

Anti- SARS-CoV-2 main protease complex (M^{pro}) activity of Palmatine

VYANKATESH JADHAV (✉ vbiophysics@gmail.com)

Department of Biophysics, D B College [ACS], Bhokar, Dist. Nanded, 431801 (MS) India, School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded - 431606 (MS) India.

Research Article

Keywords: Palmatine, SARS-CoV-2, Mpro, Homology Modelling, Docking

Posted Date: July 6th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-38145/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

The Pandemic situation caused due to SARS-CoV-2 causing Coronavirus Disease (CoVID-19) around globe. Recent, COVID-19 main protease complex (M^{Pro}), highly modulating enzyme in SARS-CoV-2 was reported for viral replication and transcription. This multifunctionality of M^{Pro} attracts for identification of potential drug target. Considering impact, *In silico* analysis was performed for Palmatine alkaloid against M^{Pro} . Naturally, present in *Tinospora cordifolia*, found effective against Cancer, HIV, viral infections, diabetics. In methods, physico-chemical analysis by ProtParam tool and Structure of M^{Pro} was predicted by SWISS-MODEL Workspace homology modeling server. Superimposition Structure and significant equal QMQE, QSQE values were found for eight highly similar templates. Structural assessment validation by Ramachandran plot (97.67% favoured), Local Quality estimate ratio (>0.6) and higher QMEAN score (y-axis). Further, docking was performed with validated M^{Pro} model by SwissDock server. Interaction with $-8.281919 \Delta G$ indicates reliable Interaction. Also, comparative docking reveals, most favoured Palmatine interaction. Thus, an attempt was made to find potent inhibitor for SARS-CoV-2, as there is no promising and specific anti-viral drug or vaccine available for prevention and treatment of infections. However, *In Vitro* studies are required. Toxicity studies reported against Palmatine for acute effect (135 mg/kg body weight) on mouse model LD₅₀.

Introduction

Corona viruses are commonly a group of RNA Viruses infecting and causing diseases in birds and mammals including Humans [1]. Belongs to subfamily *Orthocoronavirinae* in family *coronaviridae*, order *Nidovirales* [2]. This group of viruses are Positive-sense ssRNA Genome surrounded by spherical envelope with genome size ranging from 26-to 32 kb [3, 4].

Recently, new Corona virus causing threat around the globe due to Severe Acute Respiratory Syndrome Corona virus 2 (SARS-CoV-2)[5]. Humans are mostly affected by causing respiratory tract infections disease Corona virus disease 2019 identified as COVID-19[5]. Upon infection, mild to death effect were widely reported in human population [6]. As on present there is no promising and specific anti-viral drug or Vaccine available for prevention and treatment of infections cause by COVID-19 [6]. While similar variety of viruses including SARS and MERs has divesting effect on human existence during past decades [7]. Therefore, by considering present pandemic situation around the globe it was decided to develop novel and potent strategies.

In present scenario, discovery of active components from the plant and their biological function in disease control has lead to active interest in the plant products across the globe [8]. Similarly, in modern medicine many drugs are derived from plant products and their derivatives [8]. The variety of lead compounds was obtained from plant secondary metabolites for treatment of various diseases [9]. The alkaloids and flavinoid are having activity as strong analgesic effect and dietary supplement respectively [8]. The most commonly used secondary metabolites artemisinin were widely used phytomedicine [10].

In present analysis, An attempt was made to evaluating Antiviral activity of Palmatine alkaloid against COVID-19 main protease complex (M^{Pro}) of SARS-CoV-2. Basically, alkaloid was found in naturally occurring *Tinospora cordifolia* commonly known as 'Guduchi'[8]. It has been reported for its diverse application in the treatment of various diseases i.e. Cancer, HIV, viral infections, neurological, diabetics etc. [8].

By considering pandemic impact and medical urgency the computational analysis were performed for Anti-COVID-19 main protease complex (M^{Pro}) of SARS- CoV-2 by potential Palmatine alkaloid molecule. Completely characterized COVID-19 main protease complex (M^{Pro}) is submitted in PDB [11]. It is Multifunctional and highly modulating enzyme in SARS-CoV-2 replication and transcription [11]. Therefore, identified COVID-19 main protease complex (M^{Pro}) is attracting for potential drug target.

Methods

1. ProtParam tool (<https://web.expasy.org/protparam/>)

Physico-chemical parameters was analysed for given protein or user provide protein sequence, present in Swiss-Prot or TrEMBL [2]. Then FASTA Format protein Sequence of COVID-19 main protease (M^{Pro}) was provided in one-letter Code for computing parameter.

2. PDB (<https://www.rcsb.org/>)

The protein data bank is full-fledged archive worldwide for structural data for biological molecules [13]. The crystal structure of COVID-19 main protease (M^{Pro}) was referred from <https://www.rcsb.org/structure/6LU7> [14]. PDB Format structure was downloaded for further analysis with .pdb file format. FASTA format sequences were retrieved from PDB by using URL: <https://www.rcsb.org/pdb/explore/remediatedSequence.do?structureId=6LU7> [15].

3. SWISS-MODEL (<https://swissmodel.expasy.org/>)

ExpASY web server Accessible fully functional protein Structure homology modelling server. It facilitates modelling service to life research trough worldwide [16].The primary amino acid FASTA Format sequence was submitted to SWISS-MODEL workspace on June 9, 2020. Then homology modelling project (COVID-19 main protease complex (M^{Pro})) was performed.

3.1 Template Search

SWISS-MODEL template library was searched against BLAST and HHBlits for template search (SMTL, last update: 2020-06-03, last included PDB release: 2020-05-29) [17]. The primary amino acid sequence in the SMTL was BLAST against target [17].

3.2 Template Selection

The features of template-target alignments evaluate identification and quality of template. Then best quality templates were Consider for model building [17].

4.3 Model Building

ProMod3 predicted model on the target-template alignment [18]. The highly Conserved Coordinates between the target and the template are taken from the template to the model [18]. For Rebuilding of Insertions and deletions are remodelled using a fragment library [18]. Finally, force field method was applied for the satisfying geometry of the predicted model [18]. PROMOD-II is used when loop modelling with ProMod3 fails, to build model [18].

4.4 Model Quality Estimation

The global and per-residue model quality has been assessed using the QMEAN scoring function [19].

4.5 Ligand Modelling

Template structure having ligands are transferred by homology to the model, after satisfying following criteria by ligands with model: (a) Biological significance and annotation in template library, (b) Proximity, (c) Matching, (d) Conserved bonding between target and template [16]. However, ligand fails to satisfy criteria, is not included in model [16].

4.6 Oligomeric State Conservation

To model the oligomeric target sequence, annotated quaternary structure of the template is used [20]. Including, interface conservation, structural clustering, and other template aspects to reveal a quaternary structure quality estimate (QSQE). On the basis of supervised machine learning algorithm, SVM (Support Vector Machines) method.

The QSQE score prediction ranges between zero to one, indicates expected accuracy of inter linkage of chains for a model. Higher numbers is directly proportional to reliability of model [20]. This is synergetic to GMQE score for predicting correctness of resultant model at tertiary structural level [20].

4. PubChem (<https://www.ncbi.nlm.nih.gov/pccompound>)

The database contains validated fulfilled chemical informational data [21]. Accessible to pre-cluster and cross- referenced Structure of Compound by identity and similarity groups compounds were available [21]. Palmatine was searched by selecting PubChem Compound in Drop down menu in search box of NCBI Home Page

5. SwissDock (<http://www.swissdock.ch/>)

SwissDock is EADock DSS based docking service via ExPASy web server. It was used to predict protein ligand docking [22, 23].The molecular interactions that may occur between a target protein and a small

molecule were evaluated [22, 23]. SwissDock docking is based on EADock DSS Software [22, 23]. . Its algorithm consists of the following steps: Initially, various binding modes are created in box i.e. local docking or vicinity of all target cavities i.e. blind docking. Simultaneously, their CHARMM (<https://www.charmm.org//>) energies are estimated. Further, clusters computed by SwissDock were downloaded for further visualization and analysis [22]

Results

1. Physio-chemical

The analysis of COVID-19 main protease (M^{Pro}) protein using protparam tool provide precious information about proteins including number of amino acids, Molecular weight, Theoretical pl etc (Table 1). The Total number of amino acids in protein 306, while Leucine (29 (9.5%) most frequently occurring amino acid and least one is Pyl (O) & Sec (U) 0(0.0%). Molecular weight is 33796.64 (Table 1). The theoretical pl of protein is 5.95(Table 10). Total numbers of negatively and positively charged residues are 26 and 22 respectably. The molecular formula is described based on atomic combination of Carbon, Hydrogen, Nitrogen and Oxygen found in protein (Table 1). Extinction coefficients (M-1 cm-1) at 280nm were 33640 and Absorption (Abs 0.1% =1 g/l) 0.995 were observed (Table 1). The Instability index is inversely proportional to the stability of proteins, 27.65 were observed. Estimated half-life of protein was observed to be 1.9 hours (Table 1).

Table-1: Computational analysis of physicochemical parameters of COVID-19 main protease (M^{Pro}) of SARS-CoV-2 predicted using ProtParam.

Sr.No.	Parameters	COVID-19 main protease complex (M ^{pro})
1.	Theoretical pI	5.95
2.	Molecular Formula	C1499H2318 N402O445S22
3.	Total number of atoms	4686
4.	Molecular weight	33796.64
5.	Number of amino acids	306
6.	Higher No. Of Amino acid composition	Leu (L) 29 (9.5%)
7.	Extinction coefficients (M ⁻¹ cm ⁻¹) at 280 nm in water	33640
8.	Abs 0.1% (=1 g/l)	0.995

9.	Total number of charged residues Negatively(Asp + Glu)	26
10.	Total number of charged residues positively(Arg + Lys)	22
11.	Estimated half-life (Hrs.)	1.9
12.	Instability Index	27.65
13.	AliphaticIndex	82.12
14.	Grand Average of hydropathicity (GRAVY)	-0.019

2. PubChem

A plant metabolite Palmatine is alkaloid and an organic heterotetracyclic compound [25]. The Palmatine was completely characterized and structured with PubChem CID: 19009 [25]. In toxicological studies, acute effect was found in mouse up to 135 mg/kg (135 mg/kg) during LD₅₀ test [26].

3. Protein Homology Modelling

This protein (COVID-19 main protease (M^{Pro})) is already predicted and submitted in PDB with PDB Id: 6LU7 [11]. For further validation, homology modelling with SWISS-MODEL was performed. The SWISS-MODEL template library (SMTL version 2020-06-03, PDB release 2020-05-29) was searched with BLAST [17] and HHBlits [24] for identical structures matching with target sequence.

3.1 Model Building

The results for homology modelling project “COVID-19 main protease complex (M^{Pro})” submitted to SWISS-MODEL workspace on June 9, 2020, with an amino acid sequence with alignment is given in **Figure 1**.

The SWISS-MODEL template library for evolutionary related structures matching the target sequence in Table 2. For details on the template search, were described in Materials and Methods section above. Overall 386 templates were found to match the target sequence. This list was filtered by a heuristic down to eight Entries (Table 2). The highly identical eight templates are listed below with complete analysis of homology modeling parameters (Table 2). QMQE value was observed Equal (0.99) to every template (**Table 2**). While, QSQE value ranging from 0.90 to 0.95 (**Table 2**). All the entries showing significant 100 identities (**Table 2**). X-ray diffraction Method for modeling studies with different resolution ranging from 1.6Å to 2.2Å were Considered (**Table 2**). Further, all proteins were observed in homo-dimer Oligomeric State (**Table 2**). The pre requisite Library annotated Ligands were listed According to templates (**Table 2**).

Table 2: Summarizing alignment parameters for highly identical templates to establish the relationship.

Name	Description	GMQE	QSQE	Identity	Method & Resolution	Oligo State	Ligands
6y2g.1.B	Replicase polyprotein 1ab	0.99	0.95	100.00	X-ray, 2.2Å	homo-dimer	1 x GLY, 2 x O6K
6m2n.1.A	SARS-CoV-2 3CL protease	0.99	0.94	100.00	X-ray, 2.2Å	homo-dimer	2 x 3WL
6m2n.1.B	SARS-CoV-2 3CL protease	0.99	0.94	100.00	X-ray, 2.2Å	homo-dimer	2 x 3WL
7buy.1.A	SARS-CoV-2 virus Main protease	0.99	0.94	100.00	X-ray, 1.6Å	homo-dimer	2 x JRY

6m2n.2.B	SARS-CoV-2 3CL protease	0.99	0.93	100.00	X-ray, 2.2Å	homo-dimer	2 x 3WL
6wtk.1.A	3C-like proteinase	0.99	0.93	100.00	X-ray, 2.0Å	homo-dimer	2 x UED
6yz6.1.A	Main Protease	0.99	0.93	100.00	X-ray, 1.7Å	homo-dimer	2 x ACE-LEU-LEU-AR7, 2 x IMD
6wtj.1.B	3C-like proteinase	0.99	0.90	100.00	X-ray, 1.9Å	homo-dimer	2 x K36

3.2 Structural Assessment:

The Ramachandran plot structural Assessment was performed by *MolProbity* for further evaluation of model Quality [28]. Numerical values of MolProbity results are summarized with resolution at 2.20Å^o (Table 3). Significantly, 1.08 MolProbity Score, 97.67% Ramachandran Favoured value and 35 out of 6493 Bad angles were observed (Table 3).

Table 3: Protein Conformational parameters for Structural assessment by Ramchandran plot

(MolProbity).

MolProbity Score	Clash Score	Ramachandran Favoured	Rotamer Outliers	C-Beta Deviations	Bad Bonds	Bad Angles
1.08	0.32	97.67%	0.17	7	1/4780	35/6493

3.3 COVID-19 main protease (M^{Pro}) structure

The individual predicted protein structure was obtained by clicking on checkbox (Figure 3A) and can be analysed with different angle and conformational visualization models [16, 17]. Similarly, Different templates structures superposition was visualized and compared in 3D viewer (Figure 3B). Templates Selected according to target similarity coverage were found very similar and superimposed (Figure 3B). Despite, some local dissimilarity was observed due to turns and loop region (Figure 3B).

3.4 Local Quality Estimate and Comparison

The local quality estimate was performed between local similarity to target and Number of residue. Two chains are shown in different colours in target protein was illustrated (Figure 4). Here both the chains occurred abundantly between 0.8 to 1.0 Predicted Local similarity Score with increasing Residue Number

(Figure 4A). QMEAN score on y-axis indicating, quality of the model (Figure 4B). The high comparison scores obtained for high-resolution crystal structures [16, 17]. Comparison with Non-Redundant Set of PDB Structures was observed between 0.5 to 1.0 Normalized QMEAN Score (Figure 4B).

4. SwissDock

Binding modes are scored using their FullFitness and clustered. Average value of FullFitness parameter of their elements were used to Arrange clusters.[27]. The molecular docking was performed with SwissDock is based on the docking software EADock DSS, whose algorithm consists of well defined steps mentioned above in method parts [27].

The docking was performed with palmatine against COVID-19 main protease complex (M^{Pro}). Evaluation of Binding modes are scored using their Full Fitness, clustered, thermodynamically significant ΔG value and energy minimization. Clusters are then ranked according to the significant ΔG of their elements from top to bottom. [27] (Table3, Figure5 A). Out of total 32 clusters, most favourable eight cluster0 with different Cluster Rank namely 2, 1, 0, 3, 7, 6, 5 & 4 are illustrated with favourable matching parameters (Table 3, Figure 5 B). The Hydrogen bond distance in combination of assumed cluster was observed in range of 1.725 Å to 2.464 Å (Figure 5 B). The most significant interaction observed with clusterrank 2, having ΔG value -8.281919 with Energy minimization 55.8351, Full Fitness - 1441.91, SurfFull and solvFull Vales are 227.317 and -1416.54 respectively were observed (Table 3, Figure 5 C). The significant H-bond distance in single cluster interaction was ranging from 1.725 Å to 2.152 Å (Figure 5 B).

Table 4 : Summarization of highly significant values of docking parameters calculated from Chimera [29] .

S	Cluster	ClusterRank	Energy	FullFitness	InterFull	IntraFull	deltaG▲	surfFull	solvFull
V	0	2	55.8351	-1141.91	-58.835	106.148	-8.281919	227.317	-1416.54
V	0	1	55.8351	-1141.91	-58.835	106.148	-8.281919	227.317	-1416.54
V	0	0	55.8351	-1141.91	-58.835	106.148	-8.281919	227.317	-1416.54
V	0	3	55.8372	-1141.9077	-58.7986	106.102	-8.277759	227.319	-1416.53
V	0	7	56.1598	-1141.7334	-58.8424	105.912	-8.259844	227.327	-1416.13
V	0	6	56.1598	-1141.7334	-58.8424	105.912	-8.259844	227.327	-1416.13
V	0	5	56.1598	-1141.7334	-58.8424	105.912	-8.259844	227.327	-1416.13
V	0	4	56.1598	-1141.7334	-58.8424	105.912	-8.259844	227.327	-1416.13

4.1 Comparative Docking

For further validation of Ligand molecule, comparative docking was performed with two different Ligands namely, Gangerol and Berberine. The Overall different Docking parameters were calculated (Table 4). The Significant ΔG value was observed for Palmatine among the Comparative Ligand Compounds (Table 4).

Table 5: Comparative analysis of Palmatine with different ligands namely gengerol and Berberine with the help of SwissDock and UNCF

SR Compound Cluster Rank Energy Full Fitness InterFull IntraFull Delta G SurfFull SolvFull No.

1	Palmatine	2	55.83	-1141.91	-58.83	106.14	-8.28	227.317	-1416.54
2	Gengerol	2	-15	-1215.00	-46.32	8.06	-7.42	227.317	-1404.11
3	Berberine	2	48.86	-1150.10	-61.48	83.89	-7.75	226.314	-1398.82

Discussion

Palmatine is an alkaloid molecule with active principle associated with variety of biological activities of *Tinospora cordifolia* commonly named as “Guduchi” [8, 30, 31, 32]. *T. cordifolia* Belongs to family *Menispermaceae* family which is genetically diverse shrub, naturally found at tropical regions of the Indian subcontinent [30, 31, 32]. From long back it is use in traditional medicine for treatment of various disorders [31, 32]. *T. cordifolia* exhibit variety of phytochemicals derived from the plant like alkaloids, steroids, diterpenoid lactones, aliphatics, and glycosides and mixed other compounds [32]. These compounds reported to have potential biological activities in different disease conditions attracting applicability in clinical research [8]. It is anti-viral, anti-spasmodic, anti-microbial, anti-osteoporotic, anti-inflammatory, anti-arthritic, anti-allergic, and anti-diabetic conditions. Specifically, Palmatine has been studied for used separately or in mixture for the treatment of anti-viral, jaundice, Dysentery, hypertension, inflammation and liver related diseases [8, 33]. Specifically, Palmatine was reported for suppressing Dengue and yellow fever virus in dose-dependent manner [34]. However, mode of anti-viral activity of is not yet understood.

Further, targeted molecule in present study analysed with physico-chemical properties, Reveals functional acidic environment and hydrophobic nature of protein (Table 1). The distance between different templates is proportional to the pair wise sequence similarity (Figure 1). Highly identical score of predicts COVID-19 main protease complex (M^{Pro}) is evolutionary conserved. The protein predictions with individual target and template protein have shown reliable similarities (Figure 3A, B). Typically, Sequence Variability affects superposition's structure by turns and loops (Figure 3B). In other Context, they often involved in protein function, hence, they are of crucial importance in accurate modelling [27]. Structural assessment by Local estimates for model quality based on QMEAN scoring function, shown as per-residue plot (Figure 4A) and as a global score in relation to a set of high-resolution PDB structures (Z-score) as show in Figure 4B. In present study score is associated to each residue of the model (reported on the x-axis), reflecting the expected similarity to the native structure (y-axis). Residue score < 0.6 are expected to be of low quality [16, 18]. Therefore, > 0.6 score for both the chains reveals high quality of protein modelling (Figure 4A). Further, higher QMEAN score comparison value indicates quality of the model for experimental structures [16, 18] and similar results shown in Figure 4B provides reliable quality of model.

Multifunctional COVID-19 main protease complex (M^{Pro}) of SARS-CoV-2 was found to be involved in replication and transcription of viral RNA responsible for processing of polyprotein [11]. Also, host

translational Inhibition by interacting with host 40S ribosomal unit [11, 35, 36]. Also, Modulating replication and transcription of the Viral Genome [11, 35, 36]. Similarly, different molecular functions were also reported Such as, Helicase, Hydrolase, Protease, hydrolase [11, 35, 36]. Therefore, docking studies with M^{Pro} was carried Out (Table3, Figure 5). The different docking parameter indicating the reliable and promising Ligand-Protein Interaction (Table3, Figure 5). Further, the comparative docking was performed with different ligands and M^{Pro} (Table 5). Among the three ligand-target interaction, Palmatine interacts with significant ΔG value (Table 5). In Conclusion, multifunctionality of COVID-19 main protease complex (M^{Pro}) attracts for potential drug target. Therefore, an attempt was made to find potent inhibitor against SARS-CoV-2 in present pandemic. Further, *In Vitro* studies are required for confirmation of inhibitor. However, toxicity studies reported against Palmatine for acute effect (135 mg/kg body weight) on mouse model LD₅₀ [26].

Declarations

Acknowledgment

Authors are thankful to Research Guide Dr. G.B Zore, Director, CABIFF-MDRL-CoVID-19 Testing Laboratory, S.R.T.M. University for his encouragement and support. Authors also, thankful to Dr .P.A Chavan, Principal, D B College and Dear Research Lab mates, SLS, SRTM University.

Author contribution

VAJ conceived idea, planned, designed and performed bioinformatics analysis; VAJ wrote MS.

Conflict of Interest Declaration

Author wish to confirm that there are no known conflicts of interest.

References

1. Krzysztof Pyrc, Ben Berkhout & Lia van derHoek (2007) Identification of new human coronaviruses, Expert Review of Anti-infective Therapy, 5:2, 245- 253, DOI: [1586/14787210.5.2.245](https://doi.org/10.1586/14787210.5.2.245)
2. de Groot RJ, Baker SC, Baric R, Enjuanes L, Gorbalenya AE, Holmes KV, Perlman S, Poon L, Rottier PJ, Talbot PJ, Woo PC, Ziebuhr J (2011). "*Family Coronaviridae*". In King AM, Lefkowitz E, Adams MJ, Carstens EB, International Committee on Taxonomy of Viruses, International Union of Microbiological Societies. Virology Division (eds.). Ninth Report of the International Committee on Taxonomy of Viruses. Oxford: Elsevier. pp. 806–28. [doi:1016/B978-0-12-384684-6.00068-9](https://doi.org/10.1016/B978-0-12-384684-6.00068-9)
3. Saberi A, Gulyaeva AA, Brubacher JL, Newmark PA, Gorbalenya AE. A planarian nidovirus expands the limits of RNA genome size. PLoS pathogens. 2018 Nov;14(11). <https://dx.doi.org/10.1371/journal.ppat.1007314>

4. Woo, Patrick C. Y.; Huang, Yi; Lau, Susanna K. P.; Yuen, Kwok-Yung. 2010. "Coronavirus Genomics and Bioinformatics Analysis." *Viruses* 2, no. 8: 1804-1820. <https://doi.org/10.3390/v2081803>
5. Lai CC, Shih TP, Ko WC, Tang HJ, Hsueh PR. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and corona virus disease-2019 (COVID-19): the epidemic and the challenges. *International journal of antimicrobial agents*. 2020 Feb 17:105924. <https://doi.org/10.1016/j.ijantimicag.2020.105924>.
6. Shereen MA, Khan S, Kazmi A, Bashir N, Siddique R. COVID-19 infection: Origin, transmission, and characteristics of human coronaviruses. *Journal of Advanced Research*. 2020 Mar 16. <https://doi.org/10.1016/j.jare.2020.03.005>.
7. Chen T, Wu D, Chen H, Yan W, Yang D, Chen G, Ma K, Xu D, Yu H, Wang H, Wang T. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. *Bmj*. 2020 Mar 26;368. <https://doi.org/10.1136/bmj.m1091>
8. Saha S, Ghosh S. *Tinospora cordifolia*: One plant, many roles. *Ancient science of life* 2012 Apr;31(4):151. <https://dx.doi.org/10.4103%2F0257-7941.107344>.
9. Khazir J, Mir BA, Mir SA, Cowan D. Natural products as lead compounds in drug discovery. <https://doi.org/10.1080/10286020.2013.798314>
10. Efferth T, Herrmann F, Tahrani A, Wink M. Cytotoxic activity of secondary metabolites derived from *Artemisia annua* L. towards cancer cells in comparison to its designated active constituent artemisinin. *Phytomedicine*. 2011 Aug 15; 18(11):959- 69. <https://doi.org/10.1016/j.phymed.2011.06.008>
11. Jin, Z., Du, X., Xu, Y. *et al.* Structure of M^{PRO} from SARS-CoV-2 and discovery of its inhibitors. *Nature* 582, 289–293 (2020). <https://doi.org/10.1038/s41586-020-2223-y>.
12. Gasteiger E, Hoogland C, Gattiker A, Wilkins MR, Appel RD, Bairoch A. Protein identification and analysis tools on the ExPASy server. In *The proteomics protocols handbook 2005* (pp. 571-607). Humana press. <https://doi.org/10.1385/1-59259-890-0:571>
13. Bourne PE. The protein data bank. *Protein Structure: Determination, Analysis, and Applications for Drug Discovery*. 2003 Mar 18:389. <https://doi.org/10.1107/S0907444902003451>
14. <https://www.rcsb.org/structure/6LU7>. **Released:** 2020-02-05
15. Rose PW, Beran B, Bi C, Bluhm WF, Dimitropoulos D, Goodsell DS, Prlić A, Quesada M, Quinn GB, Westbrook JD, Young J. The RCSB Protein Data Bank: redesigned web site and web services. *Nucleic acids research*. 2010 Oct 29;39(suppl_1):D392-401. <https://doi.org/10.1093/nar/gkq1021>
16. Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, Heer FT, de Beer TA, Rempfer C, Bordoli L, Lepore R. SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic acids research*. 2018 Jul 2;46(W1):W296-303. <https://doi.org/10.1093/nar/gky427>

17. Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., Madden, T.L. BLAST+: architecture and applications. *BMC Bioinformatics* 10, 421- 430 (2009).
<https://doi.org/10.1186/1471-2105-10-421>
18. Guex, N., Peitsch, M.C., Schwede, T. Automated comparative protein structure modeling with SWISS-MODEL and Swiss-PdbViewer: A historical perspective. *Electrophoresis* 30, S162-S173 (2009).
<https://doi.org/10.1002/elps.200900140>
19. Studer, G., Rempfer, C., Waterhouse, A.M., Gumieny, G., Haas, , Schwede, T. QMEANDisCo - distance constraints applied on model quality estimation. *Bioinformatics* 36, 1765-1771 (2020).
<https://doi.org/10.1093/bioinformatics/btz828>
20. Bertoni, M., Kiefer, F., Biasini, M., Bordoli, L., Schwede, T. Modeling protein quaternary structure of homo- and hetero-oligomers beyond binary interactions by homology. *Scientific Reports* 7 (2017).
<https://doi.org/10.1038/s41598-017-09654-8>
21. Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, Han L, He J, He S, Shoemaker BA, Wang J. PubChem substance and compound databases. *Nucleic acids research*. 2016 Jan 4;44(D1):D1202-13. <https://doi.org/10.1093/nar/gkv951>
22. Grosdidier A, Zoete V, Michielin O. SwissDock, a protein-small molecule docking web service based on EADock DSS. *Nucleic Acids Res*. 2011;39(Web Server issue):W270- doi:10.1093/nar/gkr366.
<https://doi.org/10.1093/nar/gkr366>
23. Grosdidier A, Zoete V, Michielin O. Fast docking using the CHARMM force field with EADock DSS. *J Comput Chem*. 2011; 32(10):2149- doi:10.1002/jcc.21797. <https://doi.org/10.1002/jcc.21797>
24. Remmert, M., Biegert, A., Hauser, A., Söding, J. HHblits: lightning-fast iterative protein sequence searching by HMM-HMM alignment. *Nat Methods* 9, 173-175 (2012).
<https://doi.org/10.1038/nmeth.1818>
25. <http://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:16096>.
26. <https://chem.nlm.nih.gov/chemidplus/sid/0003486677>
27. Grosdidier A, Zoete V, Michielin O. EADock: docking of small molecules into protein active sites with a multiobjective evolutionary optimization. *Proteins: Structure, Function, and Bioinformatics*. 2007 Jun; 67(4):1010-25. <https://doi.org/10.1002/prot.21367>.
28. Chen, B., Arendall, W. B., Headd, J. J., Keedy, D. A., Immormino, R. M., Kapral, G.J., Murray, L. W., Richardson, J. S. & Richardson, D. C. (2010). *Acta Cryst.* D66, 12-21
<https://doi.org/10.1107/S0907444909042073>
29. Meng EC, Pettersen EF, Couch GS, Huang CC, Ferrin TE. Tools for integrated sequence-structure analysis with UCSF Chimera. *BMC bioinformatics*. 2006 Dec 1;7(1):339.
<https://doi.org/10.1186/1471-2105-7-339>

30. Rana V, Thakur K, Sood R, Sharma V, Sharma TR. Genetic diversity analysis of *Tinospora cordifolia* germplasm collected from northwestern Himalayan region of India. *Journal of genetics*. 2012 Apr 1;91 (1):99. <https://www.ias.ac.in/article/fulltext/jgen/091/01/0099-0103>
31. Parthipan M, Aravindhana V, Rajendran A. Medico-botanical study of Yercaud hills in the eastern Ghats of Tamil Nadu, India. *Ancient science of life*. 2011 Apr;30(4):104.
32. Upadhyay AK, Kumar K, Kumar A, Mishra Tinospora cordifolia (Willd.) Hook. f. and Thoms. (Guduchi) – validation of the Ayurvedic pharmacology through experimental and clinical studies. *International journal of Ayurveda research*. 2010 Apr;1(2):112. <https://dx.doi.org/10.4103%2F0974-7788.64405>.
33. Sharma U, Bala M, Kumar N, Singh B, Munshi RK, Bhalerao S. Immunomodulatory active compounds from *Tinospora cordifolia*. *Journal of ethnopharmacology*. 2012 Jun 14;141(3):918-26. <https://doi.org/10.1016/j.jep.2012.03.027>
34. Jia F, Zou G, Fan J, Yuan Z. Identification of palmatine as an inhibitor of West Nile virus. *Archives of virology*. 2010 Aug 1;155 (8):1325-9. <https://doi.org/10.1007/s00705-010-0702-4>
35. Rota PA, Oberste MS, Monroe SS, Nix WA, Campagnoli R, Icenogle JP, Penaranda S, Bankamp B, Maher K, Chen MH, Tong S. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. *science*. 2003 May 30; 300(5624):1394-9. DOI: 1126/science.1085952.
36. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, Hu Y, Tao ZW, Tian JH, Pei YY, Yuan ML. A new coronavirus associated with human respiratory disease in China. *Nature*. 2020 Mar;579(7798):265-9. <https://doi.org/10.1038/s41586-020-2008-3>
37. Zhang L, Lin D, Sun X, Curth U, Drosten C, Sauerhering L, Becker S, Rox K, Hilgenfeld R. Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α -ketoamide inhibitors. *Science*. 2020 Apr 24;368(6489):409-12. DOI: 1126/science.abb3405

Figures

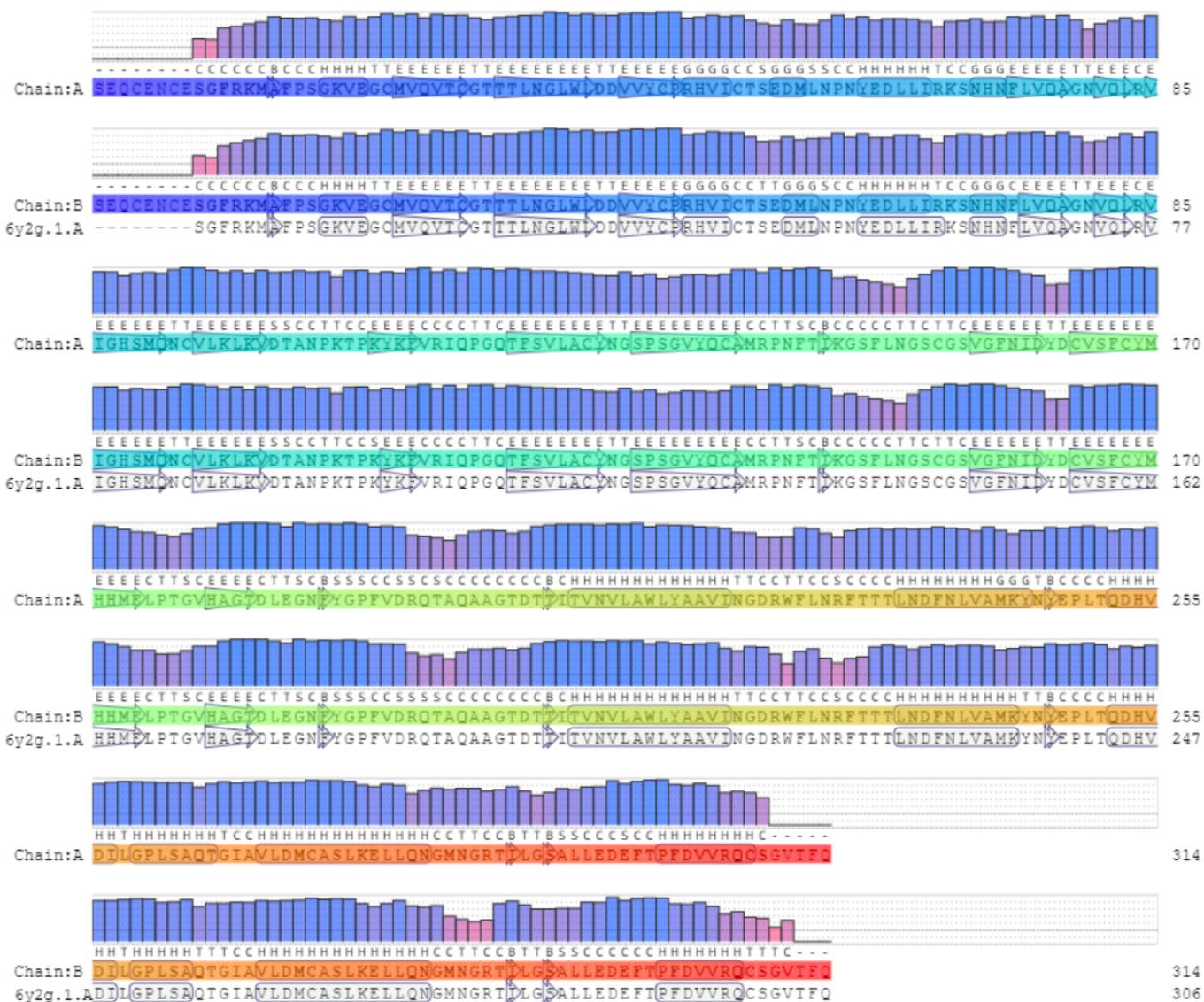


Figure 1

A predicted overview alignment for sequence similarity between the selected templates of amino acid sequence.

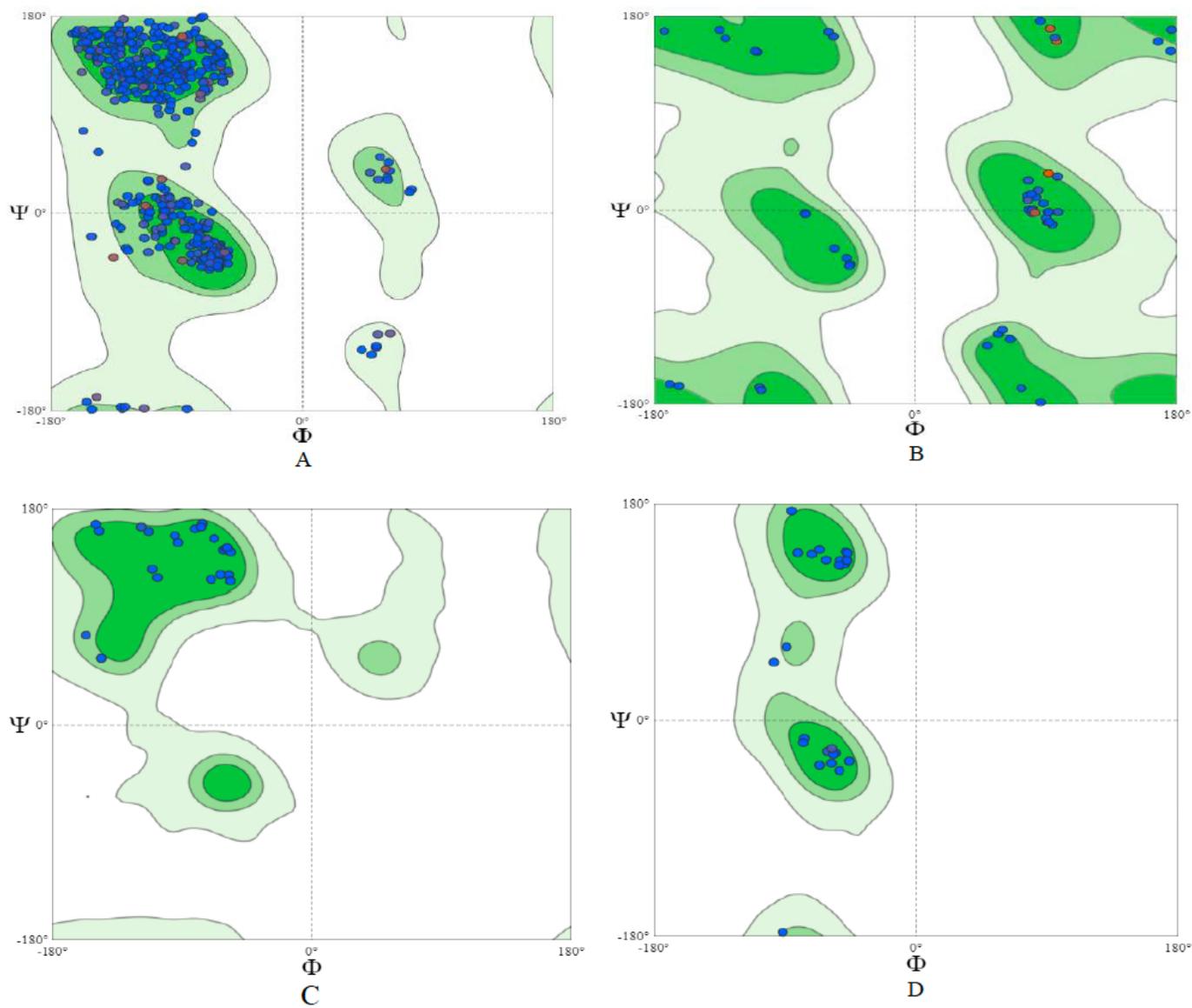


Figure 2

Ramchandran plot obtained using MolProbity for overall protein [A] and for Glycine [B], Preproline [C], Proline [D] amino acids.

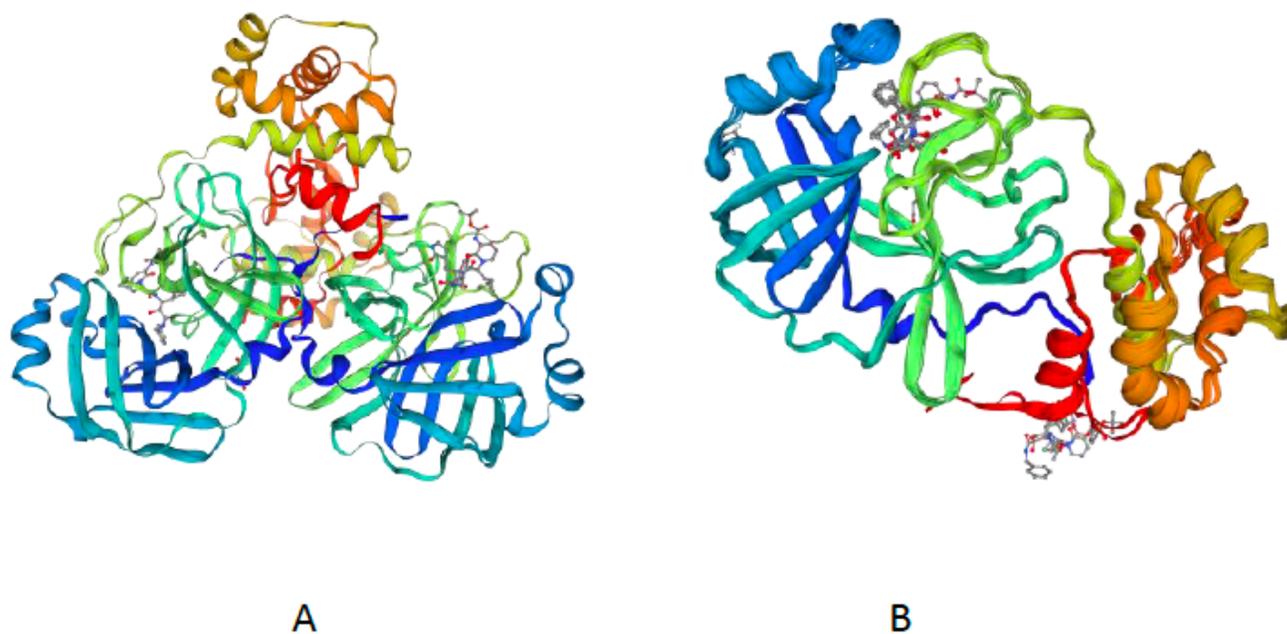


Figure 3

SWISS-MODEL Structure [A] predicted 3-Dimensional structure of COVID-19 main protease (Mpro) and [B] Superimposable Structural Alignment with different templates.

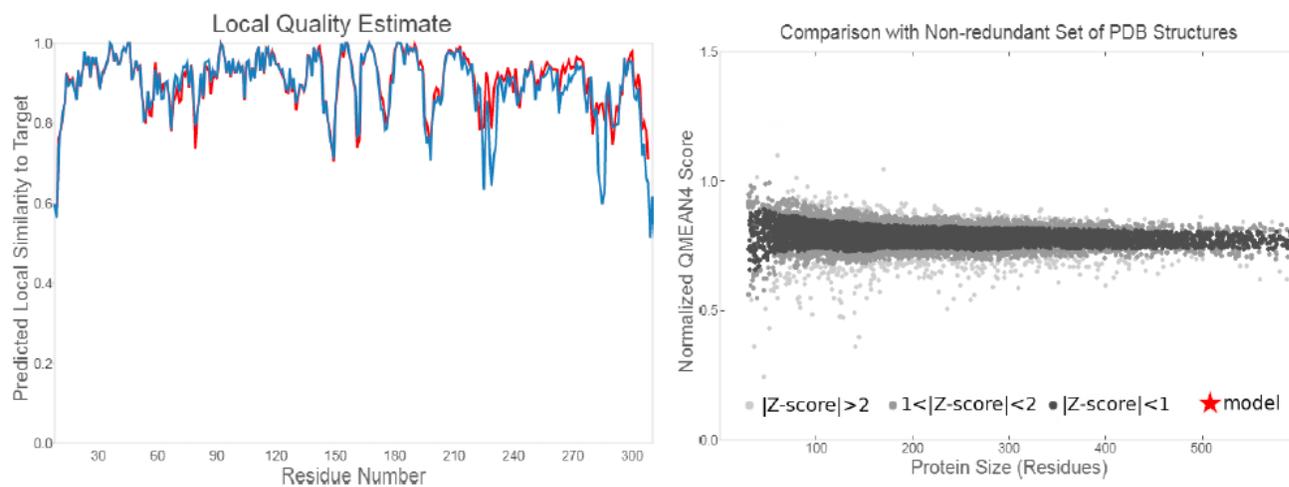


Figure 4

The two criteria were depicted for structural assessments are [A] Local Quality Estimate and [B] Comparison with Non-Redundant Set of PDB Structures.

