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Multi-target potential of Indian phytochemicals against SARS-CoV-2: A docking, molecular dynamics and MM-GBSA approach extended to Omicron B.1.1.529.

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ABSTRACT

Background: SARS-CoV-2, an emerged strain of corona virus family became almost serious health concern worldwide. Despite vaccines availability, reports suggest the occurrence of SARS-CoV-2 infection even in a vaccinated population. With frequent evolution and expected multiple COVID-19 waves, improved preventive, diagnostic, and treatment measures are required. In recent times, phytochemicals have gained attention due to their therapeutic characteristics and are suggested as alternative and complementary treatments for infectious diseases. This present study aimed to identify potential inhibitors against reported protein targets of SARS-CoV-2.

Methodology: We computationally investigated potential SARS-CoV-2 protein targets from the literature and collected druggable phytochemicals from Indian Medicinal Plants, Phytochemistry and Therapeutics (IMPPAT) database. Further, we implemented a systematic workflow of molecular docking, dynamic simulations and generalized born surface area free-energy calculations (MM-GBSA).

Results: Extensive literature search and assessment of 1508 articles identifies 13 potential SARS-CoV-2 protein targets. We screened 501 druggable phytochemicals with proven biological activities. Analysis of 6513(501 *13) docked phytochemicals complex, 26 were efficient against SARS-CoV-2. Amongst, 4,8-dihydroxysesamin and arboreal from *Gmelina arborea* were ranked potential against most of the targets with binding energy ranging between − 10.7 to − 8.2 kcal/mol. Additionally, comparative docking with known drugs such as arbidol (−6.6 to −5.1 kcal/mol), favipiravir (−5.5 to −4.5 kcal/mol), hydroxychloroquine (−6.5 to −5.1 kcal/mol), and remedesivir (−8.0 to −5.3 kcal/mol) revealed equal/less affinity than 4,8-dihydroxysesamin and arboreal. Interestingly, the nucleocapsid target was found commonly inhibited by 4,8-dihydroxysesamin and arboreal. Molecular dynamic simulation and Molecular mechanics generalized born surface area (MM-GBSA)calculations reflect that both the compounds possess high inhibiting potential against SARS-CoV-2 including the recently emerged Omicron variant (B.1.1.529).

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Conclusion: Overall our study imparts the usage of phytochemicals as antiviral agents for SARS-CoV-2 infection. Additional *in vitro* and *in vivo* testing of these phytochemicals is required to confirm their potency.

1. Introduction

COVID-19 is an infectious disease caused by the SARS-CoV-2 virus, which leads to pneumonia [1] and multi-organ failure [2]. SARS-CoV-2 was first reported in China. Later the disease was declared a pandemic by the World Health Organization (WHO) [3] due to the outbreak in December 2019. It spreads almost in 218 nations, resulting in increasing death rates. SARS-CoV-2 is an RNA virus [4] with a genomic size of 30 kbp [5] that encodes four structural proteins and 16 non-structural proteins (NSP) [6]. Among these proteins, a few are identified as potential targets for inhibiting its pathogenesis. There is no specific drug available to treat COVID-19, which may be the primary cause of increased mortality. Despite the vaccinated population, reports suggest the possible reoccurrence of COVID-19 infection due to the genetic alteration in SARS-CoV-2. Recently, a new variant of SARS-CoV-2 named Omicron (B.1.1.529) have emerged in South Africa 2021 [7] is considered one of the fast-spreading strain among SARS-CoV-2. With multiple COVID-19 waves expected around the world, improved preventive, diagnostic, and treatment measures are required.

Recent concept of repurposing the existing drugs to treat SARS-CoV-2 had proven to save time in avoiding pharmacological testing [8]. Particularly, the drugs like arbidol [9], favipiravir [10], hydroxychloroquine [11], and remedesivir [12] are repurposed to effectively handle the pandemic situation. However, the demand for these drugs during pandemic has certainly increased themortality. Also, there are reported side effects while usage of these drugs [13-16]. Considering the long run with the repeated pandemic wavesthere is a need for the development of specific drugs for SARS-CoV-2 infection. Finding suggest the successful use of traditional medicine for COVID-19 treatment has created an opportunity towards alternate therapy [17]. Among the alternate treatment strategies, phytochemical based approach against viral infection are well-known for diseases like Chikungunya, Ebola, and Zika [18-20]. Thereby, it is believed to be more suitable for the future emergency and pandemic situation. Several phytochemicals are being assessed against SARS-CoV-2 of coronavirus family. Particularly, few clinical trials for COVID-19 using Indian herbs like *Piper longum* [21], *Glycyrrhiza glabra* [22], *Tinospora cordifolia* [23], and *Withania somnifera* Aarogyam UK. [24] are in progress. Additionally, WHO also promotes the utility and development of natural medicines for human illness. As a result, it is important to look for the bioactive natural compounds against SARS-CoV-2 [25]. Several phytochemicals are being assessed based on bioinformatics approach against this virus. Most studies were focused to screen phytochemicalsagainst one or two protein targets of SARS-CoV-2. Alternatively, there are no studies reporting the capability of phytochemicals simultaneously inhibiting multiple targets of SARS-CoV-2, which may benefit any emerging strains.

In this study, 501 active molecules belonging to various Indian medicinal plants were selectedto perform molecular docking against 13 reported protein targets of SARS-CoV-2. The molecular affinity of phytochemicals bound to the protein targets have been analyzed. Among them, arboreal and 4,8-dihydroxysesamin from *Gmelina arborea* species showed significant affinity to most of the protein targets, than known drugs of SARS-CoV-2. Particularly, arboreal and 4,8-dihydroxysesamin were noticed inhibiting SARS-CoV-2 efficiently on binding to the nucleocapsid protein target. Further analysis showed conformational changes in nucleocapsid induced by arboreal and 4,8-dihydroxysesamin during the molecular dynamic (MD) simulations. Also, the MM-GBSA approach revealed that both arboreal and 4,8-dihydroxysesamin can potentially exhibit equal affinity like hydroxychloroquine and remedesivir.

2. Materials and methods

2.1. Target identification

A systematic literature search was carried out in PubMed/MEDLINE, Google Scholar, and preprint databases to identify studies reporting molecular targets for SARS-CoV-2 from 31 December 2019 (first reported case in Wuhan, China) to June 2021. Two authors (GK and DN) independently reviewed the titles and abstracts of the articles. Any duplicates in the collected articles were removed. Relevant abstracts were shortlisted and full-length papers were assessed for the eligibility by other three authors (JR and VR) and any disagreement was resolved by group discussion with the authors (SP and SSJ). The selection of article is based on inclusion and exclusion criteria. The inclusion criteria was *the article reporting molecular targets of SARS-CoV-2* and the exclusion criteria were: 1) *Studies that did not report molecular targets in SARS-CoV-2*; and 2) *Clinical, preclinical, incidence, prevalence studies, case or news reports*. From the selected articles, following information were extracted such as the first author name, country, name of the molecular targets, (Protein Data Bank) PDB IDs, name of the ligands/compounds, and any accessible ligands/compounds IDs to retrieve the structures from databases.

2.2. Protein target preparation

Based on the collected information, 13 proteins targets of SARS-CoV-2 were shortlisted. The three dimensional (3D) structures of 13 targets were retrieved from RCSB protein data bank in the PDB format. Each structure was cleaned by1) removing the water molecules, unwanted chains and co-crystallized ligands, 2) Adding missing hydrogens to the chains, 3) optimizing the H-bonds, and 4) applying force field to have stable conformation [26]. Additionally, the homology modelling was performed by following the [27] to generate nucleocapsid structure for SARS-CoV-2 Omicron variant (B.1.1.529) from the protein sequence retrieved from NCBI GenBank: (Accession no. UFO69287.1). Further the modelled structure was refined [28] for later assessment.

2.3. Phytochemical collection

The phytochemicals used in this study were collected from IMPPAT database [29]. IMPPAT is publically accessible to download the compounds structure for virtual screening. Currently, IMPPAT has more than 9500 phytochemicals with biologically relevant information. Of 9500 available structures [Accessed on: July 2021], a set of phytochemicals were selected based on the druggable properties, which include Lipinski rule of five and Oral PhysChem score. Simultaneously, the structure of known drugs such as arbidol (*131411*), favipiravir (*492405*), hydroxychloroquine (*3652*), and remedesivir (*121304016*) were collected from the NCBI-PUBCHEM

compound database. All selected molecules were imported to UCSF Chimera 1.14 for structural geometry optimization.

2.4. Molecular docking

The molecular docking was performed using Vina [30] Autodocking module in Python Prescription Virtual Screening Tool (PyRx) software [31]. Every phytochemical (n = 501) and known drugs (n = 4) were subjected to docking against each target protein to determine its binding energy (kcal/mol). Each docking was done in triplicates, to get a clear picture of the binding pose and least binding energy. Further, the top-3 phytochemicals showing least binding energy to each target was selected for further analysis.

2.5. Molecular dynamic simulation

Among top-3 phytochemicals of each target, arboreal and 4,8-dihydroxysesamin were over-represented against multiple targets. Of these targets, nucleocapsid was noticed to be inhibited by both arboreal and 4,8-dihydroxysesamin with high affinity. Following phytochemicals and target selection, MD simulations were performed using the GROMACS 2020.4 version for six nucleocapsid-ligand complexes that includes, two phytochemicals (arboreal and 4,8-dihydroxysesamin) and four drugs (arbidol, favipiravir, hydroxychloroquine, and remedesivir). The simulations were performed for up to 10 ns for each complex system. The GROMOS54a7 and single-point charge (SPC) were implemented. The topologies files for the phytochemicals and the drugs were generated using PRODRG server. All the complexes were simulated inside the cubic box with ions neutralized. In energy minimization, steps of steepest decent method (n = 5000) was adopted to resolve bad contacts and clashes in the complexes. Following the energy minimization, NVT and NPT equilibration were performed. Further simulations were performed to assess random mean square deviation (RMSD) and root-mean-square fluctuation (RMSF) between the complexes based on time frame.

2.6. MM-GBSA analysis

MM-GBSA calculates energy of the optimized proteins, ligand, and its complex. MM-GBSA was calculated for the phytochemicals (arboreal and 4,8-dihydroxysesamin) and drugs (arbidol, favipiravir, hydroxychloroquine, and remedesivir) against nucleocapsid protein using Prime MM/GBSA module of Schrodinger maestro version 11.2. Additionally, the MM-GBSA energy calculation was extended to the nucleocapsid structure of Omicron variant (B.1.1.529) generated based on homology modelling against six molecules for comparative analysis.

3. Results

3.1. Protein target identification

Based on the literature search strategy described in the methodology section, 1508 relevant articles were retrieved for the initial phase of assessment. The authors determined that the titles and abstracts contained in 90 articles were worthy for full-text reading. Among which, 23 studies were considered suitable to collect protein targets according to the inclusion/exclusion criteria.

3.2. Protein target preparation

From the selected studies, SARS-CoV-2 protein targets such as Replicase polyprotein 1a, ADP ribose phosphatase of NSP3, E-protein, NSP16, NSP1, NSP15, NSP13 helicase, NSP12, spike glycoprotein, $3CL^{pro}$, PL^{pro}, RNA-dependent RNA polymerase (RdRp), and nucleocapsid were collected. For each protein, the PDB structure was retrieved from the RCSB database using the PDB IDs mentioned in the articles. The appropriate protein structure, *6YHU* (replicase polyprotein 1a), *6VXS* (ADP ribose phosphatase of NSP3), *2MM4* (E protein), *6W61* (NSP16), *7K7P* (NSP1), *6W01* (NSP15), *6ZSL* (NSP13 helicase), *6NUR* (NSP12), *7BZ5* (Spike glycoprotein), *6LU7* ($3CL^{pro}$), *4OW0* (PL^{pro}), *6M71* (RdRp) and *6M3M* (nucleocapsid) were taken from PDB using its Accession IDs. Each structure was processed to have stable conformation before molecular docking.

3.3. Phytochemicals collection

Among the 9500 phytochemicals in IMPPAT database, 908 were identified as druggable compounds. These selected phytochemicals belong to more than 400 plant species of Indian origin. The characteristics of these phytochemicals were flavonoids, lipids, benzoxepines, naphthopyrans, coumarins, indolizidines and organooxygen compounds. Few of these phytochemicals were noticed common among the plants. After removing duplicates, a total of 501 phytochemicals were retained. For comparative analysis, the structure of known drugs such as arbidol, favipiravir, hydroxychloroquine, and remedesivir were collected. The structure of each molecule was retrieved in PDB format and processed as mentioned in methodology section.

3.4. Molecular docking

The PyRx software 0.8 version was used to perform molecular docking. A total of 6513 (501 *13) docking was performed in triplicates with 501 phytochemicals against 13 protein targets. Similarly, four drugs namely, arbidol, favipiravir, hydroxychloroquine and remedesivir were docked against 13 targets. Several binding modes for the phytochemicals and drugs across the targets were predicted. A list of 39 phytochemicals (13 targets *top-3 phytochemicals) showing least binding energy to each of the targets were collected (Table 1). Among these 39, a few phytochemicals showed least binding energy to the multiple targets. For example, arboreal showed least binding energy to NSP16 (*6W61*), NSP13 helicase (*6ZSL*), NSP12 (*6NUR*), $3CL^{pro}$ (*6LU7*), RdRp (*6M71*) and nucleocapsid (*6M3M*) (Table 1).4,8-dihydroxysesamin exhibit least binding energy to NSP13, spike glycoprotein, PL^{pro}, RdRp and nucleocapsid. Similarly, all four drugs (arbidol, favipiravir, hydroxychloroquine and remedesivir) showed the binding energy in the range between − 8.0 to − 4.5 kcal/mol (Table 2). From Table 1, arboreal and 4,8-dihydroxysesamin noticed to have high affinity to multiple proteins. Particularly, arboreal and 4,8 dihydroxysesamin, were noticed to have high affinity against nucleocapsid in common. The three dimensional molecular interactions were visualized for nucleocapsid with arboreal, 4,8-dihydroxysesamin, arbidol, favipiravir, hydroxychloroquine and remedesivir (Fig. 1-2). MD simulations were performed for the arboreal-nucleocapsid and 4,8-dihydroxysesamin-nucleocapsid complexes and compared with the four nucleocapsid-drug complexes.

3.5. Molecular dynamic simulation

Six individual MD simulations were performed to determine the structural stability of nucleocapsid with arboreal, 4,8-dihydroxysesamin, arbidol, favipiravir, hydroxychloroquine, and remedesivir. Based on RMSD and RMSF, the stability of simulated systems were evaluated. The average RMSD values for arboreal and 4,8-dihydroxysesamin complexes were 2.46 Å and 4.36 Å, respectively. Similarly, the average RMSD forarbidol, favipiravir, hydroxychloroquine, and remedesivir were ranged between 1.55 and 1.92 Å. After 500 frames, 4,8-dihydroxysesamin complex presented two higher trajectories than the drugs. One was observed between 500 and 700 frames, while the other was after 800 frames. Comparatively, arboreal

Table 1
Binding energies of top-three phytochemicals with each target protein.

Sl. NO	Protein target	Phytochemicals	Binding energy (kcal/mol)
1	Replicase polyprotein 1a	Medicagol	-8.2
		Maruquine	-8.1
		(+)-arborone	-7.9
2	ADP ribose phosphatase of NSP3	Micromelin	-7.5
		DL-Catechin	-7.4
		Alectrol	-7.4
3	E protein	Dicumarol	-7.4
		Dicumarol	-6.7
		Rhazimol	-6.7
4	NSP16	Medicagol	-8.3
		Isomaruquine	-8.2
		Arboreol	-8.2
5	NSP1	Corylidin	-7.6
		Chaparrinone	-7.3
		Gummadiol	-7.0
6	NSP15	7-O-lactoly-apigenin	-8.7
		Isoscutellarein	-8.6
		Artocarpus	-8.5
7	NSP13 helicase	Arboreol	-9.3
		4,8-dihydroxysesamin	-9.3
		Nepetaefolinol	-9.2
8	NSP 12	Hetidine	-9.4
		Gibberellins	-9.1
		Arboreol	-9.1
9	Spike glycoprotein	Scutellarein	-8.9
		4,8-dihydroxysesamin	-8.9
		Luteoforol	-8.8
10	$3CL^{pro}$	Arboreol	-8.2
		Rhein	-8.0
		Isomaruquine	-7.9
11	PL^{pro}	4,8-dihydroxysesamin	-10.3
		Medicagol	-10.3
		Nororientaline	-10.0
12	RdRp	Arboreol	-8.9
		Izmirine	-8.7
		4,8-dihydroxysesamin	-8.6
13	Nucleocapsid	4,8-dihydroxysesamin	-10.7
		Arboreol	-10.6
		Haematoxylin	-10.2

exhibited two higher fluctuations at 500 and 900 frames. Similarly, the RMSF was calculated to determine the individual residue flexibility of the system. All the six systems (nucleocapsid-arboreal, nucleocapsid-4,8-dihydroxysesamin, nucleocapsid-arbidol, nucleocapsid-favipiravir, nucleocapsid-hydroxychloroquine, and nucleocapsid-remedesivir complex) demonstrated almost a similar pattern of fluctuation during simulation. The RMSF average for nucleocapsid-arboreal, nucleocapsid-4,8-dihydroxysesamin, nucleocapsid-arbidol, nucleocapsid-favipiravir, nucleocapsid-hydroxychloroquine, and nucleocapsid-remedesivir complexes were 0.89, 1.03, 1.83, 0.81, 0.86, and 0.87 Å, respectively.

3.6. MM-GBSA analysis

The MM-GBSA is one of the most popular methods to determine the binding affinity of small compounds to protein. The evaluated binding free energy of arboreal (−33.75 kcal/mol), 4,8-dihydroxysesamin (−35.80 kcal/mol), arbidol (−34.04 kcal/mol), favipiravir (−21.05 kcal/mol), hydroxychloroquine (−35.60 kcal/mol), and remedesivir (−35.97 kcal/mol) to nucleocapsid was shown in Table 3. As compared with the drugs, arboreal and 4,8-dihydroxysesamin showed the maximum negative binding energy. Additionally, the binding free energy of arboreal, 4,8 dihydroxysesamin, arbidol, favipiravir, hydroxychloroquine, and remedesivir with nucleocapsid of Omicron variant (B.1.1.529) were − 30.11, − 34.67, − 23.38, − 18.30, − 35.68 and − 36.75 kcal/mol, respectively (Table 3).

4. Discussion

COVID-19 pandemic triggered by rapidly evolving SARS-CoV-2 remains as a threat to the human society [32]. After, sequencing genome and identifying the crucial viral proteins, scientists across the world are attempting to design effective drugs against the SARS-CoV-2. In recent times, several studies support the usage of traditional medicines for viral diseases [33]. It is anticipated that phytochemicals will produce a non-toxic inhibitory effect against SARS-CoV-2 [34].

In this study, 501 druggable phytochemicals from the IMPPAT database were selected and docked against 13 reported proteins of SARS-CoV-2 (B.1.351). Besides, four known drugs such as arbidol, favipiravir, hydroxylchloroquine and remedesivir, were used as control for this study (Table 2). Of 6513 executed docking, few phytochemicals were found efficient to interact with the viral targets. Among them, the top-3 phytochemicals for each protein target was selected based on the order of least binding energies (Table 1). For instance, spike glycoprotein was noticed to be inhibited by 4,8-dihydroxysesamin (−8.9 kcal/mol), scutellarein (−8.9 kcal/mol), and luteoforol (−8.8 kcal/mol). Whereas, RdRp was inhibited by arboreal (−8.9 kcal/mol), izmirine (−8.7 kcal/mol), and 4,8-dihydroxysesamin (−8.6 kcal/mol). Also, NSP13 was inhibited by arboreol (−9.3 kcal/mol), 4,8-dihydroxysesamin (−9.3 kcal/mol) and Nepetaefolinol (−9.2 kcal/mol). From Table 1, arboreal exhibit interactions with six targets such as NSP16, NSP13, NSP12, $3CL^{pro}$, RdRp and nucleocapsid. Likewise, 4,8-dihydroxysesamin exhibit efficient interactions with five targets such as NSP13, spike glycoprotein, PL^{pro}, RdRp and nucleocapsid. Both arboreal and 4,8-dihydroxysesamin commonly inhibit nucleocapsid with least binding energy.

Arboreal binds with nucleocapsid of SARS-CoV-2 (−10.6 kcal/mol) at GLY70, PRO163, GLN84, VAL73 and LEU162 residues (Fig. 1A). Similarly, 4,8-dihydroxysesamin with nucleocapsid of SARS-CoV-2 (−10.7 kcal/mol), depicts four hydrogen bonds with GLY70, PRO163, THR166 and LEU162, three alkyl bonds with ILE75, LEU160 and LEU162, three carbon hydrogen bonds with THR166, LEU162 and GLN161 (Fig. 1B). Whereas, arbidol exhibit interaction with nucleocapsid at THR136, GLN164, GLY70, PRO163, ILE75 and PRO81 residues (Fig. 2 A). Likewise, favipiravir (Fig. 2B), and hydroxychloroquine (Fig. 2C) interact with nucleocapsid with the binding energy between − 5.2 to − 4.5 kcal/mol. Remedesivir showed interactions with nucleocapsid at ASN76, GLU84, ILE75, PRO163, and PRO81 with binding energy(−5.3 kcal/mol) (Fig. 2D).

Table 2
Binding energies of antiviral drugs with protein targets.

Protein targets	**Hydroxychloroquinone** Binding energy (kcal/mol)	**Arbidol** Binding energy (kcal/mol)	**Favirapir** Binding energy (kcal/mol)	**Remedesivir** Binding energy (kcal/mol)
NSP16	-6.5	-6.3	-5.5	7.6
NSP13	-6.4	-5.2	-5.4	-8
NSP12	-6.2	-6.4	-4.9	7.6
$3CL^{pro}$	-6.2	-6.3	-5	-7.5
RdRp	-5.8	-6.6	-5.1	-7.6
Nucleocapsid	-5.1	-5.1	-4.5	-5.3
Spike	-5.1	-5.2	-4.7	-6.3

Fig. 1. Two dimensions interaction plot of 4, 8-dihydroxysesamin and arboreal with the nucleocapsid protein. Arboreal(A) and 4, 8-dihydroxysesamin (B) interacts with nucleocapsid targetthrough pi-sigma, hydrogen, pi-anion, alkyl interactions with their surrounding amino-acids.

Table 3
MM-GBSA calculations.

Sl. No	Protein	Ligands	dG Bind kcal/mol	dG Bind Coulomb kcal/mol	dG Bind Covalent kcal/mol	dG Bind H bond kcal/mol	dG Bind Lipo kcal/mol
1	Nucleocapsid of SARS-CoV-2(B.1.351)	Remedesivir	-36.00	-27.85	2.82	-2.63	-8.86
		Arbidol	-34.05	-131.30	3.31	-1.13	-13.21
		Favipiravir	-21.04	-17.20	0.40	-1.14	-3.28
		Hydroxychloroquine	-35. 60	-57.70	2.43	-1.79	-8.98
		4,8-dihydroxysesamin	-35.80	-11.10	0.38	-1.56	-11.39
		Arboreal	-33.74	-17.04	2.78	-3.04	-11.09
2	Nucleocapsid of SARS-CoV-2Omicron (B.1.1.529)	Remedesivir	-36.75	-12.75	3.85	-2.44	-9.37
		Arbidol	-23.38	1.04	0.87	-0.43	-10.29
		Favipiravir	-18.30	-2. 57	-0.12	-0. 65	-3.82
		Hydroxychloroquine	-35. 68	-5.32	2.843	-1.10	-12.75
		4,8-dihydroxysesamin	-34. 67	-13.07	0.93	-1. 61	-10.73
		Arboreal	-30.11	-19.11	1.26	-1.26	-10.26

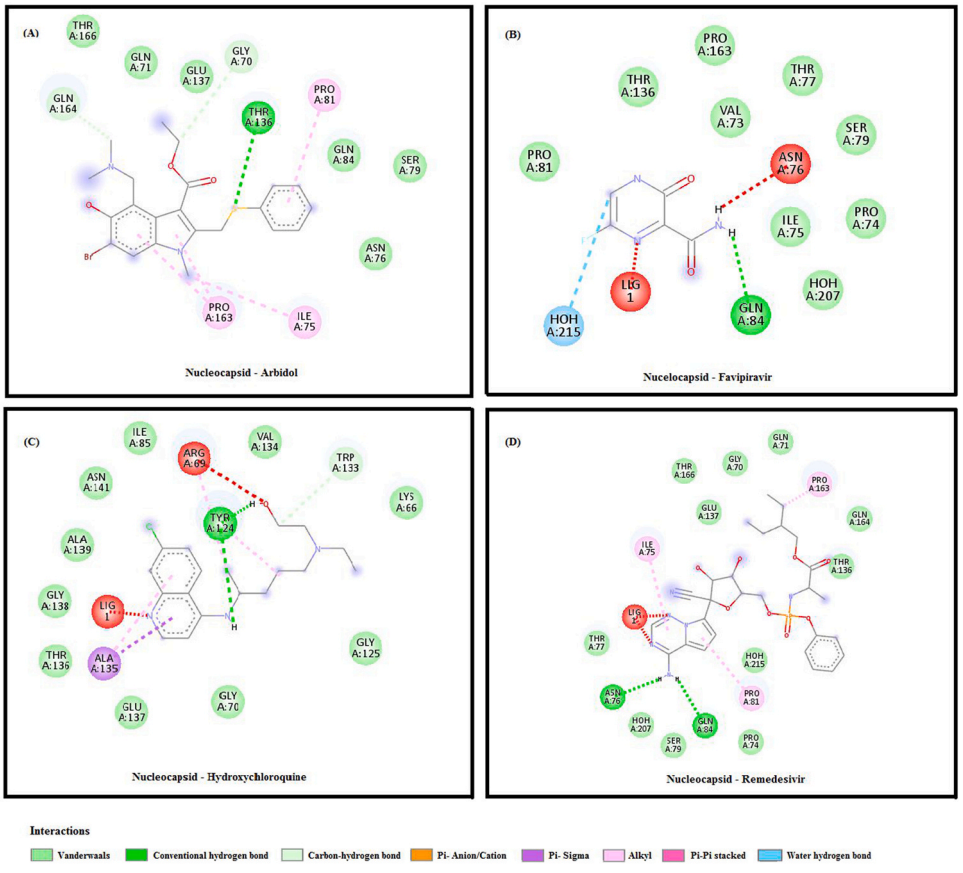

Fig. 2. Two dimensions interaction plot of antiviral drugs with the nucleocapsid protein Four drugs like, arbidol (A), favipiravir (B), hydroxychloroquine (C) and remedesivir (D) interacts with nucleocapsidthrough pi-sigma, hydrogen, pi-anion, alkyl interactions with their surrounding amino-acids.

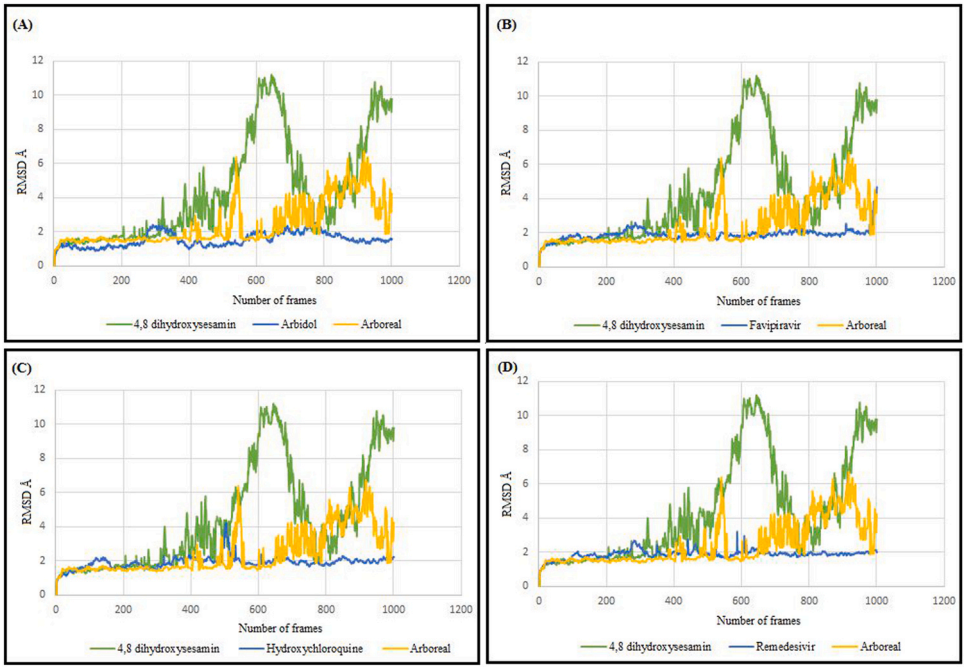

Fig. 3. RMSD analysis. Time dependence of the RMSDs of SARS-CoV-2 nucleocapsid protein after binding with arboreal, 4,8-dihydroxysesamin, arbidol, favipiravir, hydroxychloroquine, and remedesivir.

To date, several molecular docking studies were performed using phytochemicals against any of these 13 viral targets. Most studies were focused on single target with multiple phytochemicals. [28], report Chrysin, Emodin, Hesperidin, Anthraquinone, and Rhein to be efficient against spike protein with binding energies between − 8.9 to − 6.7 kcal/mol. Similar study conducted with 127 phytoconstituents from *Menthaarvensis, Coriandrum sativum* and *Ocimum sanctum* resulted in binding energies between − 10.1 to − 6.2 kcal/mol against nucleocapsid target [35]. Also, a study with 154 phytochemicals from limonoids and triterpenoids family against $3CL^{pro}$ and PL^{pro} exhibit binding energies ranging from − 9.1 to − 7.1 kcal/mol [36]. Recently, [37] executed docking with large collection of 2035 phytochemicals including alkaloids, flavonoids, lignans, carboxylic acids, polyphenols quinones, terpenoids, aurones, and chalcones against NSP13 and NSP16, which demonstrates binding energy values between − 8.6–8.1 kcal/mol. Likewise, [8] report compounds from *Hyssopus officinalis* and *Withania somnifera* showing affinity to RdRp with energy between − 9.0 to − 7.7 kcal/mol. Comparing to all the phytochemicals used in the above studies, both arboreal and 4,8-dihydroxysesamin from *Gmelina arborea*showed maximum binding affinities to most of the SARS-CoV-2 targets (Table 1). Furthermore, we have tried to explain the importance of our results using the nucleocapsid protein as a common target for both the phytochemicals with least binding energies.

Based on the docking scores, MD simulation were performed to investigate and compare the stability of nucleocapsid-arboreal and nucleocapsid-4,8-dihydroxysesamin with the nucleocapsid-drugs complexes. From Fig. 3A-D, based on the average differences in RMSD, we suggest that both phytochemicals were able to form significant interaction with the target. Similarly, RMSF was calculated to identify the fluctuations in nucleocapsid regions upon ligand binding. The RMSF plot displayed in Fig. 4A-D shows that both phytochemicals and drug complexes exhibit similar pattern with fluctuation at specific residues. Furthermore, the binding energies of arboreal, 4.8-dihydroxysesamin and drugs were evaluated using MM-GBSA. From Table 3, arboreal (−33.75 kcal/mol) and 4,8-dihydroxysesamin (−35.80 kcal/mol) exhibited equal effect like arbidol, favipiravir, hydroxychloroquine and remedesivir against nucleocapsid of SARS-CoV-2 (B.1.351). Additionally, the benefit of these phytochemicals were assessed against nucleocapsid protein of emerging Omicron variant (B.1.1.529). The arboreal (−30.11 kcal/mol) and 4,8-dihydroxysesamin (−34.67 kcal/mol) remain to have maximum negative binding energy like remedesivir and hydroxychloroquine. These natural compounds with the better binding affinity could be utilized as potential inhibitors against the nucleocapsid of COVID-19.

Hence, arboreal and 4, 8-dihydroxysesamin is found to be comparatively efficient to the well-known drugs such as remedesivir, arbidol, favipiravir, and hydroxychloroquine. Both the phytochemicals are derived from *Gmelina arborea*, a deciduous tree valued in Indian Systems of Medicine [38]. *Gmelina arborea* species has been therapeutically utilized as anti-inflammatory, anti-oxidant, anti-ulcer, anti-nociceptive, analgesic, anti-diabetic, anti-cancer and wound healing activities [38]. Yet still, there is no study carried out on the arboreal and 4,8-dihydroxysesamin against COVID-19. Our study reveals their importance as potential anti-COVID-19 compounds. However, there are few limitations in our study to be considered. First, the molecular dynamic simulation can be extended till 100 ns. Second, both the phytochemicals need to be validated *in vitro* for further confirmation. On the other hand, the strength of this study include 1) Binding affinity of phytochemicals against multiple COVID-19 targets. 2) Confirm the binding affinity of phytochemicals with other known drugs 3) Assess the utility of these efficient phytochemicals for emerging Omicron variant.

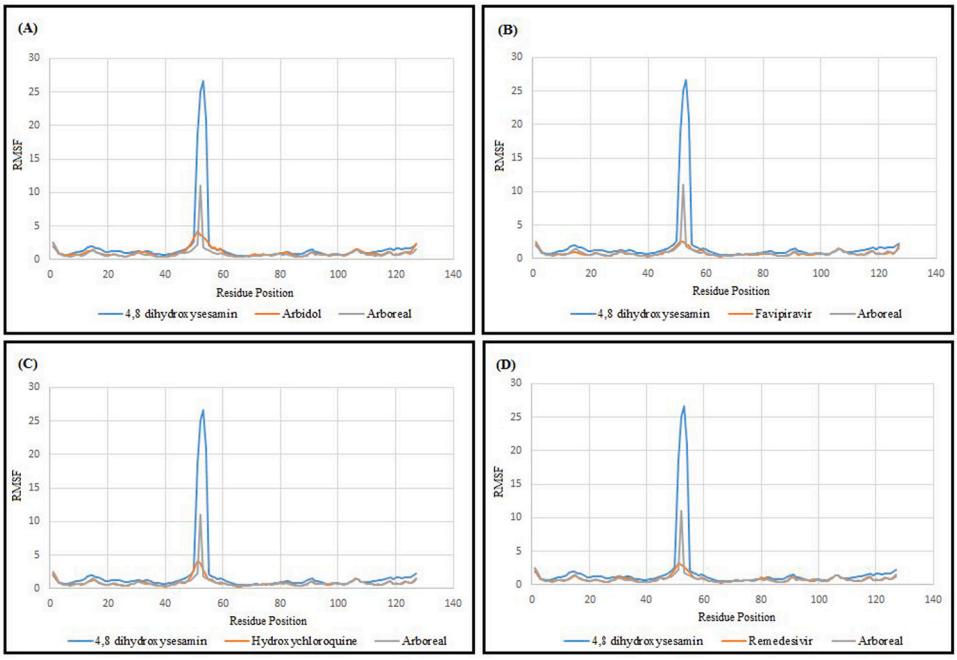

Fig. 4. RMSF analysis. Time dependence of the RMSFs of SARS-CoV-2 nucleocapsid protein after binding with arboreal, 4,8-dihydroxysesamin, arbidol, favipiravir, hydroxychloroquine, and remedesivir.

5. Conclusion

In conclusion, our molecular docking and dynamic simulation found that both arboreal and 4,8-dihydroxysesamin from Indian medicinal plants can be suggested for COVID-19 treatment. These phytochemicals/leads have potential inhibitory effect towards nucleocapsid protein of both SARS-CoV-2 (B.1.351) and emerging Omicron variant (B.1.1.529). Particularly, both the compounds have shown comparable inhibitory effect to other known inhibitors such as arbidol, favipiravir, hydroxychloroquine, and remedesivir. Overall, our *insilico* prediction provide clue for usage of arboreal and 4, 8-dihydroxysesamin for the upcoming COVID-19 strain. Further work are need to be conducted to confirm its utility against SARS-CoV-2.

Conflict of Interest

This study was not funded by any private or government organization. Authors from the four institutes declare that they have no competing interest.

References

[1] Renu K, Prasanna PL, Valsala Gopalakrishnan A. Coronaviruses pathogenesis, comorbidities and multi-organ damage - a review. Life Sci 2020;255:117839. https://doi:10.1016/j.lfs.2020.117839.
[2] Mokhtari T, Hassani F, Ghaffari N, Ebrahimi B, Yarahmadi A, Hassanzadeh G. COVID-19 and multiorgan failure: a narrative review on potential mechanisms. J Mol Histol 2020;51(6):613–28. https://doi:10.1007/s10735-020-09915-3.
[3] Şenay Ş. Coronavirus pandemic and cardiovascular issues. Turk GogusKalpDamarCerrahisiDerg. 2020;28(2):227–228. Published 2020 Apr 22. https://doi:10.5606/tgkdc.dergisi.2020.09298.
[4] V'kovski P, Kratzel A, Steiner S, Stalder H, Thiel V. Coronavirus biology and replication: implications for SARS-CoV-2. Nat Rev Microbiol 2021;19(3):155–70. https://doi:10.1038/s41579-020-00468-6.
[5] Gordon DE, Jang GM, Bouhaddou M, et al. A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. Nature 2020;583(7816):459–68. https://doi:10.1038/s41586-020-2286-9.
[6] Yadav R, Chaudhary JK, Jain N, et al. Role of structural and non-structural proteins and therapeutic targets of SARS-CoV-2 for COVID-19. Cells 2021;10(4):821. https://doi:10.3390/cells10040821.
[7] Gu H, Krishnan P, Ng DYM, et al. Probable transmission of SARS-CoV-2 omicron variant in quarantine hotel, Hong Kong, China, November 2021. Emerg Infect Dis 2022;28(2):460–2. https://doi:10.3201/eid2802.212422.
[8] Singh TU, Parida S, Lingaraju MC, Kesavan M, Kumar D, Singh RK. Drug repurposing approach to fight COVID-19. Pharm Rep 2020;72(6):1479–508. https://doi:10.1007/s43440-020-00155-6.
[9] Jie X, Hongmei Y, Ping F, Kuikui Z, Bohan Y, Rui M. Beneficial effect of Arbidol in the management of COVID-19 infection. Aging (Albany NY) 2021;13(7):9253–64. https://doi:10.18632/aging.202867.
[10] Manabe T., Kambayashi D., Akatsu H., Kudo K. Favipiravir for the treatment of patients with COVID-19: a systematic review and meta-analysis. BMC Infect Dis. 2021;21(1):489. Published 2021 May 27. https://doi:10.1186/s12879–021-06164-x.
[11] Kumar R, Sharma A, Srivastava JK, Siddiqui MH, Uddin MS, Aleya L. Hydroxychloroquine in COVID-19: therapeutic promises, current status, and environmental implications. Environ SciPollut Res Int 2021;28(30):40431–44. https://doi:10.1007/s11356-020-12200-1.
[12] Beigel JH, Tomashek KM, Dodd LE, et al. Remdesivir for the treatment of covid-19 - final report. N Engl J Med 2020;383(19):1813–26. https://doi:10.1056/NEJMoa2007764.
[13] Nojomi M, Yassin Z, Keyvani H, et al. Effect of arbidol (Umifenovir) on COVID-19: a randomized controlled trial. BMC Infect Dis 2020;20(1):954. https://doi:10.1186/s12879-020-05698-w.
[14] Kaur RJ, Charan J, Dutta S, et al. Favipiravir use in COVID-19: analysis of suspected adverse drug events reported in the WHO database. Infect Drug Resist 2020;13:4427–38. https://doi:10.2147/IDR.S287934.
[15] Gevers S, Kwa MSG, Wijnans E, van Nieuwkoop C. Safety considerations for chloroquine and hydroxychloroquine in the treatment of COVID-19. Clin Microbiol Infect 2020;26(9):1276–7. https://doi:10.1016/j.cmi.2020.05.006.
[16] Gupta AK, Parker BM, Priyadarshi V, Parker J. Cardiac adverse events with Remdesivir in COVID-19 Infection. Cureus 2020;12(10):e11132. https://doi:10.7759/cureus.11132.
[17] Ahmad S, Zahiruddin S, Parveen B, et al. Indian medicinal plants and formulations and their potential against COVID-19-preclinical and clinical research. Front Pharm 2021;11:578970. https://doi:10.3389/fphar.2020.578970.
[18] Rasool N, Bakht A, Hussain W. Analysis of inhibitor binding combined with reactivity studies to discover the potentially inhibiting phytochemicals targeting chikungunya viral replication. Curr Drug Disco Technol 2021;18(3):437–50. https://doi:10.2174/1570163817666200312102659.
[19] Kwofie SK, Broni E, Teye J, et al. Pharmacoinformatics-based identification of potential bioactive compounds against Ebola virus protein VP24. Comput Biol Med 2019;113:103414. https://doi:10.1016/j.compbiomed.2019.103414.
[20] Byler KG, Ogungbe IV, Setzer WN. In-silico screening for anti-Zika virus phytochemicals. J Mol Graph Model 2016;69:78–91. https://doi:10.1016/j.jmgm.2016.08.011.
[21] Aarogyam UK.A Pilot Study on Efficacy and Safety of Ayurveda Combination in Patients With Mild-to-Moderate COVID-19, Available from: https://clinicaltrials.gov/ct2/show/NCT04621903 (Accessed on November 29, 2021a).
[22] Adel Gomaa. Complementary Intervention for COVID-19, Available from: https://clinicaltrials.gov/ct2/show/NCT04487964 (Accesses on November 29, 2021).
[23] Aarogyam UK A Pilot Study on Efficacy and Safety of Ayurveda Combination in Patients With Mild-to-Moderate COVID-19, Available from: https://clinicaltrials.gov/ct2/show/NCT04621903 (Accessed on November 29, 2021b).

[24] Aarogyam UK Feasibility of Ayurveda in Patients With Mild-to-Moderate COVID-19: A Community-Based Participatory Research,Available from:https://clinicaltrials.gov/ct2/show/NCT04716647 (Accessed on November 29, 2021c).
[25] Kulkarni SA, Nagarajan SK, Ramesh V, Palaniyandi V, Selvam SP, Madhavan T. Computational evaluation of major components from plant essential oils as potent inhibitors of SARS-CoV-2 spike protein. J Mol Struct 2020;1221:128823. https://doi:10.1016/j.molstruc.2020.128823.
[26] Guex N, Peitsch MC. SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. Electrophoresis 1997;18(15):2714–23. https://doi:10.1002/elps.1150181505.
[27] Dong S, Sun J, Mao Z, Wang L, Lu YL, Li J. A guideline for homology modeling of the proteins from newly discovered betacoronavirus, 2019 novel coronavirus (2019-nCoV). J Med Virol 2020;92(9):1542–8. https://doi.org/10.1002/jmv.25768
[28] Basu A, Sarkar A, Maulik U. Molecular docking study of potential phytochemicals and their effects on the complex of SARS-CoV2 spike protein and human ACE2. Sci Rep 2020;10(1):17699. https://doi:10.1038/s41598-020-74715-4.
[29] Mohanraj K, Karthikeyan BS, Vivek-Ananth RP, et al. IMPPAT: a curated database of Indian Medicinal Plants, Phytochemistry And Therapeutics. Sci Rep 2018;8(1):4329. https://doi:10.1038/s41598-018-22631-z.
[30] Trott O, Olson AJ. AutoDockVina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem 2010;31(2):455–61. https://doi:10.1002/jcc.21334.
[31] Dallakyan S, Olson AJ. Small-molecule library screening by docking with PyRx. Methods Mol Biol 2015;1263:243–50. https://doi:10.1007/978-1-4939-2269-7_19.
[32] Islam MR, Hoque MN, Rahman MS, et al. Genome-wide analysis of SARS-CoV-2 virus strains circulating worldwide implicates heterogeneity. Sci Rep 2020;10(1):14004. https://doi:10.1038/s41598-020-70812-6.
[33] Mukhtar M, Arshad M, Ahmad M, et al. Antiviral potentials of medicinal plants. Virus Res 2008;131(2):111–20. https://doi:10.1016/j.virusres.2007.09.008.
[34] Woo Jackson CS, Lau JSH, et al. Herbal medicine: toxicity and recent trends in assessing their potential toxic effects. Advances in Botanical Research Vol 62. London: Academic press; 2012. p. 365–84.
[35] MuthumanickamS Kamaladevi A, Boomi P, et al. Indian ethnomedicinal phytochemicals as promising inhibitors of RNA-binding domain of SARS-CoV-2 nucleocapsid phosphoprotein: an in silico study. Front Mol Biosci 2021;8:637329. https://doi:10.3389/fmolb.2021.637329.
[36] Vardhan S, Sahoo SK. In silico ADMET and molecular docking study on searching potential inhibitors from limonoids and triterpenoids for COVID-19. Comput Biol Med 2020;124:103936. https://doi:10.1016/j.compbiomed.2020.103936.
[37] Kousar K, Majeed A, Yasmin F, et al. Phytochemicals from selective plants have promising potential against SARS-CoV-2: investigation and corroboration through molecular docking, md simulations, and quantum computations. Biomed Res Int 2020;2020:6237160. https://doi:10.1155/2020/6237160.
[38] Warrier RR, Priya SM, Kalaiselvi R. Gmelina arborea- an indigenous timber species of India with high medicinal value: a review on its pharmacology, pharmacognosy and phytochemistry. J Ethnopharmacol 2021;267:113593. https://doi:10.1016/j.jep.2020.113593.