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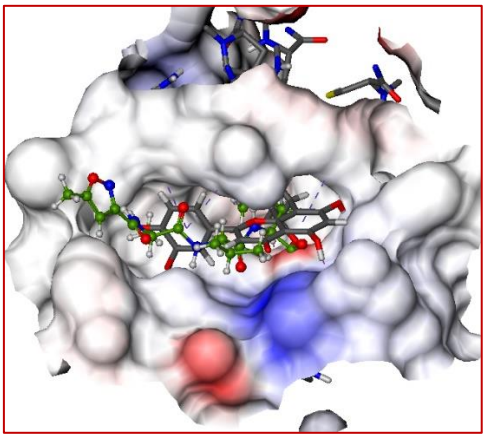

A Project Report

Submitted by

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1. Background

SARS-CoV-2 (COVID-19) is a recently identified novel strain of the SAR-CoV virus that has spread rapidly across the globe. On January 31st 2020, the World Health Organisation (WHO) declared that SARS-CoV-2 was a public emergency of international concern. Currently, there are no U.S. Food and Drug Administration approved drugs for the treatment of SARS-CoV-2. Consequently, significant efforts are being directed towards delineating pathogenesis of SARS-CoV-2 to identify potential drug targets that may contribute to the development of effective prevention and treatment strategies. Recently, several papers have reported the structure and function of proteins critical to the life cycle of SARS-CoV-2 and have the potential to be drug targets, including the spike protein, main protease (M^{pro}), papain-like proteases (PLpro), chymotrypsin-like protease (3CLpro), RNA-dependent RNA polymerase (RdRp), angiotensin-converting enzyme (ACE2) and transmembrane protease, serine 2 (TMPRSS2)(1, 2). Furthermore, the availability of protein structural information has facilitated several drug discovery efforts, with the majority focusing on repurposing or repositioning existing drug molecules as potential compounds for treating SARS-CoV-2 infection(2). This approach offers significant advantages over traditional drug development pipelines and has resulted in the discovery of promising candidates such as remdesivir, which is currently being evaluated in clinical trials.

With a plethora of medicinal plants and formulations containing these medicinal plants, traditional Indian systems of medicines like Siddha & Ayurveda have been used for the treatment of several ailments including viral and bacterial diseases(3-5). Furthermore, medicinal plant-based phytochemicals offer attractive, effective, and holistic drug action against the pathogens without much of the side effects. Plant based medicines contain a complex mixture of phytoconstituents hence unlike allopathic drugs they cannot be repurposed in emergency scenarios such as COVID-19 without experimental validation. Furthermore, the complex mixture of phytochemicals present in plant extract makes experimental studies arduous and laborious. However, if the structural knowledge of

phytoconstituents in a plant is known, this structural data can be used for *in silico* screening of these phytoconstituents against targets implicated in SARS-COV-2 infection. Hence, computational studies can be used for prioritizing the screening of selected plants for experimental studies for evaluating its efficacy against the virus.

With this understanding, the objective of the current study is to perform *in silico* screening of phytoconstituents present in medicinal plants of herbal formulation "Clevira" against some selected targets involved in the SARS-CoV-2 infectivity and replication to identify its anti-COVID-19 potential. Four targets namely the viral spike protein (S1), RNA dependent RNA polymerase (RdRp), viral proteases cysteine like protease or 3CL protease (3CLpro) and papain like protease (PLpro) that play a pivotal role in the viral infectivity and replication were selected for the *in-silico* screening on the basis of their druggability and availability of experimentally solved crystal structures for structure-based drug design.

2. Materials and Methods

2.1 Ligand preparation

The structures of 52 major phytoconstituents (table 1) in the medicinal plants incorporated in 'Clevira formulation' were downloaded from Pubchem in 3D SDF format and subsequently geometry optimized employing MMF94 forcefield in Avogadro software(6). The energy minimized structures were then saved mol-2 format and used for docking studies

Table 1: Phytoconstituents in Clevira formulation aongwith their botanical sources

Herb	Constituents	Class	PubChem CID
Andrographis paniculata	Andrographolide	Diterpene	5318517
	Bis-andrographolide	Terpene	12000062
	Ninandrographolide	Terpene	44575270
	Oroxylin A	Flavone	5320315
	Wogonin	Flavone	5281703
	Caffeic acid	polyphenol	689043
	B-Sitosterol-D-Glucoside	Phytosterol	5742590
Cyperus rotundus	α Cyperone	Sesquiterpene	6452086
	Isocyperol	Sesquiterpene	44576209
	Kobusone	Sesquiterpene	6710676
	Isokobusone	Sesquiterpene	3860435
	Cyperotundone	Sesquiterpene	12308615
Mollugo cerviana	Orientin	Flavonoid	5281675
	Vitexin	Flavonoid	5280441
Piper nigrum	Piperine	Alkaloid	638024
	B-caryophyllene	Sesquiterpene	5281515

	A-pinene	Terpene	6654
	Moupinamide (N-trans-feruloyl tyramine)	Phenol	5280537
Trichosanthes cucumerina	Bryonolic acid	Triterpenoid	472768
	Cucurbitacin B	Triterpinoid	5281316
	Cucurbitacin E	Triterpinoid	5281319
	Isocucurbitacin B	Triterpinoid	5352014
	B-Sitosterol	Phytosterol	222284
	Stigmasterol	Phytosterol	5280794
	23,24-dihydrocucurbitacin D	Triterpenoid	180535
Zingiber officinale	6 Shogaol	Phenol	5281794
	6 Gingerol	Phenol	442793
	Zingiberol	Sesquiterpene alcohol	5317270
	A-Zingiberene	Sesquiterpene	92776
	B-Bisabolene	Sesquiterpene	10104370
	Hexahydrocurcumin	diarylheptenones	5318039
	Paradols	Ketone	94378
	Limonene	Cyclohexene	22311
	Nerolidol	Sesquiterpene	5284507
	Myrcene	Monoterpene	31253
Carica Papaya	Carpaine	Alkaloid	442630
	dehydrocarpaine I	Alkaloid	131750991
	dehydrocarpaine II	Alkaloid	131750992
	p-coumaric acid	Polyphenol	637542
	Chlorogenic acid	Polyphenol	1794427
	Caricaxanthin	Xanthophyll	44554791
	Violaxanthin	Xanthophyll	448438
	Zeaxanthin	Xanthophyll	5280899
	Cardenolide	Glycoside	3035019
Melia azedarach	Meliacarpin	Alkaloid	183572
	Quercetin	Flavonoid	5280343
	Kaempferol	Flavonoid	5280863
	Rutin	Flavonoid	5280805
Vettiveria zizhanoides	Vetiveryl acetate	sesquiterpenoid	8347
	Ethyl 4-(4-methylphenyl)-4-pentenoate		589505
Tinospora cordifolia	Tinosporide	Diterpenoid furanolactone	167631
	Tinosporaside	Diterpenoid furanolactone	14194109

2.2 Retrieval of protein structure and preparation:

Four targets that play a significant role in SARS-CoV-2 infectivity and replication were selected for the docking studies. The X-ray crystal structures of four protein targets

implicated in SARS-CoV-2 infectivity and replication (table 2) were retrieved from protein databank and the 'Receptor preparation wizard' option in LeadIT module (version 2.3.2) was used to prepare the target proteins for docking studies. The binding site for docking studies were defined as amino acid residues within a radius of 8 Å around the complexed ligand for the targets PLpro, Mpro and RdRP whereas the binding site in viral spike protein (S1) was defined by the aminoacid residues in the interface of the viral spike protein (s) and ACE2.

Table 2: Xray crystal structures of viral target proteins used for the molecular docking studies

S. No.	Name of the Target Protein	PDB ID	Resolution	Co-Complexed Ligand	Residues in the Ligand Binding Site
1.	SARS CoV2 Papain-Like Proteases (PLpro)	7JRN	2.48 Å	N-[(4-fluorophenyl)methyl]-1-[(1R)-1-naphthalen-1-ylethyl]piperidine-4-carboxamide	LEU162, GLY163, ASP164, GLU167, PRO247, PRO248, TYR264, TYR268, GLN269, TYR273, THR301.
2.	SARS CoV2 main protease (Mpro)	6W63	1.46 Å	N-(4-tert-butylphenyl)-N-[(1R)-2-(cyclohexylamino)-2-oxo-1-(pyridin-3-yl)ethyl]-1H-imidazole-4-carboxamide	PHE140, LEU141, ASN142, GLY143, HIE163, HIE164, MET165, GLU166, ASP187, ASP188, GLN189, THR26, LEU27, HIS41, MET49
3.	SARS CoV2 RNA-dependent RNA polymerase (RdRP)	7BV2	2.50 Å	Remdesvir	LYS545, VAL557, SER682, THR687, ASP623, ASN691, CYS622, ASP760, ALA688, ARG555, SER759, U10, A11.
4.	SARS CoV2 spike receptor-binding domain (S protein)	6LZG	2.50 Å	ACE2 interface	ARG454, TYR473, LYS458,ARG457, ASP467, ARG454,SER469, PRO491, ILE472, GLU471, LYS458.

2.3 Molecular Docking Studies

Molecular docking studies were performed using FlexX docking algorithm(7) implemented in the LeadIT module (version 2.3.2). The phytochemicals present in the Clevira were docked into the binding site of the four targets using default settings except for options "maximum number of solutions per iteration' and the 'number of solutions per fragmentation' values which were increased to 1000. The top ranking poses for each ligand were saved for further analysis and the protein-ligand interactions in each selected docking pose were visualized using Discovery Studio Visualizer (version 20.1.0.19295)(8).

Results and Discussion

To rationalize the anti-CoVID-19 efficacy elicited by Clevira herbal formulation, we chose a *in silico* strategy for identifying the phytoconstituents that might contribute to antiviral efficacy through modulation of targets significant for viral infectivity and replication. Four targets namely the viral spike protein (S1), RNA dependent RNA polymerase (RdRp), viral proteases cysteine like protease or 3CL protease (3CLpro) and papain like protease (PLpro) that play a pivotal role in the viral infectivity and replication were selected for the *in silico* screening on the basis of their druggability and availability of experimentally solved crystal structures for structure based drug design. We also formulated a phytochemical library composed of 52 major phytoconstituents present in 10 medicinal plants (*Andrographis paniculata, Cyperus rotundus, Mollugo cerviana, Piper nigrum, Trichosanthes cucumerina, Zingiber officinale, Carica Papaya, Melia azedarach, Vettiveria zizhanoides, Tinospora cordifolia*) of Clevira formula to facilitate the *in silico* screening against the aforementioned targets. The three dimensional structures of these phytochemicals were retrieved from Pubchem online database(9).

The formulated phytochemical library was then used for virtual screening studies against the identified targets using FlexX molecular docking program in LeadIT software which uses the robust incremental construction algorithm. The results of the virtual screening studies are discussed below.

SARS CoV2 Papain-Like Proteases (PLpro)

First, we screened the Clevira database against SARS-CoV-2 Papain-Like Proteases (PLpro). PLpro is a viral cysteine protease enzyme which plays an important role in both viral entry

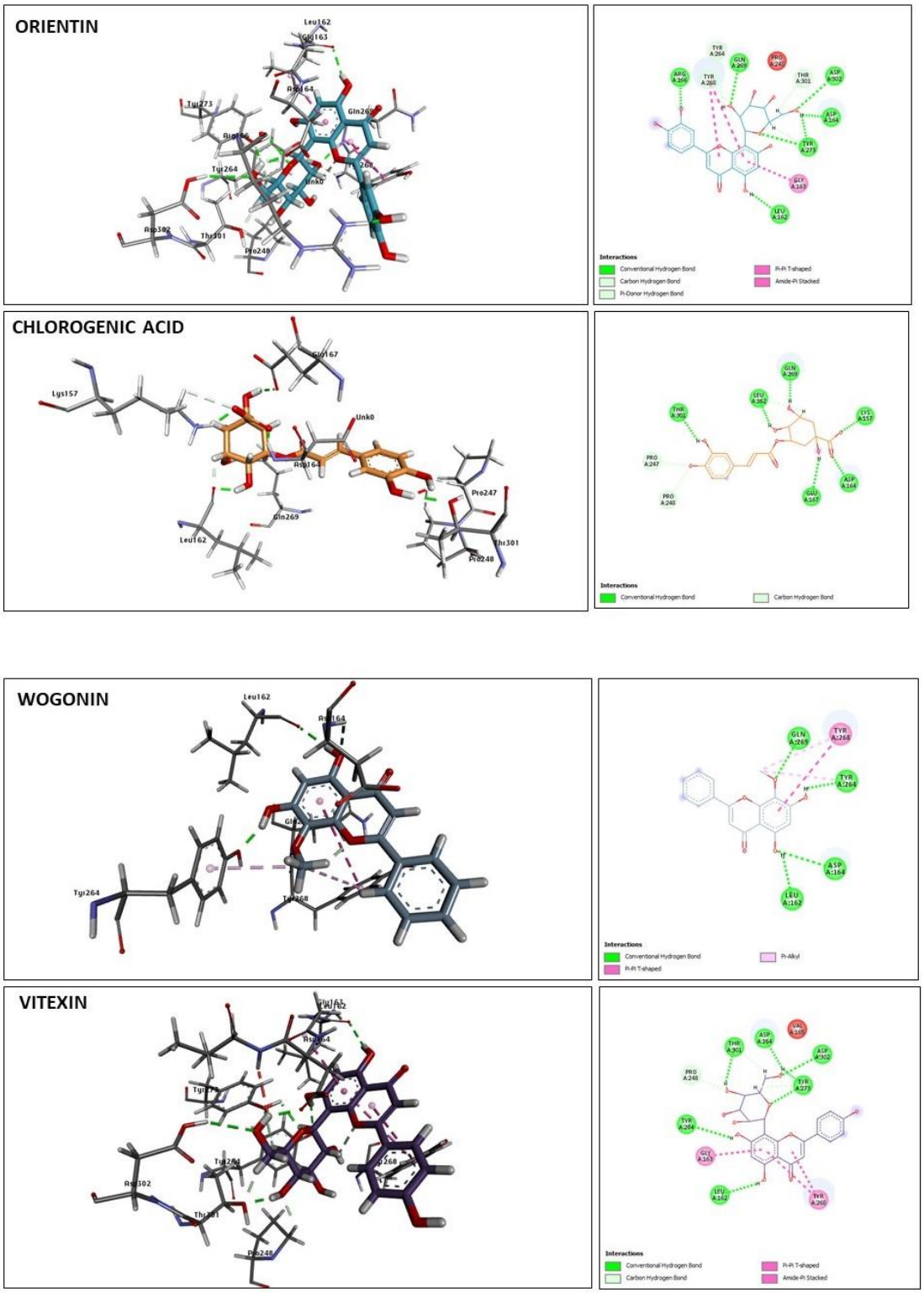
and replication stages of coronavirus life cycle(10). The results of the molecular docking studies of Clevira phytoconstitutents against PLpro is summarized in Table 3.

Table 3: Results of molecular docking studies of 52 Clevira Phytoconstituents against SARS CoV2 Papain-Like Protease.

S. NO	PHYTOCONSTITUENT	FlexX SCORE
1.	Orientin	-26.4
2.	Chlorogenic Acid	-24.0
3.	Wogonin	-22.2
4.	Vitexin	-20.9
5.	Moupinamide	-20.3
6.	Rutin	-19.8
7.	Quercetin	-19.2
8.	Hexahydrocurcumin	-18.6
9.	Kaempferol	-17.5
10.	Shogaol	-15.1

The molecular docking studies predicted Orientin, Chlorogenic acid, Wogonin and Vitexin as top scoring ligands against PLpro with docking scores of −26.4, −24.4, −22.2, and -20.9 respectively. Analysis of the docked conformation of Orientin (figure 1) in the active site of PLpro revealed multiple interactions including conventional hydrogen bonding interactions with LEU162, GLY163, ASP164, ARG166, ASP302, GLN269, TYR273 and non-conventional hydrogen bonding interactions with TYR264, TYR268, TYR273, and THR30. Additionally, Orientin also formed hydrophobic interactions with GLY163, TYR264 and TYR268. The binding conformation of Chlorogenic acid in the active site of PLpro (figure 1) shows six conventional hydrogen bonding interactions with LYS157, LEU162, ASP164, GLU167, GLN269, THR301 and three Carbon-hydrogen bonds with LEU162, PRO247 and PRO248. Wogonin adopted a binding conformation in the PLpro active site (figure 1) which facilitated formation of hydrogen bonds with LEU162, ASP164, TYR264, GLN269 and hydrophobic contacts with TYR264 and TYR268. Vitexin interacted with active site of PLpro (figure1) through a network of hydrogen bonds with LEU162, ASP164, TYR264, TYR273, THR301, ASP302 and Carbon hydrogen bonds with PRO248, TYR273, THR301.

Figure 1: Docking poses (left) of top four ranked ligands in the ligand binding site of SARS CoV2 Papain-Like Protease. (Right) Ligand interaction diagram showing important interactions between the docked ligand and the amino acids in ligand binding site of SARS CoV2 Papain-Like Protease.



SARS CoV2 main protease (Mpro)

The SARS CoV2 main protease (also called as 3CLpro or M^{pro}) is a cysteine protease that regulates the coronavirus replication complex hence is essential for viral replication(11). Molecular docking studies of Clevira database against M^{pro} enzyme revealed that four phytochemicals namely Rutin, Ninandrographolide, Quercetin, and Chlorogenic acid to be most promising M^{pro} inhibitors with docking scores of -30.5, -29.3, -28.0, and -27.0 respectively (Table 4 & figure 2).

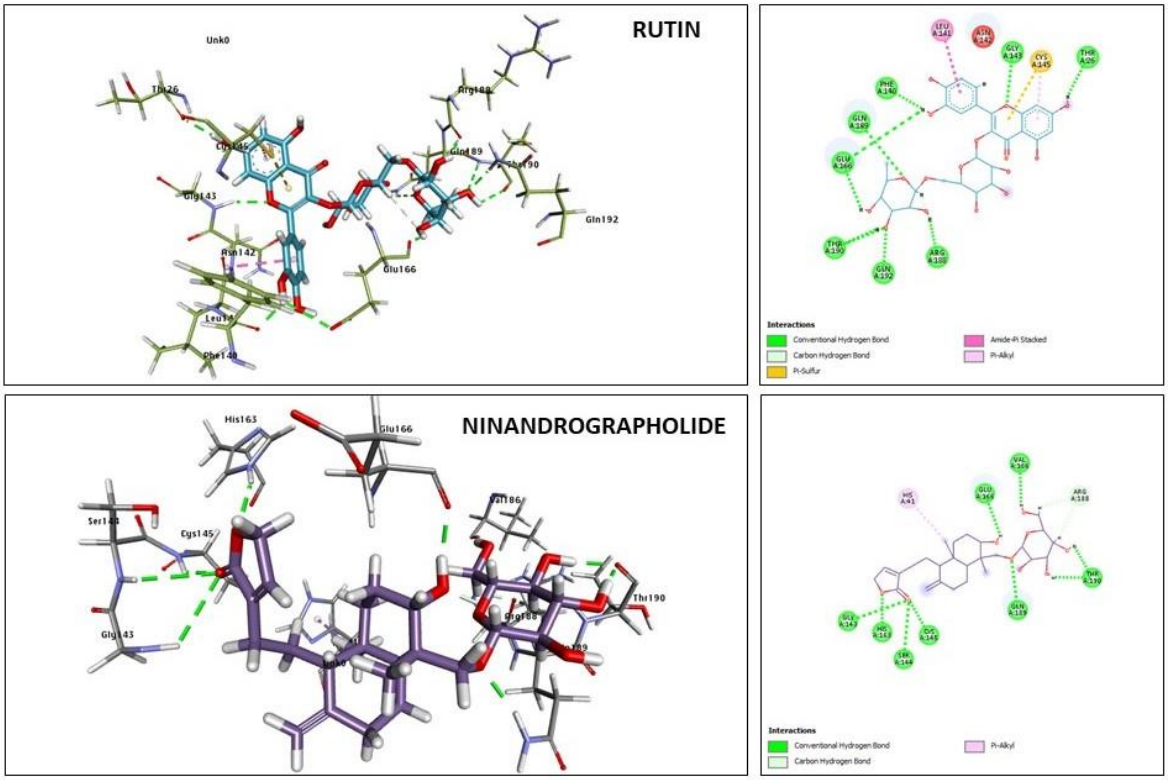
Table 4: Results of molecular docking studies of 52 Clevira Phytoconstituents against SARS CoV2 main protease.

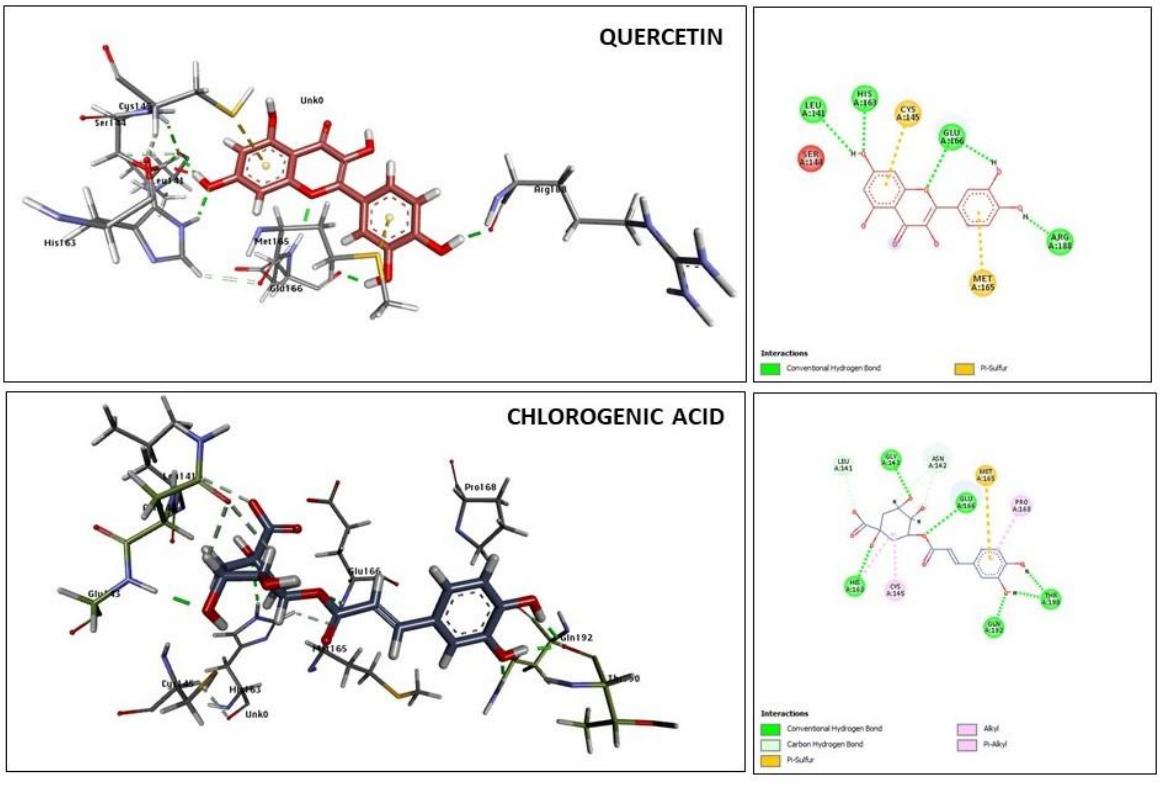
S. NO	PHYTOCONSTITUENT	FlexX SCORE
1.	Rutin	-30.5
2.	Ninandrographolide	-29.3
3.	Quercetin	-28.0
4.	Chlorogenic acid	-27.0
5.	Orientin	-25.5
6.	Kaempferol	-24.6
7.	Hexahydrocurcumin	-24.3
8.	Vitexin	-23.4
9.	Tinosporide	-23.2
10.	Wogonin	-22.7

Figure 2: Docking poses (left) of top four ranked ligands in the ligand binding site of SARS CoV2 main protease. (Right) Ligand interaction diagram showing important interactions between the docked ligand and the amino acids in ligand binding site of SARS CoV2 main protease.

Figure 2 depicts the conformation adopted by the top four scoring ligands in the binding site of 3CLpro. The top ranked molecule Rutin binds with the active site of 3CLpro through hydrogen bonding with amino acid residues THR26, PHE140, GLY143, GLU166, ARG188, GLN189, THR190, GLN192 and hydrophobic interactions with residues LEU141 and CYS145. Ninandrographolide binds with GLY143, SER144, CYS145, HIS163, GLU166, VAL186, GLN and THR190 through hydrogen bonds. Residue ARG188 and HIS41 interacted with Ninandrographolide through nonconventional hydrogen bond and hydrophobic contacts respectively. Orientin binds with the active of 3CLpro through hydrogen bonding interactions with HIS41, MET49, LEU141, GLY143, SER144, CYS145,

GLU166 and hydrophobic interactions with CYS145. The 3CLpro bound conformation of Kaemferol reveal three hydrogen bonding interactions with LUE141, HIS163, GLU166, ARG188 and pi-sulfur interactions with CYS145 and MET165.





RNA-dependent RNA polymerase (RdRp)

RNA-dependent RNA polymerase (RdRp) is the vital component of coronaviral replication and transcription machinery, and it is also the primary target for the antiviral drug remdesivir(12). RdRp have been shown to exist as heterotrimeric complex with nsp7 and nsp8 in its active form (12). The *in-silico* screening of Clevira database against the recently solved X-ray structure of the nsp12 protein resulted in the identification of four phytochemicals, namely Ninandrographolide, Rutin, Quercetin and Tinosporide as potential inhibitors of the enzyme. The docking scores for Ninandrographolide, Rutin, Quercetin and Tinosporide are -30.9, -29.5, -27.1 and 26.9 respectively (Table 4).

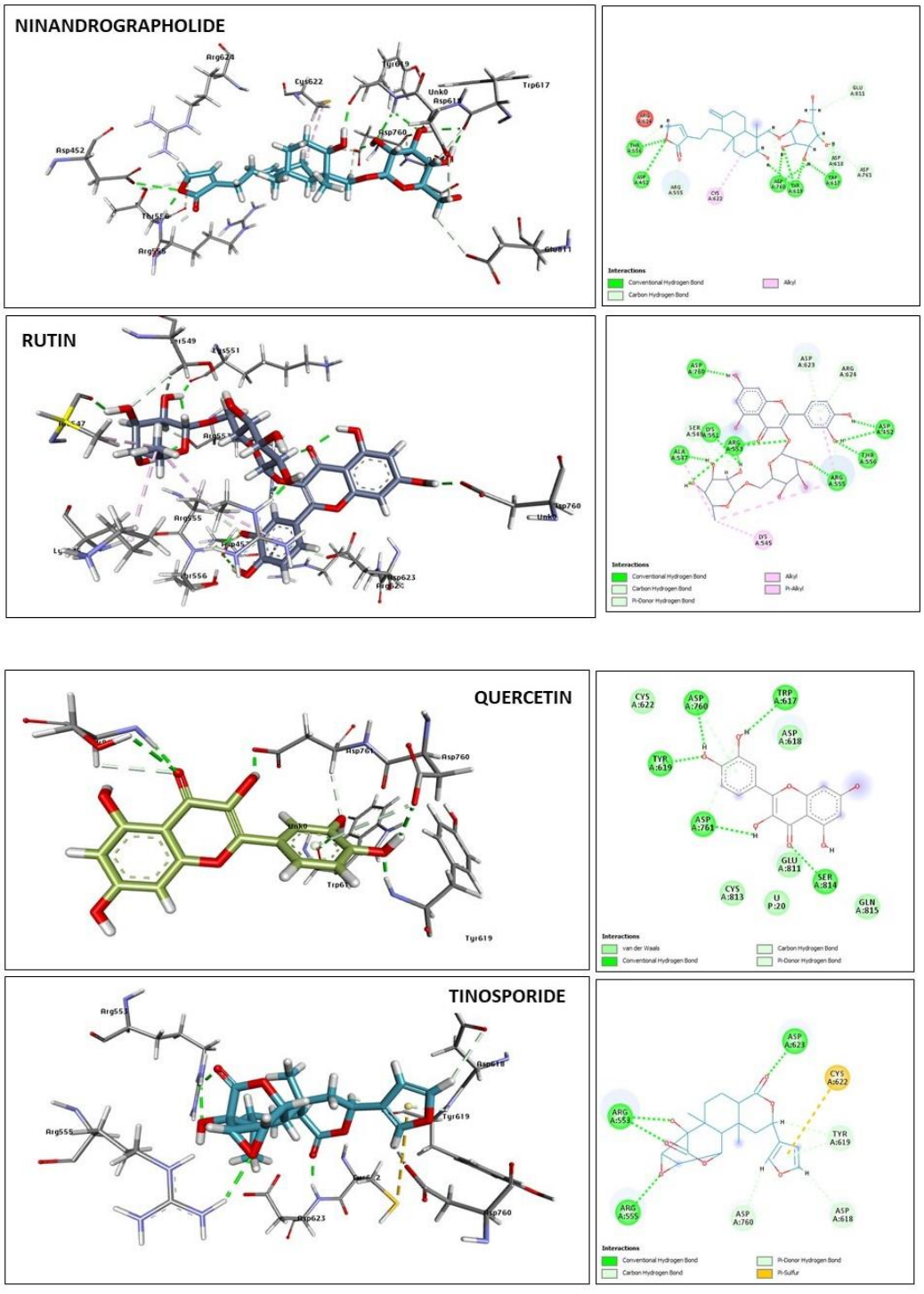
Table 4: Results of molecular docking studies of 52 Clevira Phytoconstituents against SARS CoV2 RNA-dependent RNA polymerase.

S. NO	PHYTOCONSTITUENT	FlexX SCORE
1.	Ninandrographolide	-30.9
2.	Rutin	-29.5
3.	Quercetin	-27.1
4.	Tinosporide	-26.9
5.	Bis-andrographolide	-26.6
6.	Orientin	-25.8
7.	Caffeic acid	-25.8
8.	Dehydrocarpaine II	-25.5
9.	Moupinamide	-25.4
10.	Hexahydrocurcumin	-23.4

Analysis of the docking results (figure 3) provided structural insights into the binding mode of the four topscoring ligands in the ligand binding site of RdRp. As shown in figure 3, the top-ranking ligand Ninandrographolide was found to interact with the amino acid residues within the active site of the RdRp through a network of nine conventional hydrogen bonds with five residues ASP452, THR556, TRP617, TYR619 and ASP760. In addition, the molecule also forms six nonconventional hydrogen bonds with residues ARG555, THR556, ASP618, ASP760, ASP761, GLU811 and one hydrophobic contact with CYS622. The second topscoring ligand Rutin interacts with the active site of RdRp forming nine conventional hydrogen bonds with ASP542, ALA547, LYS551, ARG553, ARG555, THR556, ASP760 and six nonconventional hydrogen bonds with SER549, ARG553, ASP623

and ASP624. The molecule also exhibited nonpolar interactions with amino acid residues LYS545, ALA547 and ARG555.

Figure 3: Docking poses (left) of top four ranked ligands in the Remdesvir binding site of SARS CoV2 RNA-dependent RNA polymerase. (Right) Ligand interaction diagram showing important interactions between the docked ligand and the amino acids in ligand binding site of SARS CoV2 RNA-dependent RNA polymerase.



The flavone derivative quercetin bound with active site of RdRp through conventional hydrogen bonding interactions with TRP617, TYR619, ASP761, ASP761, and SER814. The compound also formed hydrophobic interactions with ASP618, CYS622, GLU811, CYS813, and GLU 815.

Tinosporide binds in the active site of RdRp through four conventional hydrogen bonds with ARG553, ARG555, ASP623 and four nonconventional hydrogens bonding interaction with ASP618, TYR619 and ASP760. The molecule also formed pi-sulfur bond with CYS622.

SARS CoV2 spike receptor-binding domain (S protein)

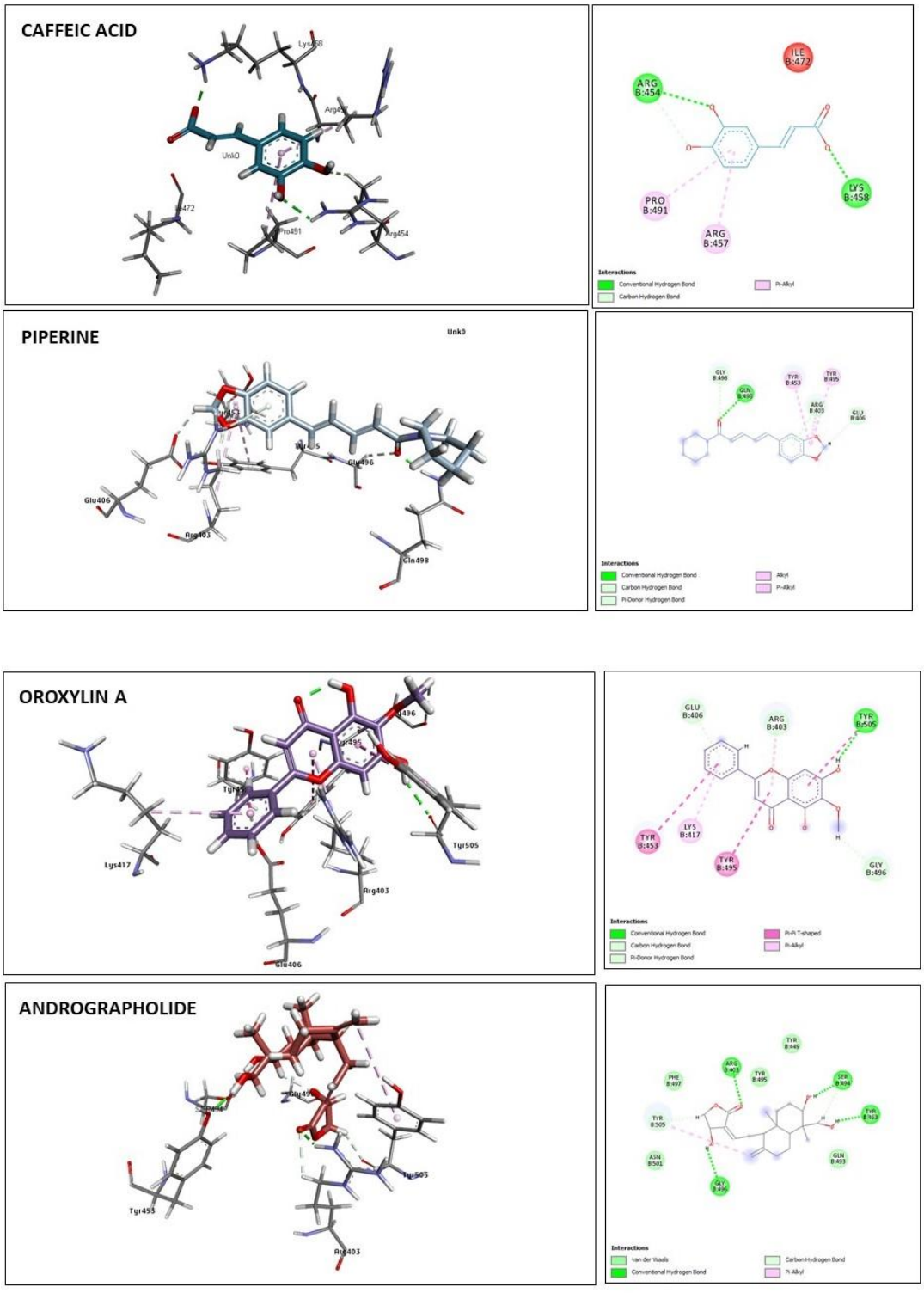
The spike (S) protein of SARS-CoV-2 plays a key role in the receptor recognition and cell membrane fusion process(1). It is composed of two subunits, S1 and S2(1). The S1 subunit contains a receptor-binding domain that recognizes and binds to the host receptor angiotensin-converting enzyme 2, while the S2 subunit mediates viral cell membrane fusion by forming a six-helical bundle via the two-heptad repeat domain(1). The receptor binding domain (RBD) of the spike protein, which is mainly responsible for binding of the virus to the receptor was targeted in the molecular docking studies. The results of the docking studies are summarized in table 5.

Table 4: Results of molecular docking studies of 52 Clevira Phytoconstituents against SARS CoV2 spike receptor-binding domain (S protein).

S. NO	PHYTOCONSTITUENT	FlexX SCORE
1.	Caffeic acid	-18.0
2.	Ninandrographolide	-14.8
3.	Piperine	-14.8
4.	Oroxylin A	-14.7
5.	Andrographolide	-13.8
7.	Wogonin	-13.6
8.	Vitexin	-12.6
9.	Orientin	-11.9
10.	Hexahydrocurcumin	-11.6

Among the 52 phytochemicals docked against ACE2 binding interface of spike protein, four phytochemicals namely Caffeic acid, Piperine, Oroxylin A and Andrographolide were predicted to show best binding affinity as indicated by the docking scores.

Figure 4: Docking poses (left) of top four ranked ligands in the ACE2 binding interface of SARS CoV2 S protein. (Right) Ligand interaction diagram showing important interactions between the docked ligand and the amino acids in ACE2 binding interface of SARS CoV2 S protein.



As shown in Figure 4, the top scoring ligand caffeic acid interacts with Arg454, LYS458 through hydrogen bonding and also shows pi-alkyl interaction with amino acid residues PRO491 and ARG457, whereas, Piperine formed conventional hydrogen bond with GLU498, and non-conventional hydrogen bonds with ARG403, GLU406 and GLY496. In addition, Piperine also showed hydrophobic interactions with TYR453 and TYR495. Oroxylin A, an O-methylated flavone present in *Andrographis paniculata* interacted with amino acid residues GLU406, GLY496 and TYR505 through hydrogen bonding. The molecule also exhibited non-polar interactions with LYS417, TYR453, TYR495, and TYR505. Andrographolide, a phytoconstituent present in *Andrographis paniculata* also demonstrated good computational binding affinity towards RBD of spike protein which is facilitated by a network of hydrogen bonds with ARG403, TYR453, SER494, GLY496 and TYR505. The phytochemical also exhibited van der waal's interaction with amino acid residues TYR449, GLN493, TYR495, PHE497, and ASN501.

Conclusion

To conclude, the present study employed *in silico* approach for screening of phytoconstituents of medicinal plants present in "Clevira" herbal formulation against some selected targets involved in the SARS-CoV-2 infectivity and replication to identify its anti-COVID-19 potential. A total of 52 phytoconstituents present in medicinal plants incorporated in the Clevira formulation were screened for their inhibitory potential through molecular docking studies against four targets namely the viral spike protein (S1), RNA dependent RNA polymerase (RdRp), SARS CoV2 main protease (3CLpro) and papain like protease (PLpro) that play a pivotal role in the SARS-CoV-2 infectivity and replication cycle. The results obtained show several of the phytoconstituents present in 'Clevira' herbal formulation showed good computational binding affinity against the four targets and some of the phytoconstituents were observed to show computational binding affinity to more than one of the four targets investigated. The phytoconstituents Orientin, Chlorogenic Acid, Wogonin, and Vitexin showed excellent computational affinity to SARS CoV2 Papain-Like Protease whereas the phytoconstituents Rutin, Ninandrographolide, Quercetin, and Chlorogenic acid exhibited good computational affinity to SARS CoV2 main protease. Four phytoconstituents namely Ninandrographolide, Rutin, Quercetin, Tinosporide were predicted to have good binding affinity to SARS CoV2 RNA dependent RNA polymerase and four phytoconstituents namely Caffeic acid, Ninandrographolide, Piperine and Oroxylin A are predicted to efficiently bind to receptor binding domain of SARS CoV2 spike protein (S1). All these

results obtained are indicative of therapeutic potential of phytoconstituents present in Clevira herbal formulation against SARS-CoV-2 hence merits further experimental validation the against the four targets and SARS CoV2 which would ultimately prove the therapeutic efficacy of Clevira against SARS-CoV-2 infection.

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