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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 (IMPAT) 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 651(501 *13) docked phytochemicals complex, 26 were efficient against SARS-CoV-2. Amongst, 4,8-dihydroxy-sesamin 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 remdesivir (−8.0 to −5.3 kcal/mol) revealed equal/less affinity than 4,8-dihydroxy-sesamin and arboreal. Interestingly, the nucleocapsid target was found commonly inhibited by 4,8-dihydroxy-sesamin 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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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 remdesivir [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 waves there 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 Arogyam 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 phytochemicals against 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 selected to 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-dihydroxysemis 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-dihydroxysemis 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-dihydroxysemis during the molecular dynamic (MD) simulations. Also, the MM-GBSA approach revealed that both arboreal and 4,8-dihydroxysemis can potentially exhibit equal affinity like hydroxychloroquine and remdesivir.

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 (CK and PN) 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 SJ). 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, pre-clinical, 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. UFO692871). Further the modelled structure was refined [28] for later assessment.

2.3. Phytochemical collection

The phytochemicals used in this study were collected from IMPAT database [29]. IMPAT is publically accessible to download the compounds structure for virtual screening. Currently, IMPAT 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 remdesivir (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] Auto-docking 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-dihydroxy sesamin were over-represented against multiple targets. Of these targets, nucleocapsid was noticed to be inhibited by both arboreal and 4,8-dihydroxy sesamin 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-dihydroxy sesamin) and four drugs (arbidol, favipiravir, hydroxychloroquine, and remdesivir). 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-dihydroxy sesamin) and drugs (arbidol, favipiravir, hydroxychloroquine, and remdesivir) 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, NSP6, NSP1, NSP15, NSP13 helicase, NSP12, spike glycoprotein, 3CLpro, PLpro, 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 (3CLpro), 4OW0 (PLpro), 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, benzenes, 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 remdesivir 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 remdesivir 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), 3CLpro (6LU7), RdRp (6M71) and nucleocapsid (6M3M) (Table 1). 4,8-dihydroxy sesamin exhibit least binding energy to NSP13, spike glycoprotein, PLpro, RdRp and nucleocapsid. Similarly, all four drugs (arbidol, favipiravir, hydroxychloroquine and remdesivir) showed the binding energy in the range between −8.0 to −4.5 kcal/mol (Table 2). From Table 1, arboreal and 4,8-dihydroxy sesamin noticed to have high affinity to multiple proteins. Particularly, arboreal and 4,8 dihydroxy sesamin, were noticed to have high affinity against nucleocapsid in common. The three dimensional molecular interactions were visualized for nucleocapsid with arboreal, 4,8-dihydroxy sesamin, arbidol, favipiravir, hydroxychloroquine and remdesivir (Fig. 1-2). MD simulations were performed for the arboreal-nucleocapsid and 4,8-dihydroxy sesamin-nucleocapsid complexes and compared with the four nucleocapsid-drug complexes.
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-remdesivir 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-remdesivir complexes were 0.89, 1.03, 0.81, 0.96, 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 remdesivir (~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 arbidol, 4,8-dihydroxysesamin, arbidol, favipiravir, hydroxychloroquine, and remdesivir 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, hydroxychloroquine and remdesivir, 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), imizirine (~8.7 kcal/mol), and 4,8-dihydroxysesamin (~8.6 kcal/mol). Also, NSP13 was inhibited by arboreal (~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, 3CLpro, RdRp and nucleocapsid. Likewise, 4,8-dihydroxysesamin exhibit efficient interactions with five targets such as NSP13, spike glycoprotein, PLpro, RdRp and nucleocapsid. Both arboreal and 4,8-dihydroxysesamin commonly inhibit nucleocapsid with least binding energy.

Arbooreal 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. 2A). Likewise, favipiravir (Fig. 2B), and hydroxychloroquine (Fig. 2C) interact with nucleocapsid with the binding energy between ~5.2 to ~4.5 kcal/mol. Remdesivir showed interactions with nucleocapsid at ASN76, GLU84, ILE75, PRO163, and PRO81 with binding energy (~5.3 kcal/mol) (Fig. 2D).

### Table 1

| Sl. NO | Protein target | Phytochemicals | Binding energy (kcal/mol) |
|-------|----------------|----------------|--------------------------|
| 1     | Replicase      | Medicagol       | ~8.2                     |
|       |     polyprotein 1a | Maruquine       | ~8.1                     |
|       |                | (+)-arboreol    | ~7.9                     |
| 2     | ADP ribose     | Micromelin      | ~7.5                     |
|       | phosphatase of NSP3 | 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                     |
|       |                | Arboreal        | ~8.2                     |
| 5     | NSP1           | Corydlin        | ~7.6                     |
|       |                | Chaparrinone    | ~7.3                     |
|       |                | Gummadiol       | ~7.0                     |
| 6     | NSP15          | 7-O-lactoly-apigenin | ~8.7                   |
|       |                | Isscutellarein  | ~8.6                     |
|       |                | Artocarpus      | ~8.5                     |
| 7     | NSP13 helicase | 4,8-dihydroxysesamin | ~9.3                   |
|       |                | Nepetaefolinol  | ~9.2                     |
| 8     | NSP12          | Hetidine        | ~9.4                     |
|       |                | Gibberellins    | ~9.1                     |
|       |                | Arboreal        | ~9.1                     |
| 9     | Spike glycoprotein | Scutellarein   | ~8.9                     |
|       |                | 4,8-dihydroxysesamin | ~8.9                   |
|       |                | Luteoforol      | ~8.8                     |
| 10    | 3CLpro         | Arboreal        | ~8.2                     |
|       |                | Rhein           | ~8.0                     |
|       |                | Isomaruquine    | ~7.9                     |
| 11    | PLpro          | 4,8-dihydroxysesamin | ~10.3                   |
|       |                | Medicagol       | ~10.3                     |
|       |                | Noronientaline  | ~10.0                    |
| 12    | RdRp           | Arboreal        | ~8.9                     |
|       |                | Imizirine       | ~8.7                     |
| 13    | Nucleocapsid   | 4,8-dihydroxysesamin | ~8.6                   |
|       |                | Arboreal        | ~10.6                    |
|       |                | Haematoxylin    | ~10.2                    |

### Table 2

| Protein targets | Hydroxychloroquine Binding energy (kcal/mol) | Arbidol Binding energy (kcal/mol) | Favipiravir Binding energy (kcal/mol) | Remdesivir 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                                 |
| 3CLpro          | ~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                                 |
Arboreal (A) and 4, 8-dihydroxysesamin (B) interacts with nucleocapsid target through 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) | Remdesivir    | -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-20micron (B.1.1.529) | Remdesivir    | -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. 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 target through pi-sigma, hydrogen, pi-anion, alkyl interactions with their surrounding amino-acids.

Fig. 2. Two dimensions interaction plot of antiviral drugs with the nucleocapsid protein. Four drugs like, arbidol (A), favipiravir (B), hydroxychloroquine (C) and remdesivir (D) interacts with nucleocapsid through pi-sigma, hydrogen, pi-anion, alkyl interactions with their surrounding amino-acids.
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 3CLPRO and PLpro 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, arbidol, favipiravir, hydroxychloroquine, and remdesivir.

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Hence, arboreal and 4, 8-dihydroxysesamin is found to be comparatively efficient to the well-known drugs such as remdesivir, 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. 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 remdesivir.
Phytochemicals/leads have potential inhibitory effect towards medicinal plants can be suggested for COVID-19 treatment. These found that both arboreal and 4,8-dihydroxyseamin from Indian as arbidol, favipiravir, hydroxychloroquine, and remdesivir. Overall, our insilico prediction provide clue for usage of arboreal and 4,8-dihydroxyseamin for the upcoming COVID-19 strain. Further work are need to be conducted to confirm its utility against SARS-CoV-2.

5. Conclusion

In conclusion, our molecular docking and dynamic simulation found that both arboreal and 4,8-dihydroxyseamin 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 remdesivir. Overall, our insilico prediction provide clue for usage of arboreal and 4,8-dihydroxyseamin 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.

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