INNOVARE JOURNAL OF MEDICAL SCIENCES

Vol 10, Issue 5, 2022

ISSN - 2321-4406

Research Article

A MOLECULAR DOCKING STUDY: TARGETING COVID-19 (SARS-COV-2) MAIN PROTEASE USING ACTIVE PHYTOCOMPOUNDS FROM *TERMINALIA ARJUNA*

SHUBHAM WANARASE, SHIVAM CHAVAN, AND SAMEER SHARMA*

Department of Bioinformatics, BioNome, Bengaluru, India. Email: info@bionome.in

Received: 6 August 2022, Revised and Accepted: 14 September 2022

ABSTRACT

Objective: COVID-19 is transmissible disease triggered by SARS-CoV-2 strain of coronavirus family. It infected a million of people worldwide. Hence, an attempt was made to identify natural compounds from *Terminalia arjuna*, having multiple medicinal values in Indian Ayurveda, to prevent the disease, using molecular docking, drug likeness prediction and ADME analysis.

Methods: SARS-CoV-2 main protein was retrieved from the PDB database. The ligands with poor binding and molecules that can affect docking were removed and docking is done with PyRx tool. ADME and drug likeness analysis were done using Swiss-ADME and Admetlab web server.

Results: Ramachandran plot analysis shows the statistical distribution of the combinations of the backbone dihedral angles ϕ and ψ of the protein. Molecular docking studies show five compounds from *T. arjuna,* which have potential binding affinity to resist the main protease M^{pro} by preventing proteolytic cleavage, translation, and replication of virus. ADMET profile and drug likeness prediction showed that, among these five compounds Triterpenoid and N-Desmethyl Sildenafil were safe and possess the drug-like properties.

Conclusion: The present study suggests that Triterpenoid and N-Desmethyl Sildenafil have specific binding affinity and they could inhibit main protease M^{pro} and also helps to manage the therapeutic strategies against COVID-19.

Keywords: SARS-CoV-2, COVID-19, M^{pro} protein, Non-structural proteins, Viral replication, *Terminalia arjuna*.

 DOI: http://dx.doi.org/10.22159/ijms.2022v10i5.46122. Journal homepage: https://innovareacademics.in/journals/index.php/ijms

INTRODUCTION

The COVID-19 pandemic resulted in the numerous of deaths around the world. Even various effective and safe vaccines have been approved, the problem is not completely solved for those who are living in underprivileged areas which lack of vaccines and insufficient medical infrastructure. In accordance to July 21, 2022, as per the WHO (World Health Organization), globally, there have been greater than 564 million confirmed cases of COVID-19, including about 6.3 million deaths [1]. In India, according to Ministry of Health and Family Welfare, 43.3 million cases have been stated, among which 43 million cases are recovered, 1.5 lakh active cases and 5.2 lakh deaths [2].

Coronavirus (CoV) is a group of related RNA viruses which are threaten to mammals and birds. It causes infection in respiratory tract, range from mild to lethal. It belongs to family Coronaviridae which infects vital organs such as respiratory, enteric, hepatic, and nervous system [3,4]. CoV is single stranded RNA virus which is categorized in four genera, namely, alpha, beta, gamma, and delta. Among these genera, alpha-CoV and beta-Cov are known to be infect humans [5]. Among coronavirus group, SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus-2) has highest 3–4% mortality rate in humans. They are large, spherical particles having diameter ranges between 80 and 120 nm [6]. Bats are thought to be natural hosts of SARS-CoV-2, due to their zoonotic origin and genetic resemblance [7]. It is enclosed within an envelope consisting number of proteins such as Spike (S), Envelop (E), Membrane (M), and Nucleocapsid (N). These proteins help virus to identify receptor of host cell, fusion, and further transmission within the hosts. The lipid bilayer envelop protects the virus outside the host cell. It is still apparent how SARS-CoV-2 causes organ damage on a pathological level [8].

Proteases are important and well-studied proteins in CoV [9]. Proteases are necessary for poly-proteins which are translated from viral RNA [10]. Blocking of viral replication can be achieved by inhibit the activity of this viral proteases. The first strategy is to prevent the virus from attaching it on ACE-2 receptors of the host by blocking the function of Spike proteins. However, the main proteases (M^{pro}) are being searched as it translates the viral RNA into functional polyproteins which alter normal physiology in already infected cases [11,12]. M^{pro}, also known as 3C-like protease, formally is known as 3-cymotrypsin-like protease. It is main protease dimer consisting cluster of five helices, six stranded antiparallel β-sheets which hold substrate binding site at middle. Major of coronaviridae genome encodes two polyproteins, namely, pp1a [13] and pp1ab [14,15]. These polyproteins are cleaved and transformed in non-structural proteins (NSPs), with the help of two protease $3CL^{pro}$ and PL^{pro} (Papain like protease). M^{pro} is necessary in addition to Papain-like protein for releasing polyproteins at 11 specific sites to synthesize proteins that are translated from the viral RNA [16,17]. However, there is no other human protease with the cleavage specificity of same sites, inhibitors of these targets more likely to be non-toxic. Hence, M^{pro} has better target for inhibition of viral replication. Potential protease inhibitors, include the antivirals Nelfinavir, Remdesivir, Lopinavir, and Ritonavir along with α-ketoamide, are effective as a medicine to defeat the novel coronavirus [18,19]. Other than these, some combination of anti-viral such as Lopinavir, Ritonavir, Favipiravir [11], Anti-malarial such as Chloroquine, Hydroxychloroquine [20], immunosuppressants, steroids, and antibodies from plasma of recovered patients [21] and corticosteroid like Dexamethasone [22] therapies are used to control COVID-19.

Ayurveda is known as "The Life of Science," an ancient system of medicine in Indian subcontinent; based on principle of life, health, and healing system. In an Ayurvedic literature, a number of rejuvenating treatments which provide biological nourishment to the tissue cells were described [23]. According to ayurveda, stem and bark of *T. arjuna* has multiple therapeutic properties such as cardiotonic, anticancer,

antiviral, antibacterial, antifungal, hypercholesteremic, hypolipidemic, and anti-coagulant [24]. For therapeutic purpose, the bark of *T. arjuna* is the main medicinal part. It contains minerals such as calcium and magnesium. Phenolic compounds such as terminic acid and arjunolic acid, glycosides such as arjunetic and arjunosides I-IV, flavons, tannins, oligomeric Proanthocuanidins, β-sitosterol, and Casuarinin, phenolic acids such as ellagic and gallic acid and lactones are the major phytocompounds found in Arjuna bark. Arjuna's terminalia bark is utilized in various of ways, such as juice, powder, decoction, and more. When taken in the prescribed dose, all Arjuna formulations are remarkably safe and well tolerated by the majority of people [25]. Arjuna bark powder consumption over a long period of time has no adverse side effects and is probably safe for the kidneys and liver. Clinical and laboratory parameters indicate no sign of significant change [26,27]. With consideration of the need of effective antiviral agent against SARS-CoV-2, *T. arjuna* with its cardioprotective ability and inhibitory action against catalase may be a promising candidate against the virus [24]. In present study, an attempt was made to identify new active and stable inhibitors against coronavirus main protease M^{pro} from total 215 different active phytoconstituents from *T. arjuna*.

METHODS

Protein preparation

The three-dimensional crystal structure of M^{pro} protein (PDB ID: 6LU7) of COVID-19 (SARS-CoV-2) was retrieved from the RCSB (Research Collaboratory for Structural Bioinformatics) Protein Data Bank (https://www.rcsb.org/) [28,29]. It has single chain A consisting total 306 amino acids. It has a crystal resolution 2.16 Ð. Protein crystal structures are prepared prior docking to optimization of hydrogen bonds and removal of atomic clashes. Protein preparation was done using standard protocol of Discovery studio visualizer 21.1. Water molecules and heteroatoms from the proteins were removed followed by the addition of the polar hydrogen. Further, active site prediction of prepared protein was done.

Ramachandran plot

Ramachandran plot is a plot of torsional angles phi and psi of the amino acids contained in a peptide. Ramachandran plot analysis was done using the web-based Ramachandran plot server of Zlab (https://zlab.umassmed.edu/bu/rama/) [30] and MolProbity Server (http://molprobity.biochem.duke.edu/index.php) [31]. The PDB file of Protein (6LU7) was uploaded and analysis of Ramachandran plot were run with outliers that are labeled by residue type, residue number, and chain and displaying all the labels.

Ligand selection

For the documentation of potential inhibitors of COVID-19 M^{pro}, total 215 active phytocompounds from *T. arjuna* (Arjuna plant) were retrieved from the different literatures. Structures of phytocompounds were retrieved from PubChem compound database (https://pubchem.ncbi.nlm.nih.gov/) in the 3D SDF (Three-Dimensional Structure Data File) format [32]. Ligand preparation was carried out by optimization of ligand, energy minimization, and conversion of ligands to 3D PDB format using PyRx tool [33].

Molecular docking

The molecular docking method allows us to characterize how small molecules behave in the binding site of target proteins and to better understand basic biochemical processes by simulating the interaction between a small molecule and a protein at the atomic level [33]. PyRx-virtual screening tool software was used for molecular docking study [34]. Using PyRx tool, all 215 active phytocompounds of *T. arjuna* were docked with COVID-19 M^{pro} (PDB ID: 6LU7). For docking study, prepared receptors and ligand files were selected to set the target. Protein was loaded and converted into macromolecule for docking; then, ligands were imported using open-babel tab in tool and prepared [35]. After defining protein and ligand molecules, grid box was defined by maximizing to check all the possibilities of ligand to bind with protein. After adjusting all the things, docking was started by clicking on forward button. After completion of docking, we got a table consisting binding affinity of each ligand. Top 5 ligands were selected for further study based on the highest binding affinity of the ligand. Selected top 5 compounds were saved in PDB file format. 2D-3D interactive visualization study was done with the help of Discovery studio visualizer 21.1 [36].

ADME analysis

The term ADME or "absorption, distribution, metabolism, and excretion," in pharmacokinetics and pharmacology, refer to how a drug is disposed of within an organism. The performance and pharmacological activity of the compound as a drug are affected by the four criteria, because they all have an impact on drug levels and the kinetics of drug exposure to tissues. Sometimes toxicity is also taken in a consideration, called an ADMET [37]. In this research study, top 5 compounds having highest binding affinity were taken for the drug likeness test and ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) analysis. Drug-likeness and ADMET analysis were done using SWISS-ADME (http://www.swissadme.ch/) [38] and ADMETLAB (https://admetmesh.scbdd.com/service/evaluation/index) [39]. Boiled-Egg analysis was also carried out with SWISS-ADME tool [40]. Lipinski rule of five was considered for ADME analysis. Lipinski's rule of five forecasts whether a drug likeness would be successful or not when a molecule complies with two or more of the conditions,

a) Molecular mass < 500 Dalton,
b) logP < 4.15,
c) H-bond donor < 5,
d) H-bond acceptor < 10 and
e) 40 < molar refractivity < 130.

RESULTS

Ramachandran plot

In the Ramachandran plot, considering protein geometry, we found 230 favored rotamers and 295 Ramachandran favored 87.45% and 97.04%, respectively, which indicates the good protein quality. There were no poor rotamers and outliers. The Rama distribution Z-score for the protein was observed, which was –1.96 ± 0.41. Considering low resolution criteria, there was 3 CaBLAM outliers, which is <1% (Fig. 1). Z-scores for whole residues, helices, sheets, and loops were scaled independently, as shown in Table 1.

Molecular docking

Molecular docking study revealed that various active phytocompounds from *T. arjuna* show significant binding affinity with SARS-CoV-2 M^{pro}. Table 2 shows the list of top 5 phytocompounds from *T. arjuna* having highest binding affinity with M^{pro}.

Table 1: Z-score of protein 6LU7

S. No.	Type of residues	Z-score	No. of residues
1.	Whole Residues	–1.96	304
2.	Helices	–1.71	83
3.	Sheets	–1.34	38
4.	Loop	–1.04	183

Table 2: Top 5 phytocompounds from *T. arjuna* having highest binding affinity with M^{pro}

S. No.	PubChem compound ID	Name of phytocompound	Binding energy (Kcal/mol)
1.	CID_135452901	Hydroxyhomo Sildenafil	–8.7
2.	CID_10494	Oleanolic acid	–8.5
3.	CID_71597391	Triterpenoids	–8.3
4.	CID_135455980	N-Desmethyl Sildenafil	–8.3
5.	CID_2336	Benzo[A] Pyrene	–8.3

On the basis of results obtained from molecular docking studies using PyRx, we found that out of 215 compounds from *T. arjuna* 15 compounds show significant binding affinity (greater than 8Kcal/mol) with SARS-CoV-2 M^{pro}. From docking results, we selected top 5 compounds (Table 2) for Drug-likeness Prediction and ADME analysis.

Molecular visualization

Interactions between receptor-ligand of top 5 phytocompounds having highest binding affinity were visualized using Discovery Studio Visualizer 21.1. Docked ligands were saved in PDB file format using PyRx and then opened with purified protein SARS-CoV-2 M^{pro}. Different 2D and 3D interactions of phytocompounds with SARS-CoV-2 M^{pro} were observed. In diagram of 2D interactions, we observed the different interactions such as van-der Waal forces, conventional and carbon hydrogen bonds, π-sulfur interactions, alkyl and π-alkyl interactions, π-π T-shaped interactions, and unfavorable interactions.

Hydroxyhomo sildenafil

Hydroxyhomo Sildenafil forms different 2D-3D interactions with M^{pro}. It includes conventional hydrogen bonding with residues Thr 45, Ser 46 and Gly 143; carbon hydrogen bonding with Arg 188 residue only; π-sulfur bonding with Cys 145 and His 41; alkyl and π-alkyl bond with Met 49, Met 165, His 163, and His 172. Few van-der Waals interactions also formed by remaining residues, as shown in Fig. 2.

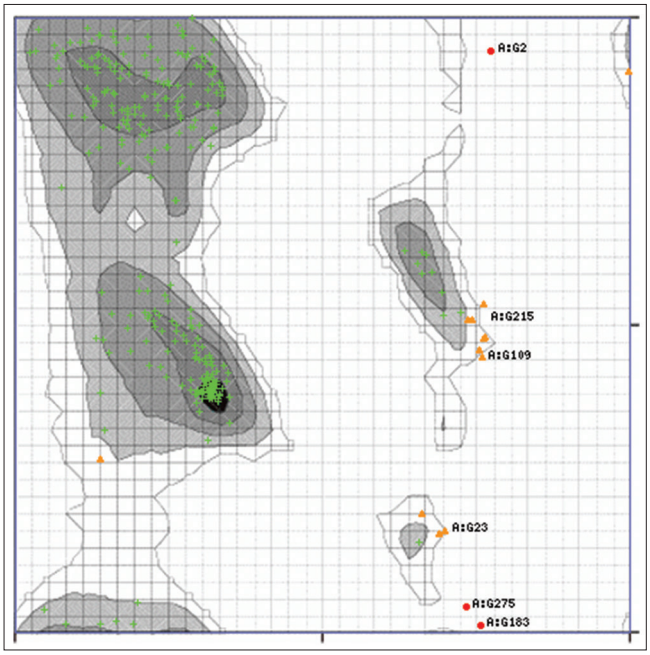

Fig. 1: Ramachandran Plot analysis of M^{pro}

Oleanolic acid

Oleanolic acid forms different 2D-3D interactions with Mpro. It includes conventional hydrogen bonding with residue Asp 289 only and also forms van-der Waals with other residues, as shown in Fig. 3.

Triterpenoids

Triterpenoids forms different 2D-3D interactions with Mpro. It includes conventional hydrogen bonding with two residues Thr 199 and Arg 131. It also forms one unfavorable acceptor-acceptor reaction with Asp 197. It also forms van-der Waals with other residues as shown in Fig. 4.

N-desmethyl sildenafil

N-Desmethyl Sildenafil forms different 2D-3D interactions with Mpro. It includes conventional hydrogen bonding with residue Gly 143; π-sulfur bonding with Cys 145; alkyl and π-alkyl bond with Met 49, Met 165, His 172, and His 163. Few van-der Waals interactions also formed by remaining residues, as shown in Fig. 5.

Benzo[A]Pyrene

Benzo[A]Pyrene forms different 2D-3D interactions with Mpro including π-π T-shaped interaction with only Phe 294 residue; π-Alkyl interactions with Val 104 and Ile 106 residues; and also forms van-der Waals with other residues, as shown in Fig. 6.

Drug-likeness prediction and ADMET analysis

Based on five aspects, Lipinski's rule of five helps to distinguish between compounds that are drug-like and non-drug like molecules. Drug-likeness prediction for the best docked compounds was done with Lipinski's rule of five and ADME analysis was performed using Swiss-ADME web server and ADMETLAB 2.0. Boiled-Egg analysis was also carried out using Swiss-ADME tool (Table 3). Furthermore, with the help of Swiss-ADME tool, Boiled-egg analysis was carried out for the prediction of passive brain access (BBB) and gastrointestinal absorption (HIA) of selected phytocompounds (Fig. 7).

In addition, best-docked compounds were analyzed within the standard scale for their water solubility (LogS), human intestinal absorption (HIA), Blood–Brain Barrier (BBB), Permeability Glycoprotein substrate (Pgp-sub), carcinogenic effects, and Lipinski's rule validation (Table 4).

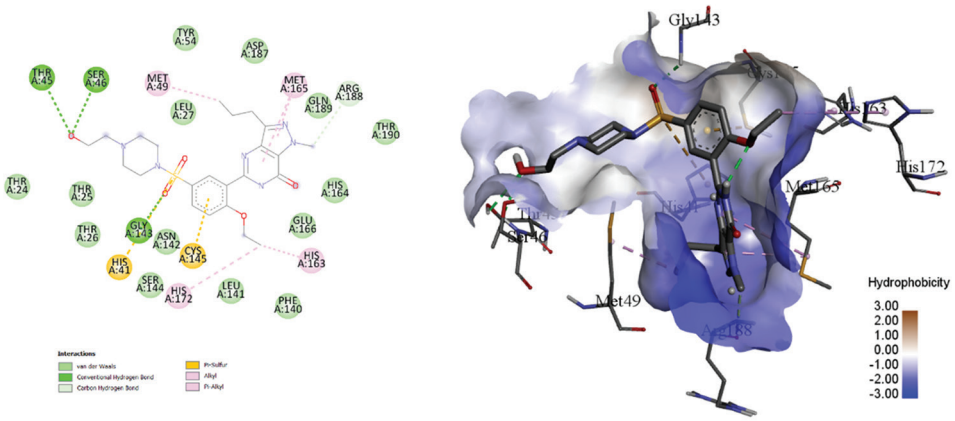

Fig. 2: 2D and 3D diagram of interactions of Hydroxyhomo Sildenafil with SARS-CoV-2 M^{pro}

Table 3: ADME analysis of best docked compounds based on Lipinski's rule

S. No.	Ligand Name	Molecular weight (g/mol)	H-Bond donor	H-Bond acceptor	LogP	Molar refractivity
1.	Hydroxyhomo Sildenafil	504.60	2	11	0.66	140.52
2.	Oleanolic acid	456.70	2	3	5.82	136.36
3.	Triterpenoids	472.66	4	5	3.85	133.99
4.	N-Desmethyl Sildenafil	460.55	2	8	0.98	129.65
5.	Benzo[A] Pyrene	252.31	0	0	6.32	87.65

Table 4: ADME analysis using Admetlab 2.0

S. No.	Ligand Name	LogS	HIA	Pgp-sub	BBB	Carcinogenicity	Lipinski's Rule
1.	Hydroxyhomo Sildenafil	−3.622	0.005	0.994	0.224	0.762	2 violations
2.	Oleanolic acid	−4.086	0.022	0	0.694	0.037	1 violation
3.	Triterpenoids	−3.699	0.084	0.001	0.84	0.525	0 violation
4.	N-Desmethyl Sildenafil	−3.543	0.004	0.999	0.15	0.301	0 violation
5.	Benzo[A] Pyrene	−8.053	0.027	0.791	0.586	0.936	1 violation

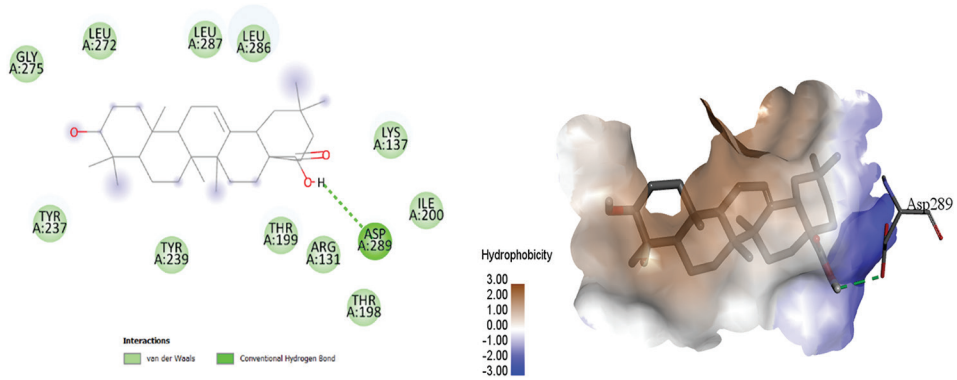

Fig. 3: 2D and 3D diagram of interactions of Oleanolic acid with SARS-CoV-2 M^{pro}

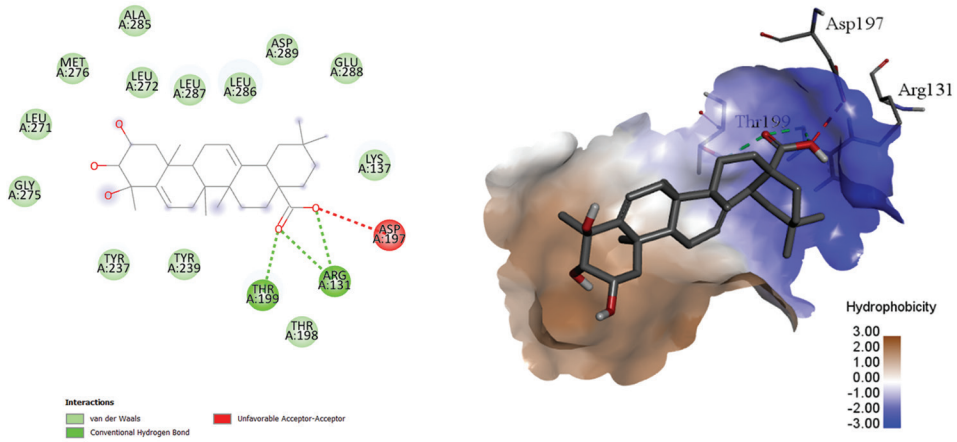

Fig. 4: 2D and 3D diagram of interactions of Triterpenoid with SARS-CoV-2 M^{pro}

DISCUSSION

In modern medicine, COVID-19 is treated with anti-viral and corticosteroid therapies individually and in combination too [22]. Natural phytocompounds from medicinal plants such as *T. arjuna* can be used, as they are less toxic than synthetic compounds. *In silico* methods such as molecular docking, ADME analysis, and molecular dynamic simulation shown to be beneficial for further research to analyze the binding affinity, interactions, and stability of the ligands with target. In addition to affecting the host's immunity, SARS-CoV-2 M^{pro} also promotes the proteolytic maturation of viral RNA into functional proteins such as RNA polymerases and ribonucleases. It helps in the translation of viral proteins to form some NSPs leads to viral transcription and replication. Hence, SARS-CoV-2 M^{pro} can be considered as significant target. Ramachandran plot shows the statistical distribution of the combinations of the backbone dihedral angles ϕ and ψ.

The present study revealed the role of phytocompounds from *T. arjuna* which has multiple medicinal properties according to Ayurveda. The research study states that some of the phytocompounds from *T. arjuna* have been found effective against COVID-19. For research

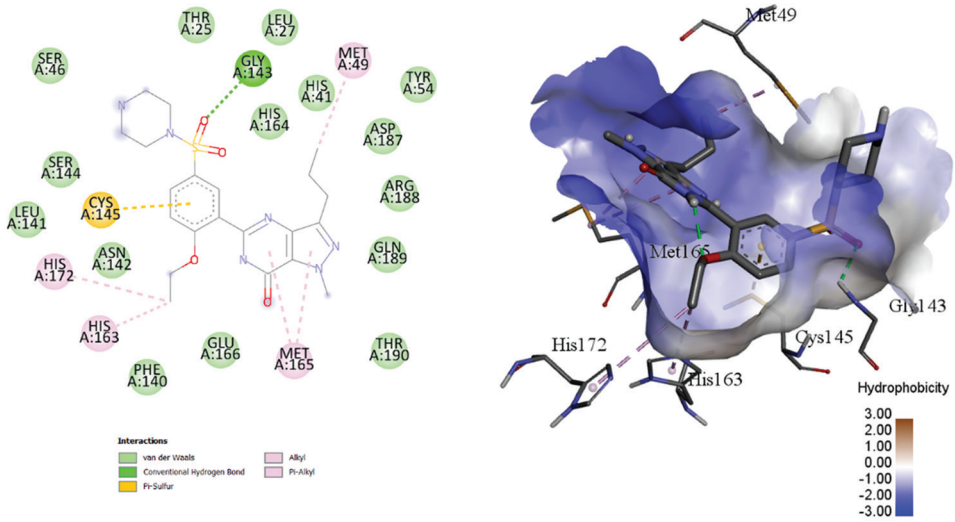

Fig. 5: 3D diagram of interactions of N-Desmethyl Sildenafil with SARS-CoV-2 M^{pro}

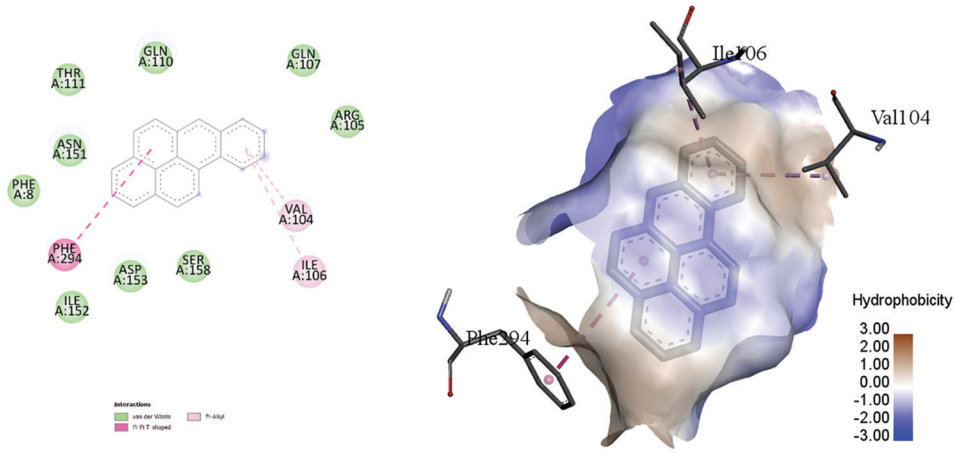

Fig. 6: 3D diagram of interactions of Benzo[A]Pyrene with SARS-CoV-2 M^{pro}

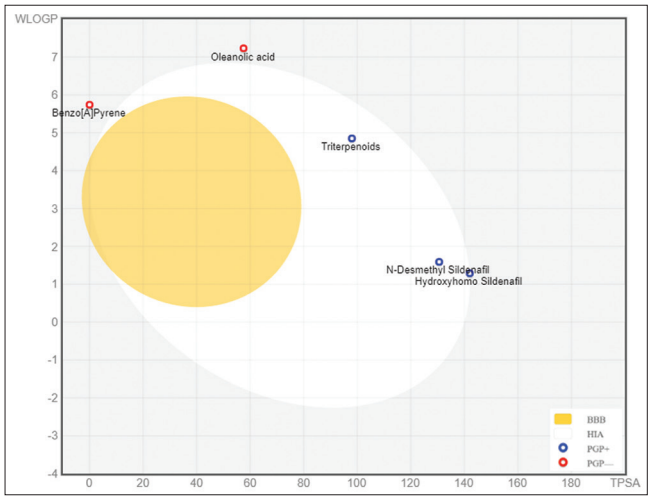

Fig. 7: Boiled-Egg analysis: Hydroxyhomo Sildenafil, Oleanolic acid, Triterpenoids, N-Desmethyl Sildenafil, Benzo[A]Pyrene

study, SARS-CoV-2 M^{pro} protein (6LU7) was analyzed for Ramachandran plot to validate the purity of the protein, and there were no outliers and poor rotamers observed. Z-Score of protein observed was –1.91 for whole residues. For *in silico* study, binding affinity of all the 215 phytocompounds was checked against SARS-CoV-2 protein (PDB ID: 6LU7) using molecular docking approach. Among them, five phytocompounds from *T. arjuna,* namely, Hydroxyhomo Sildenafil, Oleanolic acid, Triterpenoids, N-Desmethyl Sildenafil, and Benzo[A] Pyrene shown significant binding affinity. After molecular docking study, all the five compounds were further studied for ADME analysis to validate the drug likeness and toxicity. The binding of these phytochemicals with M^{pro} helps in cleavage of polyproteins to slow down the viral transcription and replication. Among these identified phytocompounds, Triterpenoid and N-Desmethyl Sildenafil can be predicted as potential inhibitors based on their significant binding affinity, Drug-likeness properties and ADMET prediction. These phytocompounds are helps to prevent interactions of viral protein into host cell and also found safe and effective against COVID-19 without toxicity.

CONCLUSION

The present study is accomplished with the aim to find out natural phytocompounds from *T. arjuna* as a curative against COVID-19. It is a disease triggered by SARS-CoV-2 virus which was turned into a global pandemic in 2020. M^{pro} protein of SARS-CoV-2 has significant potential and is a requisite target to prevent COVID-19. M^{pro} is a main protease which is important for growth of virus and proteolytic cleavage being a transit point for entry of the virus into host. Targeting main protease

with natural phytocompounds may inhibit viral entry, which, hence, resists further replication and propagation. From the present study, we can conclude that, two phytocompounds from *T. arjuna* predicted to suppress the action of SARS-CoV-2 M^{pro} by prevent the translation of viral proteins which assist in damaging host cells. These phytocompounds have high potential inhibition and best binding affinity with M^{pro}. The best-docked phytocompounds with drug-like properties, safe ADMET profile, and effective may help to develop optimized COVID-19 inhibitors and can be override as anti-COVID-19 Ayurvedic therapeutics.

ACKNOWLEDGMENT

We thank BioNome to provide support in training as well as in research projects.

AUTHORS' CONTRIBUTIONS

All the authors have contributed equally to the paper.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

FUNDING

Nil.

REFERENCES

1. World Health Organization. Coronavirus. (COVID-19) Dashboard. Geneva: World Health Organization. Available from: https://www.covid19.who.int [Last accessed on 2022 Aug 05].
2. Ministry of Health and Family Welfare, Government of India. New Delhi: Ministry of Health and Family Welfare, Government of India Gov.in. Available from: https://www.mohfw.gov.in [Last accessed on 2022 Aug 05].
3. Chan JF, Li KS, To KK, Cheng VC, Chen H, Yuen KY. Is the discovery of the novel human betacoronavirus 2c EMC/2012 (HCoV-EMC) the beginning of another SARS-like pandemic? J Infect 2012;65:477-89.
4. Zumla A, Chan JF, Azhar EI, Hui DS, Yuen KY. Coronaviruses-drug discovery and therapeutic options. Nat Rev Drug Discov 2016;15:327-47.
5. Yi Y, Lagniton PN, Ye S, Li E, Xu RH. COVID-19: What has been learned and to be learned about the novel coronavirus disease. Int J Biol Sci 2020;16:1753-66.
6. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, *et al*. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med 2020;382:727-33.
7. Ye ZW, Yuan S, Yuen KS, Fung SY, Chan CP, Jin DY. Zoonotic origins of human coronaviruses. Int J Biol Sci 2020;16:1686-97.
8. Wang L, Wang Y, Ye D, Liu Q. Review of the 2019 novel coronavirus (SARS-CoV-2) based on current evidence. Int J Antimicrob Agents 2020;55:105948.
9. Khan SA, Zia K, Ashraf S, Uddin R, Ul-Haq Z. Identification of chymotrypsin-like protease inhibitors of SARS-CoV-2 via integrated computational approach. J Biomol Struct Dyn 2021;39:2607-16.
10. Hilgenfeld R. From SARS to MERS: Crystallographic studies on coronaviral proteases enable antiviral drug design. FEBS J 2014;281:4085-96.
11. Khan RJ, Jha RK, Amera GM, Jain M, Singh E, Pathak A, *et al*. Targeting SARS-CoV-2: A systematic drug repurposing approach to identify promising inhibitors against 3C-like proteinase and 2'-O-ribose methyltransferases. J Biomol Struct Dyn 2021;39:2679-92.
12. Elmezayen AD, Al-Obaidi A, Şahin AT, Yelekçi K. Drug repurposing for coronavirus (COVID-19): *In silico* screening of known drugs against coronavirus 3CL hydrolase and protease enzymes. J Biomol Struct Dyn 2021;39:2980-92.
13. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, *et al*. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. bioRxivorg 2020;367:1260-3.
14. Mengist HM, Fan X, Jin T. Designing of improved drugs for COVID-19: Crystal structure of SARS-CoV-2 main protease Mpro. Signal Transduct Target Ther 2020;5:67.
15. Mittal L, Kumari A, Srivastava M, Singh M, Asthana S. Identification of potential molecules against COVID-19 main protease through structure-guided virtual screening approach. J Biomol Struct Dyn 2021;39:3662-80.
16. Anand K, Ziebuhr J, Wadhwani P, Mesters JR, Hilgenfeld R. Coronavirus main proteinase (3CL pro) structure: Basis for design of anti-SARS drugs. Science 2003;300:1763-7.
17. Zhang L, Lin D, Sun X, Curth U, Drosten C, Sauerhering L, *et al*. Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors. Science 2020;368:409-12.
18. De Oliveira OV, Rocha GB, Paluch AS, Costa LT. Repurposing approved drugs as inhibitors of SARS-CoV-2 S-protein from molecular modeling and virtual screening. J Biomol Struct Dyn 2021;39:3924-33.
19. Mothay D, Ramesh KV. Binding site analysis of potential protease inhibitors of COVID-19 using autodock. Virusdisease 2020;31:194-9.
20. Muralidharan N, Sakthivel R, Velmurugan D, Gromiha MM. Computational studies of drug repurposing and synergism of lopinavir, oseltamivir and ritonavir binding with SARS-CoV-2 protease against COVID-19. J Biomol Struct Dyn 2021;39:2673-8.
21. Kreil TR, Farcet MR. Immunoglobulins and virus antibody titers: Of past needs, current requirements, and future options. Transfusion 2018;58:3090-5.
22. World Health Organization. Welcomes Preliminary Results about Dexamethasone Use in Treating Critically ill COVID-19 Patients? Who. int. Geneva: World Health Organization. Available from: https://www.who.int/news-room/detail/16-06-2020-who-welcomes-preliminary-results-about-dexamethasone-use-in-treating-critically-ill-covid-19-patients [Last accessed on 2022 Aug 05].
23. Singh RH, Narsimhamurthy K, Singh G. Neuronutrient impact of ayurvedic rasayana therapy in brain aging. Biogerontology 2008;9:369-74.
24. Arumugam S, Dash SS, Mitra K, Doble M, N.Gummadi S. Potential of *Terminalia* Arjuna as a promising phytoremedyagainst COVID-19: DPPH scavenging, catalase inhibition and molecular docking studies. ChemRxiv 2020;137.
25. Subramaniam S, Subramaniam R, Rajapandian S, Uthrapathi S, Gnanamanickam VR, Dubey GP. Anti-atherogenic activity of ethanolic fraction of *Terminalia* Arjuna bark on hypercholesterolemic rabbits. Evid Based Complement Alternat Med 2011;2011:487916.
26. Dwivedi S, Jauhari R. Beneficial effects of *Terminalia* Arjuna in coronary artery disease. Indian Heart J 1997;49:507-10.
27. Ram A, Lauria P, Gupta R, Kumar P, Sharma VN. Hypocholesterolaemic effects of *Terminalia* Arjuna tree bark. J Ethnopharmacol 1997;55:165-9.
28. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, *et al*. The protein data bank. Nucleic Acids Res 2000;28:235-42.
29. Islam R, Parves MR, Paul AS, Uddin N, Rahman MS, Mamun AA, *et al*. A molecular modeling approach to identify effective antiviral phytochemicals against the main protease of SARS-CoV-2. J Biomol Struct Dyn 2021;39:3213-24.
30. Anderson RJ, Weng Z, Campbell RK, Jiang X. Main-chain conformational tendencies of amino acids. Proteins 2005;60:679-89.
31. Williams CJ, Headd JJ, Moriarty NW, Prisant MG, Videau LL, Deis LN, *et al*. MolProbity: More and better reference data for improved all-atom structure validation. Protein Sci 2018;27:293-315.
32. Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, *et al*. PubChem in 2021: New data content and improved web interfaces. Nucleic Acids Res 2021;49:D1388-95.
33. Mcconkey BJ, Sobolev V, Edelman M. The performance of current methods in ligand-protein docking. Curr Sci 2002;83:845-55.
34. Trott O, Olson AJ. Autodock vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem 2010;31:455-61.
35. O'Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR. Open babel: An open chemical toolbox. J Cheminform 2011;3:33.
36. Biovia DS. Discovery Studio Modeling Environment. San Diego: Dassault Systemes; 2015. Available from: https://www.scirp.org/(S(351jmbntv-nsjt1aadkposzje))/reference/referencespapers.aspx?referenceid=2450411 [Last accessed on 2022 Aug 05].
37. Wikipedia Contributors. ADME. Wikipedia, The Free Encyclopedia; 2022. Available from: https://en.wikipedia.org/w/index.php?title=ADME&oldid=1087865915 [Last accessed on 2022 Aug 05].
38. Daina A, Michielin O, Zoete V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep 2017;7:42717.
39. Xiong G, Wu Z, Yi J, Fu L, Yang Z, Hsieh C, *et al*. ADMETlab 2.0: An integrated online platform for accurate and comprehensive predictions of ADMET properties. Nucleic Acids Res 2021;49:W5-14.
40. Daina A, Zoete V. A BOILED-Egg to predict gastrointestinal absorption and brain penetration of small molecules. ChemMedChem 2016;11:1117-21.