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RESEARCH ARTICLES

In-silico docking studies of selected phytochemicals against papain like protease of SARS-Cov-2

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Abstract

The SARS-Cov-2 virus, which is evolving continuously and causing adverse effects throughout the world, needs an effective drug molecule for its treatment. There are several receptors of SARS Cov-2 which are targeted for its inhibition by many lead molecules both in-vitro and in-vivo. Papain like Protease (PLpro) is one of the two SARS-Cov-2 proteases that can be used as a drug target for SARS Cov-2. It is a coronavirus enzyme that plays a role in the cleavage and maturation of viral polyproteins, assembly of the replicase-transcriptase complex and disruption of host responses. PLpro has also been linked to the cleavage of proteinaceous post translational modifications on host proteins as a means of evading antiviral immune responses. Structure-based drug discovery can be one of the effective methods to screen for various molecules against the target receptors. In this study, PLpro of SARS CoV-2 was chosen as the target for docking. Forty phytochemicals from various plant sources and four synthetic drugs have been screened for their inhibitory potential against PLpro using *AutoDock Vina*. Phytochemicals such as Tinosponone, Rhoifolin, Rosmanol, Berberin, Nimbin and two other existing drugs Elbasvir and Declatasvir showed higher inhibitory potential in terms of higher binding affinities. ADME and toxicity analysis were also performed to predict the pharmacokinetics and drug likeliness properties. It was concluded from the study that Tinosponone possesss potential inhibitor property of papain-like proteases (PLpro) of SARS CoV-2. Tinosponone from the plant *Tinospora cordifolia* had a binding affinity of − 9.3 kcal/mol and obeyed the Lipinski rules, making it an effective lead molecule for treating SARS CoV-2. Molecular Dynamics simulation of Tinosponone with PLpro has proved the stability and validity of the binding with RMSD value in range of 0.2 nm when it was run for 50 ns using GROMACS. Therefore, Tinosponone could be considered as a potential inhibitor of PLpro of SARS CoV-2.

Keywords Papain-like protease (PLpro) · Tinosponone · Docking · SARS CoV-2 · MD simulation

Introduction

Severe Acute Respiratory Syndrome (SARS) Coronavirus, a novel coronavirus (2019-nCoV), also known as SARS-CoV-2, posed a huge threat to the world since 2019. The Centre for Disease Control and Prevention (CDC) tracked the outbreak of SARS-CoV-2 in Wuhan, China. The virus spread across the globe and leads to a pandemic situation with significant mortality rates that differed from country to country. People with heart disease, diabetes, lung disease, HIV, and pregnant women are at an increased risk of developing a serious illness or contracting COVID-19. The virus does not travel through the air, but rather through the respiratory droplets or aerosols of an infected individual (Dwarka et al. 2020). SARS CoV-2 is a member of the *Coronaviridae* family of the *Nidovirales* order. It is classified into four genera and SARS CoV-2 is closely related

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to the *Betacoronavirus* genus. Genome sequencing revealed higher similarity with the previous SARS CoV (2003 outbreak). Both viruses share the same membrane-bound exopeptidase Angiotensin converting enzymes (ACE2) as the host receptor to enter the cells (Chakraborty and Member 2020; Silva et al. 2020). Different structural proteins such as spike proteins play the main role in attachment to host receptors. Apart from the structural proteins, different proteases also play a major role in viral replication and immune evasion of the host. Different cysteine proteases: chymotrpsin-like proteases or main proteases (3CLpro), papain-like proteases (PLpro) are important non-structural proteins. The 3CL-like protease (3CLpro) hydrolyzes viral polyproteins to create functional proteins, which are required for coronavirus replication.Papain-like protease is essential for the release of NSP 1–16 from open reading frames ORF1a and ORF 1ab, which forms large polyproteins ppa1a and ppa1ab (Li et al. 2020). Papain-like protease (PLpro) is a SARS CoV-2 enzyme that is required for digesting viral polyproteins to generate a functional replicase complex that allows the virus to spread. It negatively regulates the host immune response by its deubiquitinating and deISGylating effect (Shah et al. 2020; Shin et al. 2020; Mohanraj et al. 2018).

Computer aided drug design tools such as molecular docking and molecular dynamic simulation help us to find the potential inhibitors against various viral targets in lesser time (El-hoshoudy 2020; Shawk et al. 2020; Meng et al. 2020). Plant-based immune modulatory compounds could reduce the effect of inflammatory response and cytokine storm (Abdellatiif et al. 2021; Pall et al. 2020; Vardhan and Sahoo 2020). Several antiviral inhibitors have been found to be more effective against the SARS CoV-2 virus (Alfaroa et al. 2020). Phytochemicals boost immunity and protect against illness (Zhu et al. 2020). This study discusses the results of docking studies of various phytochemicals against SARS CoV-2's papain-like protease (PLpro).

Material and methods

Preparation of protein

The crystal structure of papain-like protease of severe acute respiratory virus -2 (SARS-CoV-2) PDB ID 6W9C was retrieved from PDB database https://doi.org/10.2210/pdb6W9C/pdb and it was used in the present study. It has a resolution of 2.70 Å and molecular weight of 107.81 kDa. It has three chains A, B and C with a total sequence length of 317 amino acids. Energy minimization of the retrieved structure was performed using the SW*ISS PDB* viewer. It helps to repair the distorted geometrics by moving atoms to release internal constraints and is used to minimise the protein energy (Kodchakorn et al. 2020; Tripathi et al. 2011; Trott and Olson 2010).

Preparation of ligand

Antiviral compounds derived from plant resources with variety of traditional applications were screened from a variety of phytochemical databases such as IMPPAT https://cb.imsc.res.in/imppat/ (Indian Medicinal Plants, Phytochemicals and Therapeutics) (Ni et al. 2020; Swain et al. 2020). Compounds with anti-viral properties were screened based on ADME and drug-likeliness properties using SWISS ADME server. (http://www.swissadme.ch/) (Meyer-Almes 2020; Garg et al. 2020). Based on the results, forty phytoligands were chosen for the study. As a control, five commonly recommended synthetic antiviral drugs such as Elbasvir, Cefpiramide, Cefpiramide, Daclatasvir and Delamanid were also docked with PLpro (Sharma et al. 2020; Swain et al. 2020). The structure of phytoligands and drugs was obtained from Pubchem database (https://pubchem.ncbi.nlm.nih.gov/) in SDF format. The phytoligands were then converted from SDF to PDB format using the online SMILES converter. (https://cactus.nci.nih.gov/translate/). ChemSketch was used to convert the 2D structure of certain ligands into a 3D format. (https://www.acdlabs.com/resources/free-chemistry-software-apps/chemsketch-freeware/#chemsketch_modal) (Ferreira et al. 2015). Various phytoligands and synthetic drugs were docked with the target protein, Papain-like protease of SARS CoV-2 using *Autodock Vina* in blind docking mode (Venkateshana et al. 2020).

Molecular dynamic simulation of Tinosponone

The complex of Tinosponone with Papain-like protease was prepared for Molecular Dynamic simulation and run for 50 ns using GROMACS (version 2022.1) through Google Colab pro (Salsbury 2010; Dutt and Roy 2020). Topology generation and energy minimization of ligand were performed and energy minimization of ligand was performed using the Google colab pro GPU server.

Results and discussion

Molecular docking analysis

The binding energies of forty phytoligands against SARS-CoV-2 papain-like protease (6W9C) ranged from − 9.3 kcal/mol (Tinosponone) to − 2.5 kcal/mol (Methyl Rosmarinate), while synthetic anti-viral drugs ranged from − 9.1 kcal/mol (Bictegravir) to − 13.0 kcal/mol (Elbasvir). Tinosponone and Hespiridin had the highest binding affinity for the PLpro. Because the latter's drug-likeliness had some flaws, Tinosponone from *Tinospora cordifolia* was further subjected to MD simulation to study its binding stability. The list of various phytochemicals, their sources and their docking score were given in Table 1

The pharmacokinetic properties of the selected ligands with preferred docking scores is given in Table 2. Out of five ligands and a drug, Nimbin and Rhoifolin did not meet the required properties as Nimbin's molecular weight was greater than 500 Da and Rhoifolin's number of hydrogen acceptors was greater than 10. Tinosponone, Berberine, Rosamanol and Elbasvir satisfied all rules of Lipinski.

Prediction of the active site of PLpro

The probable active sites or binding pockets of the four viral proteins were identified with PyMOL (Bharadwaj et al. 2020; Yuan et al. 2017). Five phytochemical molecules were analyzed for the identification of the active site of the target molecule which is listed in Table 3. The target protein PLpro contains

Table 1 Docking scores of Phytochemicals with source and PUBCHEM ID

S no.	Phytochemicals	PUBCHEM ID	Source	Docking score (kcal/mol)
1	Luteolin	5280445	*Chrysanthemum indicum*	– 7.9
2	Thebaine	5324289	*Papaversomniferum L*	– 7.9
3	Rosmanol	13966122	*Salvia Rosmarinus*	– 8.3
4	Tinosponone	15215479	*Tinosporacordifolia*	– 9.3
5	Berberine	2353	*Berberis vulgaris*	– 8.2
6	Chrysin	5281607	*Radix scutellariae*	– *7.6*
7	Chrysoeriol	5280666	*Artemisia vulgaris*	– 7.8
8	Chrysophanol	10208	*Rheum rhabarbarum*	– 7.7
9	Cirsimaritin	188323	*Coelogynecristata*	– 7.7
10	Curcumin	969516	*Curcuma longa*	– 7.5
11	Emodin	3220	*Polygonum Cuspidatum*	– 7.7
12	Hespiridin	10621	*Citrus fruits*	– 9.1
13	Magnoflorine	73337	Anamirtacocculus	– 8.1
14	Nimbin	108058	*Azadirachta indica*	– 8.3
15	Piperine	638024	*Piper nigrum*	– 7.6
16	Quarcetin	5280343	*Onions,cherries, Broccoli & citrus fruits*	– 7.9
17	Allicin	5036	*Allium sativum*	– 4
18	Cinnamaldehyde	637511	*Cinnamomumzeylancium*	– 3.9
19	Scutellarin	185617	*Scutellarialateriflora*	– 7.9
20	Myricetin	5281672	Leaves of *Myricarubra*	– 3.5
21	Amentoflavone	5281600	*Ginkgo biloba*	– 4.1
22	Ramelteon	208902	Sleep-agent medication	– 6.4
23	Pectolinarin	168849	*Linaria vulgaris*	– 4.8
24	Licoleafol	11111496	*Glycyrrhizauralensis*	– 3.3
25	Methyl Rosmarinate	6479915	*Rosmarinus officinalis L*	– 2.5
26	Artemisinin	68827	*Artemisia annua*	– 3.1
27	Prulifloxacin	947	*Antibacterial fluoroquinolone*	– 2.9
28	Calceolarioside	5273566	*Lepisorous contortus*	– 4.4
29	Terrestrimine	102335850	*Tribulus terrestrias L*	– 7.1
30	Ellagic acid	5281855	Berries and pomegranate	– 5
31	Tryptanthrin	73549	*Wrightia tinctoria*	– 8.4
32	Caffeic acid	689043	*Bark of eucalyptus globulus*	– 2.7
33	Rhoifolin	5282150	*Boehmeria nivea*(leaf)	– 9.2
34	Colistin	5311054	*Bacillus colistinus*	– 6.2
35	Dieckol	3008868	*Ecklonia cava*	– 5.4
36	Glycyrrhizin	3495	*Glycyrrhiza glabra L*	– 4.5
37	Vasicinone	442935	*Justicia adhatoda*	– 6.8
38	Andrographolide	5318517	*Andrographi spaniculata*	– 8
39	Apigenin	5280443	*Matricaria chamomilla*	– 7.7
40	Oxytocin	439302	*Fig*	– 5.1

Table 2 ADME Analysis of selected compounds

S no.	Ligand	Mol. Wt (g/mol)	log pH	No of H donors	No of H acceptors	Drug likeliness
1	Rosmanol	346.42	2.88	3	5	Yes
2	Berberine	336.36	2.53	0	4	Yes
3	Nimbin	540.60	3.23	0	9	No
4	Rhoifolin	578.62	− 0.66	8	14	No
5	Tinosporinine	342.34	2.89	0	6	Yes
6	Elbasvir	880.02	5.50	4	9	Yes

three chains A, B and C. The binding complex of Tinosponone with the active site of PLpro of SARS CoV-2 is shown in Fig. 1.

Table 3 Active site of selected phytochemicals with A, B and C chain of PLpro

Ligands	Rosmanol	Berberine	Nimbin	Rhoifolin	Tinosponone
Docking Score (kcal/mol)	− 8.3	− 8.2	− 8.3	− 9.2	− 9.3
A Chain	–	Gly 160	Glu 161	–	P 247
B Chain	Gly 160	–	–	–	–
C Chain	Thy 158	Gly 160	–	Gly 271	P 248

Molecular dynamic simulation of Tinosponone with PLpro

The molecular dynamic simulation was carried out for the docked complex of Tinosponone with papain-like protease using GROMACS (version 2022.1) through Google Colab pro. The conformation of the molecule binding of the compound was found to be stable with a mean RMSD value deviation in the range of 0.2 nm when simulation was done for the period of 50 ns using Google colab pro. RMSD value was plotted as the function of time frame was plotted as shown in Fig. 2. RMSF fluctuation of binding of Tinosponone with PLprowas shown in Fig. 3 (Silva et al. 2020). The number of hydrogen bonds formed during 50 ns Molecular dynamic simulation was shown in Fig. 4

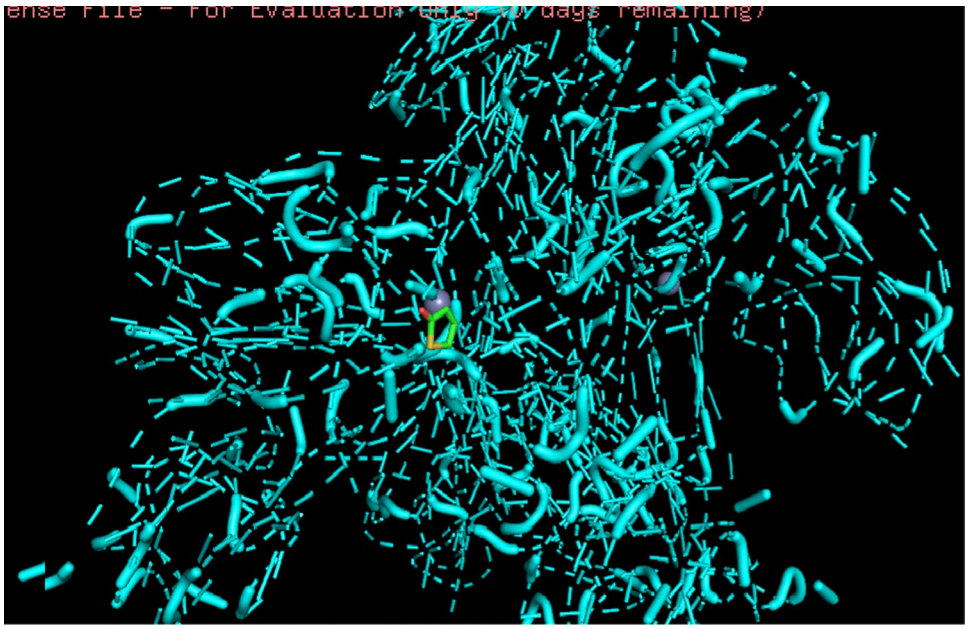

Fig. 1 Active site predicted using PyMOL for the docked structure of Tinosponone with PLpro of SARS CoV-2

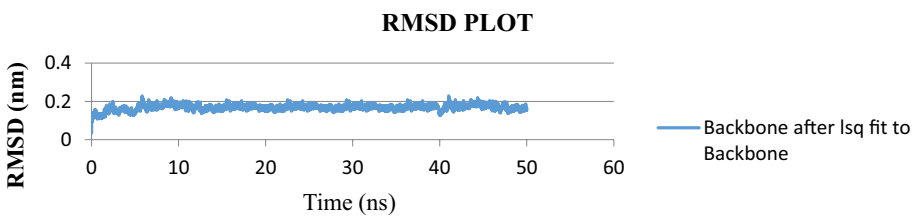

Fig. 2 Time dependence RMSD plot of binding of Tinosponone with PLpro using MD simulation

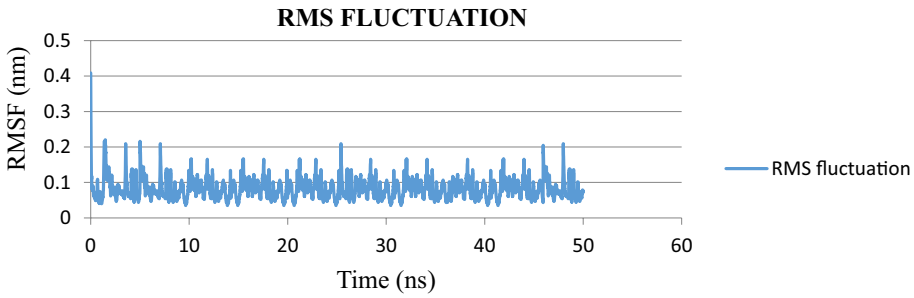

Fig. 3 Time dependence RMS fluctuation plot of binding of Tinosponone with PLpro using MD simulation

Discussion

In the current study, Tinosponone derived from the plant *Tinospora cordifolia* showed better binding activity with the binding energy of − 9.6 kcal/mol using *Autodock Vina*, Similarly in another study by Krupanidhi et al. (2020), Tinosponone showed a better binding affinity with 3CL pro also with binding activity of − 7.7 kcal/mol (Krupanidhi et al. 2020). Results of molecular dynamic simulation with Papain protease of SARS CoV-2 with Tinosponone also indicates the stable binding when the simulation was performed in the 50 ns range (Table 4).

Table 4 Affinity of docked Tinosponone with SARS CoV-2 PLpro in different docking modes

Docking mode	Affinity (kcal/mol)	Distance from best mode	
		rmsd l.b	rmsd u.b
1	− 9.3	0.000	0.000
2	− 9.1	2.767	4.895
3	− 8.9	2.752	4.867
4	− 8.2	3.175	5.338
5	− 8.0	1.798	2.402

Conclusion

Virtual screening of SARS CoV-2 papain-like protease (PDB ID: 6W9C) with various phytoligands demonstrated the five phytoligands, Tinosponone, Rhoifolin, Rosmanol, Berberin, and Nimbin with the best inhibitory potential in terms of higher binding affinities. Tinosponone had a binding affinity of − 9.3 kcal/mol and obeyed all Lipinski rules, making it a potential inhibitor for SARS Cov-2 PLpro. Tinosponone's binding site with the target PLpro was identified as P246 on chain C of SARS CoV-2's papain-like protease (PLpro). Therefore, Tinosponone could be used as a potential inhibitor of papain like protease of SRS CoV-2 based on further in-vitro and in-vivo investigations.

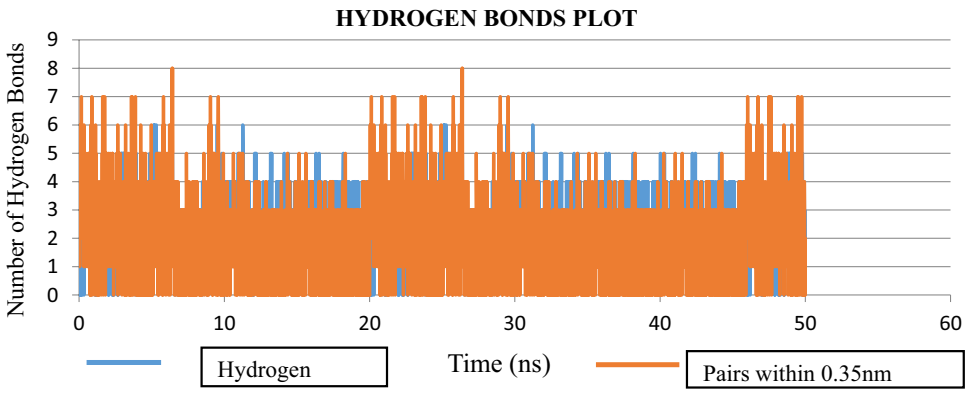

Fig. 4 Number of Hydrogen Bonds of Tinosponone binding with PLpro in 50 ns MD simulation

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Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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