(C)C)C=C1	NO
14.	Linalol	CC(=CCCC(C)(C=C)O)C	NO
15.	Beta-Humulene	CC1=CCC(C=CCC(=C)CCC1)(C)C	NO
16.	Beta-Cymene	CC1=CC(=CC=C1)C(C)C	NO
17.	6-Tridecene	CCCCCCC=CCCCCC	NO
18.	Tridecane	CCCCCCCCCCCCC	NO
19.	β-Elemene	CC(=C)C1CCC(C(C1)C(=C)C)(C)C=C	NO
20.	Zingiberene	CC1=CCC(C=C1)C(C)CCC=C(C)C	NO
21.	α-Santalene	CC(=CCCC1(C2CC3C1(C3C2)C)C)C	NO
22.	α-Bergamotene	CC1=CCC2CC1C2(C)CCC=C(C)C	NO
23.	1-Tridecene	CCCCCCCCCCCC=C	NO
24.	1 -Tetradecanol	CCCCCCCCCCCCCCO	NO
25.	Pentadecane	CCCCCCCCCCCCCCC	NO
26.	β-Bisabolene	CC1=CCC(CC1)C(=C)CCC=C(C)C	NO

27.	(—)-α-Panasinsen	CC1=CCCC2(C13CC(C3CC2)(C)C)C	NO
28.	Hexadecane	CCCCCCCCCCCCCCCC	NO
29.	8-Heptadecene	CCCCCCCCC=CCCCCCCC	NO
30.	1-Heptadecene	CCCCCCCCCCCCCCCC=C	NO
31.	Octadecane	CCCCCCCCCCCCCCCCCC	NO
32.	1-Nonadecene	CCCCCCCCCCCCCCCCCC=C	NO
33.	Z-5-Nonadecene	CCCCCCCCCCCCC/C=C\CCCC	NO
34.	Nonadecane	CCCCCCCCCCCCCCCCCCC	NO
35.	Eicosane	CCCCCCCCCCCCCCCCCCCC	NO
36.	Docosane	CCCCCCCCCCCCCCCCCCCCCC	NO
37.	Piperonal	C1OC2=C(O1)C=C(C=C2)C=O	NO
38.	Cinnamoylglycine	C1=CC=C(C=C1)C=CC(=O)NCC(=O)O	NO
39.	Cinnamicacid	C1=CC=C(C=C1)C=CC(=O)O	NO
40.	Cinnamylcinnamate	C1=CC=C(C=C1)C=CCOC(=O)C=CC2=CC=CC=C2	NO
41.	Spongia-13(16),14-dien-19-oicacid	CC12CCCC(C1CCC3(C2CCC4=COC=C43)C)(C)C(=O)O	NO
42.	Caryophylleneoxide	CC(C)C1CCC(=O)C=C1	NO
43.	2—Cyclohexen-1-one,4-(1-methylethyl)	CC(C)C1CCC(=O)C=C1	NO
44.	Cyclopentanol,1-methyl	CC1(CCCC1)O	NO
45.	7—Heptadecyne,17-chloro	CCCCCCC#CCCCCCCCCCCl	NO
46.	13—Octadecenal	CCCCC=CCCCCCCCCCCCC=O	NO
47.	Isolongifolene,9,10—dehydro	CC1(C=CC=C2C13CCC(C3)C2(C)C)C	NO
48.	Dibenzylidene-d-glucose	C1C2C(C(C3C(O2)OC(O3)C4=CC=CC=C4)O)OC(O1)C5=CC=CC=C5	YES
49.	Ledol	CC1CCC2C1C3C(C3(C)C)CCC2(C)O	NO
50.	Hexanal	CCCCCC=O	NO

Molecular Docking Using Arguslab Software

In this Argus lab docking analysis seven Compounds were Docked based on Argus Rigid Docking methods and six Compounds were Docked based on GA Rigid Docking Method. The following compound shows favourable binding energies towards the 8CSA protein. These docking results suggest that the mentioned compound has strong binding affinity for the virulent **8CSA** protein. by the Argus lab Results these Compounds are 15 Chosen out of 50 Compounds

Table: Argus lab best Binding Energy pose in of 8CSA

S.NO	Chemical Compounds	Pubchem id	Arugus lab Binding Energy (Kcal/mol)
1.	Napthalene	931	-7.28021 Kcal/mol
2.	1 -Tetradecanol	8209	-6.95909 Kcal/mol
3.	Cyclohexene	8079	-6.15486 Kcal/mol
4.	Piperonal	8438	-5.71003 Kcal/mol
5.	Beta-Cymene	10812	-7.10527 Kcal/mol
6.	Tridecane	12388	-6.85174 Kcal/mol
7.	Pentadecane	12391	-6.99635 Kcal/mol
8.	Cinnamicacid	16821	-7.55758 Kcal/mol
9.	1-Tridecene	17095	-7.9212 Kcal/mol
10.	7—Heptadecyne,17-chloro	557030	-8.89049 Kcal/mol
11.	Cinnamoylglycine	709625	-6.67232 Kcal/mol
12.	Cinnamylcinnamate	1550890	-12.001 Kcal/mol
13.	6-Tridecene	5364429	-7.07359 Kcal/mol
14.	13—Octadecenal	5364497	-6.66464 Kcal/mol
15.	β-Bisabolene	10104370	-6.57673 Kcal/mol

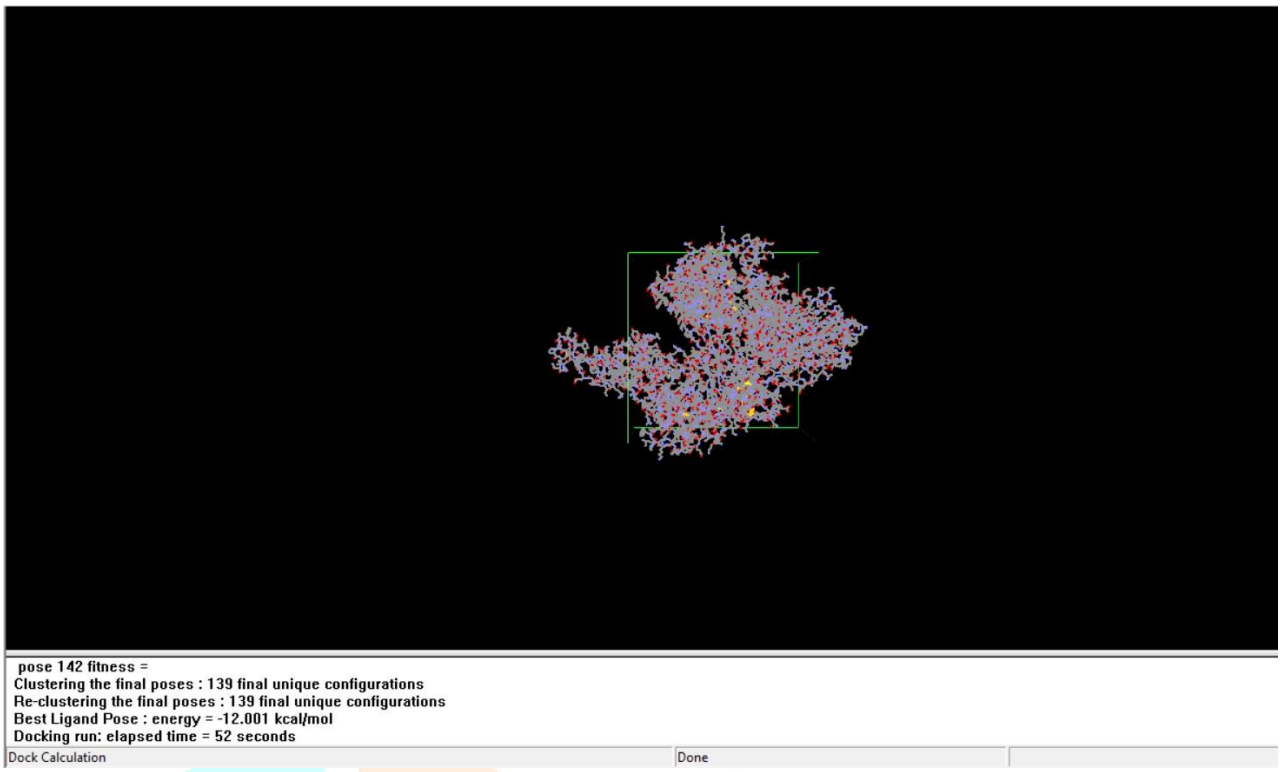


Figure: Cinnamyl cinnamate A Chain **Argus lab Binding Energy -12.001 Kcal/mol**

As a result of the above docking study, we identified fours compounds **Cinnamylcinnamate PubChem id 1550890 in A Chain (**-12.001 **Kcal/mol),** possesses favourable binding energies. In this study of bioactive compounds from Long Pepper, The Compounds were investigated for the ability to treat the viral protein. The results obtained from the present study support the traditional use of betel leaves as an antiviral herb against SARS COV-2 virus. However, to extrapolate the results of present study to clinical trial and drug development, further invitro and *invivo* experimental studies are warranted.

CONCLUSION

Using computational approaches, four phytocompounds from *Piper longum* were identified with favorable binding affinities toward the SARS-CoV-2 viral protein (PDB ID: 8CSA). Additionally, 50 phytoconstituents from *Piper longum* leaves were screened based on Lipinski's Rule of Five, out of which 38 compounds met the criteria for oral drug-likeness. These 38 compounds were further evaluated using web-based tools for toxicity and pharmacokinetic properties. Molecular docking was performed using Argus lab software, and the most promising compounds were re-docked for validation. The findings support the potential of *Piper longum* as a source of antiviral agents against SARS-CoV-2.

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