ORIGINAL ARTICLE

# Computational insights of phytochemical-driven disruption of RNA-dependent RNA polymerase-mediated replication of coronavirus: a strategic treatment plan against coronavirus disease 2019

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## Abstract

The current pandemic of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has raised global health concerns. RNA-dependent RNA polymerase (RdRp) is the prime component of viral replication/proliferation machinery and is considered to be a potential drug target against SARS-CoV-2. The present study investigated the anti-RdRp activity of phytochemicals against SARS-CoV-2 infection. Virtual ligand screening was carried out to determine the potent compounds against RdRp. Molecular docking and an MD Simulation study were employed to evaluate the spatial affinity of selected phytochemicals for the active sites of RdRp. Structural stability of target compounds was determined using root mean square deviation computational analysis and drug-like abilities were investigated using ADMET. Bond distances between ligand and receptor were marked to predict the strength of interaction. Aloe, azadirachtin, columbin, cirsilineol, nimbiol, nimbocinol and sage exhibited the highest binding affinities and interacted with active sites of RdRp, surpassing the ability of chloroquine, lamivudine, favipiravir and remdesivir to target the same. All the natural metabolites exhibited stable conformation during MD Simulation of 101 ns at 310 K. Kinetic, potential and electrostatic energy were observed to be least in the case of natural metabolites in comparison with synthetic analogues. Deviations and fluctuations were observed to be structurally least in target phytochemicals. Physiochemical and biological properties of these compounds further validated their drug-like properties. Non-bonded distance was found to be short enough to form hydrogen bonding or hydrophobic interactions, which revealed that these target compounds can strongly bind with RdRp. The study found potential phytochemicals to disrupt the replication domain of SARS-CoV-2 by hindering RdRp. We therefore anticipate that the current findings could be considered as valuable for the development of an efficient preventive/therapeutic expedient against COVID-19.


**Keywords:** ADMET, coronavirus disease 2019, molecular docking, RNA-dependent RNA polymerase, root mean square deviation, severe acute respiratory syndrome coronavirus 2

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## Introduction

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) first emerged in Wuhan, China in December 2019 and since then has become a global pandemic. SARS-CoV-2 is a member of betacoronavirus genus and exhibits 94.6% sequence homology with conserved domains of other members belonging to the *Coronaviridae* family, namely severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) [1,2]. Cough, fever and shortness of breath were observed to be the prominent pathological symptoms associated with SARS-CoV-2 infection. Higher frequency of hospitalization, mortality rate and the non-availability of preventive/therapeutic treatment strategies have posed a serious threat to the world population during the current outbreak [3,4].



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The pathophysiology of COVID-19 has not yet been fully explored. SARS-CoV-2 is a single-stranded positive sense RNA virus with viral RNA genome, employing a multi-subunit replication and transcription set-up. The genome size of SARS-CoV-2 is expected to be in the range of 29.8–29.9 kb. The 5′ end of the genome comprises of *orf1ab*, which encodes the orf1ab polyprotein whereas the genes lying at the 3′ end encode for structural proteins such as surface (S), envelope (E), membrane (M) and nucleocapsid (N) proteins. RNA-dependent RNA polymerase (RdRp) plays a vital role in viral replication and proliferation, which makes several copies of its RNA genome [5–8]. Gao *et al* in 2020 revealed that RdRp exists as a complex with nsp12 (residues S1 to Q932) and along with certain other smaller nsp7 (residues S1 to Q83) and nsp8 (residues A1 to Q198) polymerases on the basis of cryo-electron microscopy. RdRp resembles a right-hand structure with fingers, thumb and palm-like domains. Therefore, RdRp could be considered as a prominent drug target for SARS-CoV-2 [9]. A new therapeutic approach to target RdRp could yield promising results to overcome the virus outbreak. The present focus is keenly on the development of novel therapeutic option comprising antiviral drugs and vaccines [10,11]. Several national and international research organizations are currently enrolled in development of vaccines to prevent or treat COVID-19, but till now no effective treatment strategy is available to target the virus outbreak. The present study aimed to analyse the therapeutic potential of phytochemicals in disrupting or hindering the conserved domain of RdRp responsible for SARS-CoV-2 replication and proliferation through *in silico* study.

## Materials and methods

### Protein and ligand structure preparation

After screening a set of medicinal plants on the basis of an ancient medicinal text, 15 different natural moieties—belonging to *Tinospora cordifolia*, *Azadirachta indica*, *Ocimum sanctum*, *Origanum vulgare*, *Salvia officinalis*, *Allium sativum*, *Melissa officinalis*, *Zingiber officinale*, *Aloe vera* and *Curcuma longa*—were selected on the virtue of their ability to exhibit anti-viral activity against several viral strains [12] and to be used to target COVID-19 using computational studies. Molecular details of the chosen compounds were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov), Zinc Database in multiple formats such as pdb, mol2 and sdf. Three-dimensional (3D) structures of selected ligands were generated using canonical SMILES through the RBPS Web Portal. Crystallographic data of ligands were examined in Cartesian coordinates. The protein structure of SARS-CoV-2 RdRp was retrieved from the protein data bank with PDB ID 6M71 (https://www.rcsb.org/structure/6M71). 3D structures were visualized using UCSF Chimera to obtain actual insights of protein structure.

### Experimental settings for molecular docking

A Lamarckian genetic algorithm with 250 000 energies were implemented to compute the binding affinity of natural moieties with RdRp to predict their efficiency in targeting replication of SARS-CoV-2. By using the AutoDock Tool of the AutoDock 4.2.6 package, a molecular docking study was carried out. 3D structures of protein and ligands were saved in PDBQT format. Ligands were placed in grid boxes of varying dimensions for each docking process. Different grid points for autogrid maps were set to be 71 × 53 × 25 Å and 77 × 46 × 25 Å for RdRp interaction with ligands. While carrying out the process, torsion degree of freedom was also defined and to attain the highest number of poses, 20 different modes were chosen with exhaustiveness of 8. Docking at different sites may help in determination of the best possible ligand–receptor interaction. Docked poses of ligands can be aligned over the receptor by using UCSF Chimera as the visualization tool to analyse the ligand–receptor interaction and to anticipate the affinity of ligands to target the receptors [13,14]. Binding energy can be computed as: $\Delta G_{Binding} = G_{Complex} - G_{Protein} - G_{Ligand}$.

### Molecular dynamic simulation

Using NAMD software, molecular dynamic simulation of docked protein–ligand complexes was carried out to predict their stability. CHARMM36 force field settings were chosen to run the MD simulation. With the help of visual molecular dynamic (VMD) software, protein structure files were obtained to run the process. Protein–ligand complexes were further subjected tor solution in cubic water boxes which contain transferable intermolecular interactions. Subsequently, box size was selected to ensure the distance of 5 Å between protein and box edges. Initially MD simulation for 50 000 steps of steepest descent with minimized energy were run using NAMD. Furthermore, the present system was subjected for simulation by NVT set-up. For the optimum run, temperature of 310K and 10 ns of simulated time duration were fixed. After the completion of the run, the simulated complexes were visualized using VMD and were further analysed to predict any possible change in the dynamics of protein–ligand complexes. The trajectories of simulated complexes were plotted to predict their stability and electrostatic, kinetic and potential energy were computed using VMD and NMAD software [15].

### Root mean square deviation computation

Root mean square deviation (RMSD) values represent the flexibility of protein structure and thereby reflect its mobility in trajectory. Higher RMSD values point to the higher mobility of

ligands and vice versa. During the ligand–receptor interaction, the ligand poses generated using AutoDock Tools were analysed to determine their structural stability on the basis of their deviation from the native ligand pose. By using RMSD algorithms, results obtained from docking experiments can be validated and it will potentially help in enhancing docking performance [16]. RMSD can be calculated as:

$$\mathrm{RMSD} = \sqrt{\frac{1}{N}\sum_{i=1}^{N} d_i^2}$$

### Active site prediction

UCSF Chimera was used for the visualization of ligand–receptor binding pose. Docked structure of ligands with RdRp were analysed and the amino acid residues involved in ligand–receptor interaction were studied. Amino acid residues involved in interaction in proximity of 5 Å were depicted using the command line. Inhibitory potential of natural metabolites was further compared with synthetic anti-viral and anti-malarial compounds currently available on the market to fight against the disease. Similarly, a comparison of the active site of RdRp targeted by natural moieties and synthetic analogues was carried out [17].

### Bond distance determination between active site and ligands

Using UCSF Chimera, docked structures were visualized and bond distances between the active site and ligands were computed to predict the type of bond formation and to analyse the strength of the interaction between RdRp and the respective ligands. The presence of either hydrogen or hydrophobic bonds can be predicted based upon the bond distance [18].

### ADME toxicity prediction

Computational prediction of pharmacological and biological activity of ligands was carried out on the basis of Lipinski's Rule of Five. Assessment of absorption, dissolution, metabolism and excretion were performed virtually using the ADMETlab Web Server. AMES Toxicity, lethal dosage and skin sensitization parameters were also calculated [19].

## Results

### Protein and ligand 3D structure preparation for molecular docking

Canonical SMILES of selected ligands obtained from PubChem were loaded onto the RBPS Web Portal and their respective 3D structures were obtained, which were then converted into monomeric units in both mol2 and pdf file format. Popularly known as an anti-malarial drug, chloroquine was used as reference synthetic analogue for the comparison along with anti-viral agents such as favipiravir, remdesivir and lamivudine. 3D structure of RdRp with PDB ID 6M71was taken from the RCSB Web Portal and analysed using UCSF Chimera and saved in pdb format. Both the ligands and receptors were energy minimized and saved in PDBQT format using AutoDock Tool to carry out molecular docking at different receptor sites, thereby predicting the active site and binding affinities.

### Molecular docking study

Using AutoDock Tool, phytochemical ligands were docked with RdRp. Docked compounds were ranked on the basis of maximum occupancy of binding pockets along with minimum Gibbs free energy. Ligands were docked with RdRp at different grid points (71 × 53 × 25 Å and 77 × 46 × 25 Å) and the binding affinities were represented as kcal/mol. Docked poses with less than 5 kcal/mol binding affinity represented negligible binding affinity and were not considered for further evaluation. From a long list of phytochemicals only seven were observed to exhibit remarkable inhibitory activity. Aloe, azadirachtin, nimbiol, nimbocinol, cirsilineol, columbin and sage—belonging to *Aloe vera*, *Azadirachta indica*, *Ocimum sanctum*, *Tinospora cordifolia* and *Salvia officinalis*, respectively—were observed to bind with RdRp with binding affinities of −7.1, −8.2, −7.3, −7.4, −6.9, −7.5 and −8.7 kcal/mol at the active site (Table 1). Binding affinities exhibited by these phytochemicals were better than those of the synthetic analogues chloroquine, lamivudine, favipiravir and remdesivir.

**TABLE 1. Binding affinities of ligands at 71 × 53 × 25 Å and 77 × 46 × 25 Å grid points RdRP of SARS-CoV-2 on the basis of molecular docking**

| Ligands | RdRp (X: 71 Y: 53 Z: 25) Binding affinity (kcal/mol) | RdRp (X: 77 Y: 46 Z: 25) binding affinity (kcal/mol) |
|---|---|---|
| Columbin | −7.5 | −6.7 |
| Remdesivir | −7.3 | −6.4 |
| Nimbin | −6.7 | −6.3 |
| Nimbiol | −7.3 | −6.8 |
| Nimbocinol | −7.4 | −7.2 |
| Jatrorrhizine | −6.5 | −6.7 |
| Chloroquine | −5.8 | −5.1 |
| Favipiravir | −5.1 | −5.4 |
| Lamivudine | −5.0 | −5.9 |
| Azadirachtin | −8.2 | −7.1 |
| Cirsilineol | −6.9 | −6.8 |
| Orientin | −6.7 | −6.9 |
| Carvacrol | −5.3 | −4.7 |
| Sage | −8.7 | −7.3 |
| Ajoene | −3.7 | −3.7 |
| Citronellal | −3.7 | −4.0 |
| Gingerol | −5.8 | −4.9 |
| Aloe | −7.1 | −6.7 |
| Curcumin | −6.5 | −5.4 |

When RdRp was docked at 71 × 53 × 25 Å grid points; aloe and azadirachtin exhibited binding affinities of −7.1 and −8.2 kcal/mol whereas at 77 × 46 × 25 Å grid points, binding affinities were −6.7 and −7.1 kcal/mol, respectively. Similarly, nimbiol and nimbocinol exhibited binding affinities of −7.3 and −7.4 kcal/mol at 71 × 53 × 25 Å grid points whereas at 77 × 46 × 25 Å grid points, the results were −6.8 and −7.2 kcal/mol, respectively (Fig. 1). From the above mentioned results it can be clearly predicted that RdRp at 71 × 53 × 25 Å grid points offered the best binding site for the ligands and the active site

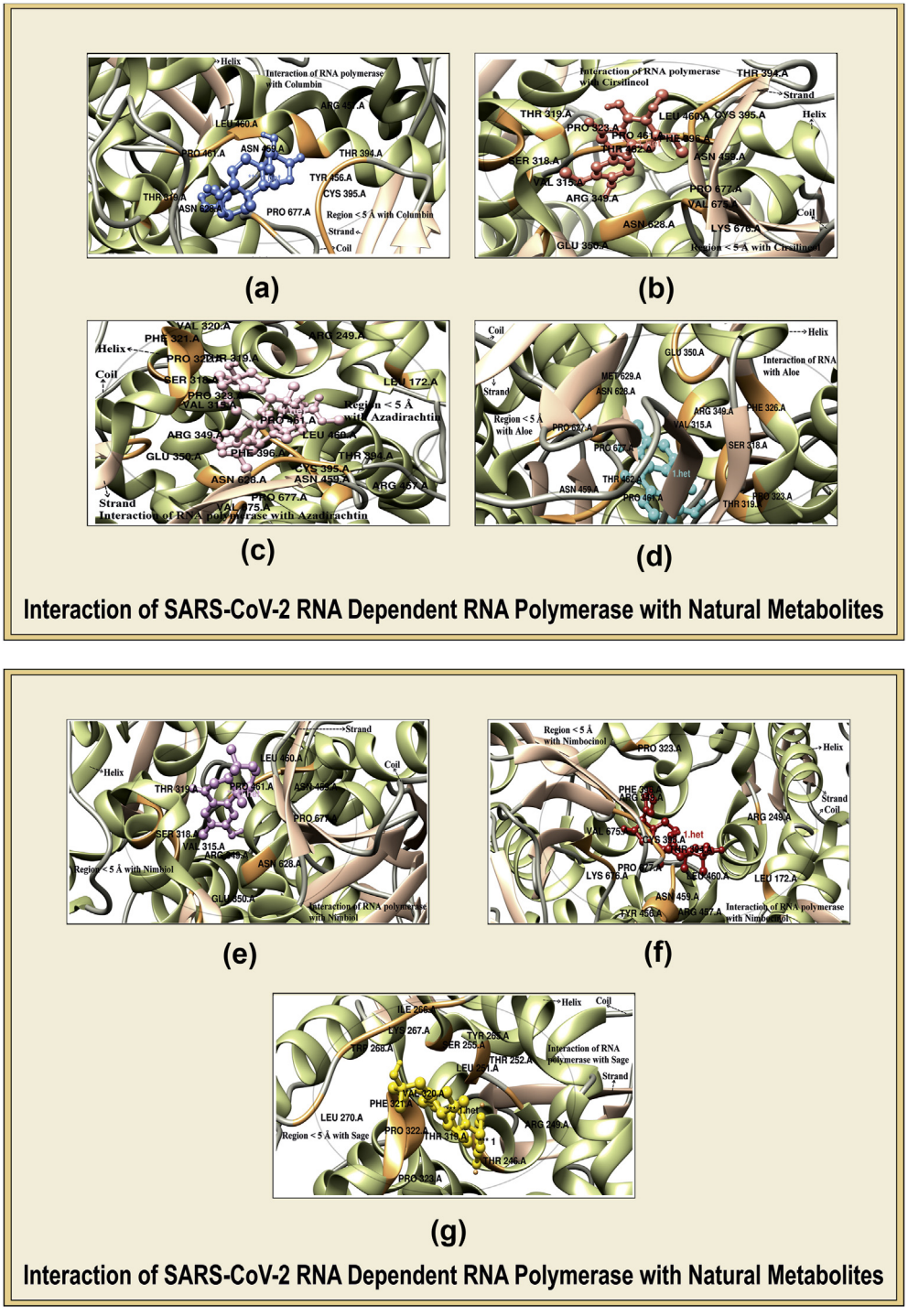


FIG. 1. Interaction of SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) with natural metabolites (a) RdRp-Columbin; (b) RdRp-Cirsilineol; (c) RdRp-Azadirachtin; (d) RdRp-Aloe; (e) RdRp-Nimbiol; (f) RdRp-Nimbocinol; (g) RdRp-Sage at its potential active sites.

can be considered as a potential target for the possible drug candidates involved in targeting RdRp to fight against the current SARS-CoV-2 outbreak.

### Molecular dynamic study and computation of free energy

MD Simulation of RdRp SARS-CoV-2 with aloe, nimbiol, nimbocinol, sage, azadirachtin, cirsilineol and columbin were carried out using NAMD software, and favipiravir and remdesivir were used as references for the study. MD Simulation was carried out for 101 ns at 310K. From the results it was observed that all the natural metabolites in complex with RdRp SARS-CoV-2 were stable for 101 ns with the receptor. From trajectory analysis it can be easily predicted that all of the above-mentioned compounds remained stable during the simulation process and exhibited similar patterns to that of the synthetic analogues remdesivir and favipiravir (Fig. 2). Furthermore kinetic, potential and electrostatic energy was computed to compare their binding affinities and the results obtained from the simulation study were observed to be in concordance with those of the molecular docking study (Table 2). All the natural metabolites selected for the study exhibited better results in comparison to the synthetic analogues.

### Docked conformer stability analysis

The flexibility and overall stability of docked complexes were further predicted using RMSD. Docked conformers obtained from the AutoDock Tool after carrying out the molecular docking process were evaluated for their residual deviation and fluctuation with respect to the reference pose. RMSD values near or equivalent to 3 Å were considered as 'near native' to

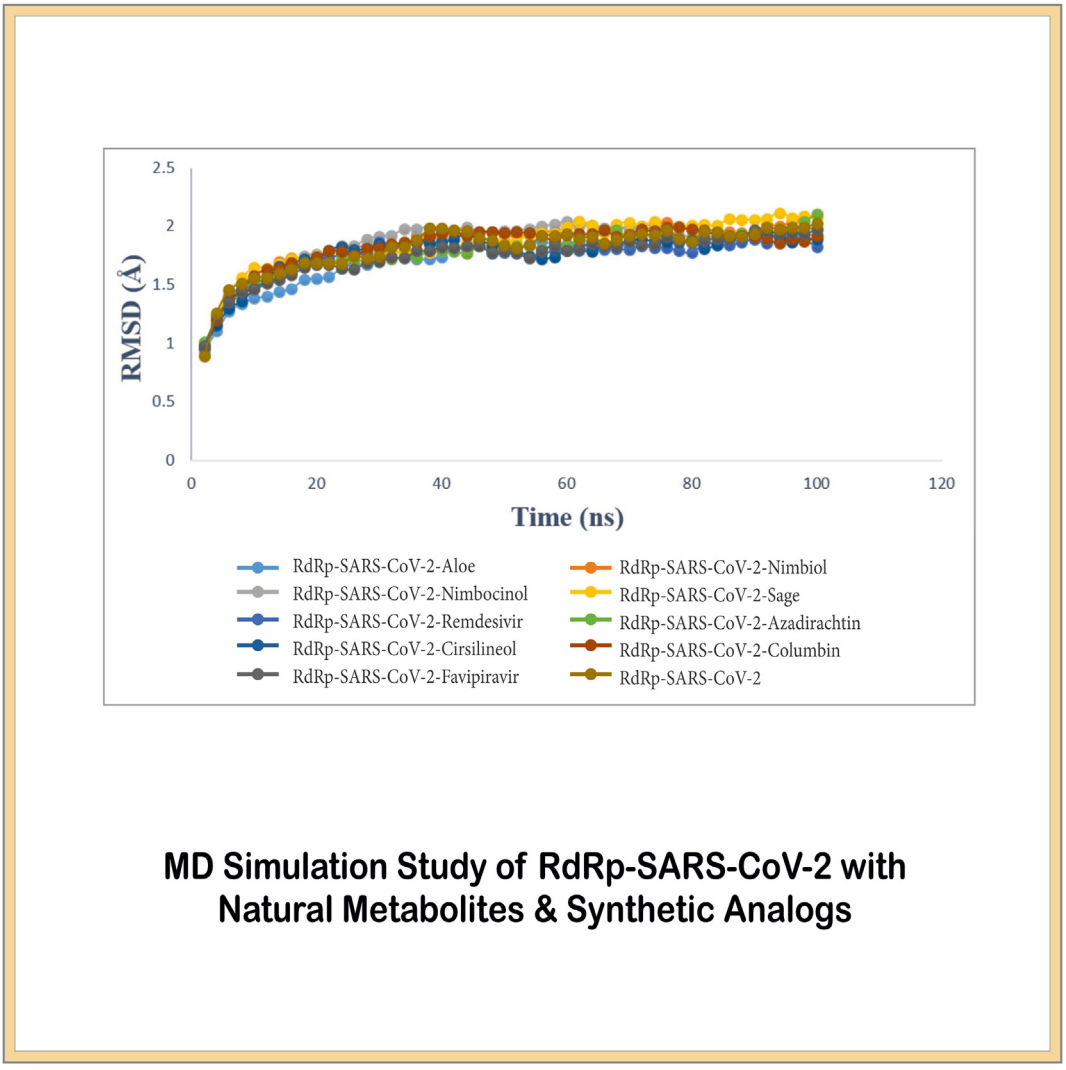


FIG. 2. Root mean square deviation trajectory of RdRp-SARS-CoV-2 with natural metabolites and synthetic analogues at 310K for 101 ns.

**TABLE 2. Binding free energy values of RdRp-SARS-CoV-2 with natural metabolites and synthetic analogues on the basis of MD simulation**

| | | Energy components (Kcal/mol) | | | |
|---|---|---|---|---|---|
| **Target** | **Ligand** | **Electrostatic** | **Potential** | **Kinetic** | **Total energy** |
| RdRp-SARS-CoV-2 | Aloe | −1 980 545.80 | −1 802 959.50 | 102 185.16 | −1 700 774.3 |
| | Nimbiol | −1 951 887.50 | −1 778 055.45 | 101 141.23 | −1 676 914.22 |
| | Nimbocinol | −1 981 441.04 | −1 804 788.62 | 102 605.82 | −1 702 182.80 |
| | Sage | −1 954 628.76 | −1 780 082.43 | 101 354.45 | −1 678 727.98 |
| | Azadirachtin | −1 944 937.73 | −1 770 931.91 | 100 689.44 | −1 670 242.47 |
| | Cirsilineol | −1 875 672.94 | −1 707 548.84 | 97 332.16 | −1 610 216.68 |
| | Columbin | −1 947 025.60 | −1 773 557.88 | 101 032.44 | −1 672 525.44 |
| | Remdesivir | −1 632 577.75 | −1 759 498.32 | 100 252.22 | −1 559 246.09 |
| | Favipiravir | −1 765 526.65 | −1 697 812.34 | 96 746.76 | −1 401 065.58 |

'native pose'. Ligands that exhibited highest binding affinity with RdRp were selectively used for analysing their potency to produce stable docked conformer. Results revealed that aloe, azadirachtin, nimbiol, nimbocinol, cirsilineol, columbin and sage exhibited RMSD of 1.3, 3.2, 1.8, 1.6, 1.5, 1.3 and 2.8 Å, respectively. Except for azadirachtin and sage, the docked poses of the above-mentioned ligands can be significantly considered as 'near native' pose with respect to their 'reference pose' (Fig. 3). Higher binding affinity does not assure stable docked conformer. Azadirachtin and sage showed slightly higher RMSD values but the results obtained from the MD Simulation study supported their efficiency to bind and target RdRp. However, other parameters also need to be explored to determine their stability index and because they exhibited high binding affinity and remained stable during the MD Simulation studies, their potential to inhibit RdRp cannot be ruled out just because of higher RMSD values.

### RdRp active site residues

Putative ligand binding site of RdRp was identified using UCSF Chimera. The region within a 5 Å proximity of where the respective ligands bound to the receptor domain was selected and the amino acid residues constituting that particular region were predicted to determine which one of them was involved in the interaction with receptor; this will also help in predicting the targeted site to tackle the current pandemic. Identification

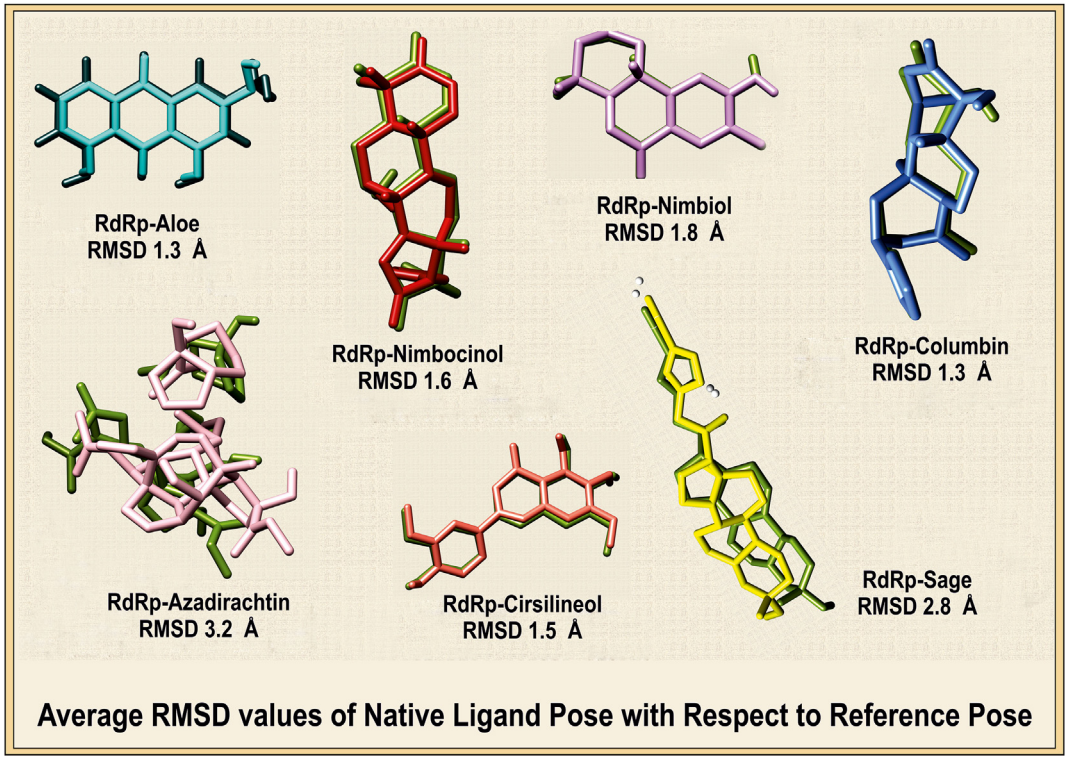


**FIG. 3.** Ligand pose of docked structure aligned over its native pose with it average root mean square deviation values.

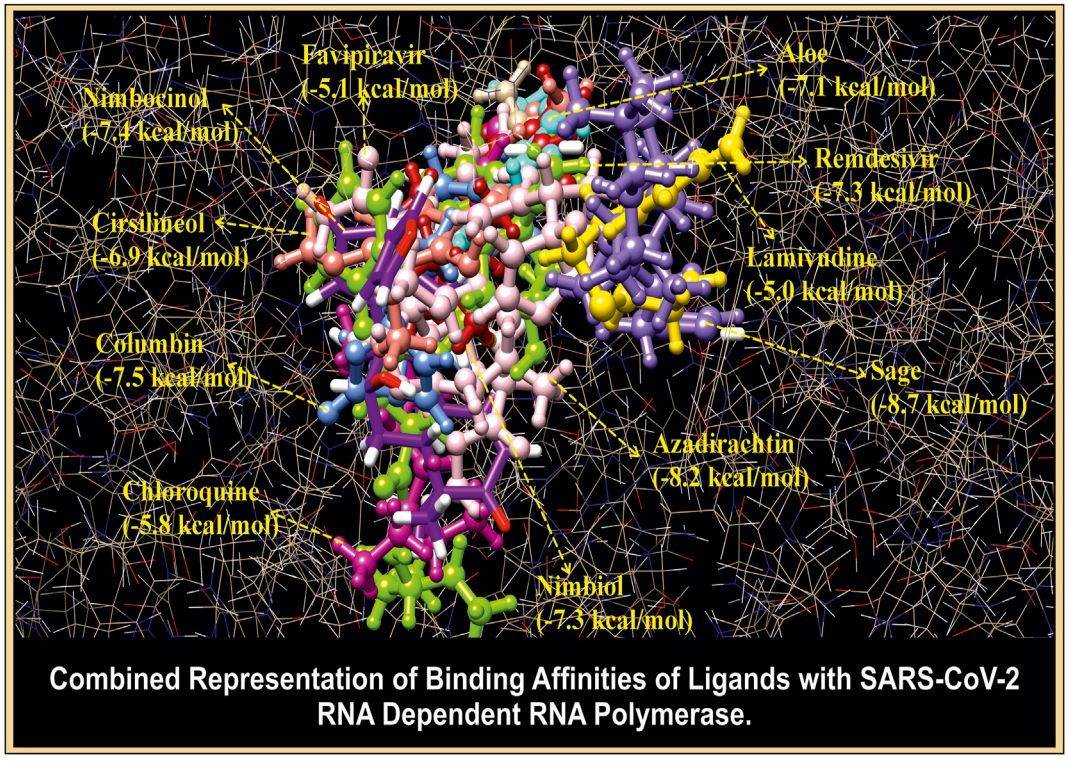


**FIG. 4.** Binding affinities of natural and synthetic analogues with RNA-dependent RNA polymerase (RdRp) at 71 × 53 × 25 Å grid points to draw a comparison between ability of columbin, cirsilineol, aloe, azadirachtin, nimbiol, nimbocinol and sage with currently available drugs on the market named as chloroquine, favipiravir, lamivudine and remdesivir to target replication and proliferation of SARS-CoV-2.

of amino acid residues involved in the docking process provides further biological insight for future drug discoveries and the availability of cryptic pockets in RdRp may help in analysing the possible space for drug binding. RdRp at 71 × 53 × 25 Å grid points forms the binding site with aloe, azadirachtin, columbin, cirsilineol, nimbiol, nimbocinol and sage comprising GLU350, PRO677, THR362, PHE326, ASN459; LEU460, GLU350, SER318, ARG349, PHE396; LEU460, ASN459, PRO461, THR319, ASN628; LEU460, SER318, PRO461, THR319, VAL315; LEU460, SER318, VAL315, PRO461, ASN628; PHE396, LEU460, VAL675, CYS395, THR394; VAL320, PHE321, THR319, LEU251 and SER255, with binding affinities of −7.1, −8.2, −7.5, −6.9, −7.3, −7.4 and −8.7 kcal/mol, respectively. Whereas chloroquine, favipiravir, lamivudine and remdesivir form the binding site with RdRp amino acid residues that include LEU460, PRO677, CYS395, TYR456, ASN628; PRO461, ASN628, GLY678, PRO677, SER664; TRP268, SER255, LYS267, VAL320, THR319; VAL675, ARG457, PRO169, SER664, LEU172, with binding affinities of −5.8, −5.1, −5.0 and −7.3 kcal/mol, respectively, at 71 × 53 × 25 Å grid points (Fig. 4). All the above mentioned seven phytochemicals exhibited higher binding affinity than the chemical analogues with RdRp and even the active site residues involved in the interaction were observed to be similar, which clearly revealed that these natural moieties may play a pivotal role in targeting RdRp to control the SARS-CoV-2 outbreak.

### Bond distance prediction

Determination of the presence of bond type, either hydrogen bond or other non-covalent interactions, may help in predicting the potency of phytochemicals to target RdRp. Side chains of amino acid residues configuring the active site of RdRp act as electron donors and thereby form either hydrogen bonds or are involved in hydrophobic interactions. A hydrogen bond is formed when the bond distance between the amino acid residues and ligand is 3.5 Å whereas a distance of 4.5 Å leads to hydrophobic or non-covalent interactions. Phytochemicals exhibiting high binding affinity with predicted catalytic domain of RdRp were further evaluated to analyse the bond strength between ligands and receptors. Average bond distance between the aloe, cirsilineol, nimbiol, nimbocinol, sage and respective amino acid residues of RdRp were observed to be less than 3.5 Å. These ligands represented hydrogen bond interactions, thereby exhibiting maximum and strong interactions with the receptor in comparison to azadirachtin and columbin, which showed a bond distance up to 4.5 Å. These two ligands were

found to be sharing hydrophobic or non-covalent interactions with RdRp (Table 3).

### ADMET

The ADMET (Absorption, Dissolution, Metabolism, Excretion, Toxicity) -based drug scanning tool ADMETlab Web Server predicted the physiochemical and biological properties of the selected potential inhibitors against RdRp. Aloe is a dihydroxyanthroquinone with molecular weight 270.24 g/mol, Log P value of 1.365; contains three hydrogen bond donor atoms and five hydrogen bond acceptor atoms. Other potential inhibitors azadirachtin, cirsilineol, columbin, nimbiol, nimbocinol and sage are a terpenoid, flavone, diterpenoid, terpenoid and triterpenoids (nimbocinol and sage) with molecular weights of 720.7, 344.3, 358.4, 272.4, 408.5, 409.6 g/mol; Log P value of 0.203, 2.89, 2.53, 4.37, 4.8, 4.3; the compound contains 3, 7, 1, 1, 1 and 1 hydrogen bond donor atoms and 16, 7, 6, 2, 4 and 4 hydrogen bond acceptor atoms, respectively (Table 4). To further validate the drug-like properties, all the above-mentioned inhibitors were subjected to ADMETlab Web Server to determine their absorption, distribution, metabolism and excretion. All four parameters were investigated on the basis of several thresholds. All seven predicted inhibitors of RdRp passed the ADMETlab threshold of drug capability. On further evaluation of toxicological parameters all the ligands were observed to be negative in the AMES mutagenicity test and being non-skin sensitizing ruled out their possibility to induce any skin-related allergic reactions (Table 5).

## Discussion

Despite making several efforts to discover an anti-viral drug against COVID-19, no US Food and Drig Administration-approved preventive/therapeutic drug has yet reached the market. Rather, the scientific community is still struggling to identify the prime target in SARS-CoV-2 to control the current outbreak. SARS-CoV-2 comprises a multi-subunit replication and transcription machinery. Non-structural proteins formed by the cleavage of viral polyproteins together configure the replication and transcription mechanism of the virus. RdRp facilitates the synthesis of SARS-CoV-2 and thereby plays a pivotal role in its survival and transmission. RdRp may be considered as a potential drug target for several drugs competing to eliminate the complication induced by the viral attack. Gao *et al.* in 2020 released the structure of the RdRp from SARS-CoV-2 and emphasized its role as an efficient target to control the outbreak by inhibiting virus proliferation [9].

**TABLE 3. Non-bonded distance between the ligand and active site amino acid residues thereby depicting hydrogen bond or hydrophobic interactions between the ligands and receptors**

| Ligands | Amino acid residues | Bond distance (Å) |
|---|---|---|
| Aloe | ASN 459 | 3.5 |
| | ARG 349 | 2.4 |
| | VAL 315 | 3.4 |
| | PRO 677 | 3.7 |
| | GLU 350 | 3.5 |
| Azadirachtin | PRO 677 | 3.9 |
| | PRP 471 | 4.7 |
| | THR 394 | 4.3 |
| | ASN 459 | 3.3 |
| | ARG 249 | 4.8 |
| Cirsilineol | ASN 628 | 2.4 |
| | PRO 461 | 3.6 |
| | VAL 675 | 3.5 |
| | LEU 480 | 3.6 |
| | PRO 323 | 3.7 |
| Columbin | CYS 395 | 3.7 |
| | PRO 461 | 3.2 |
| | ARG 457 | 4.4 |
| | THR 394 | 4.6 |
| | ARG 349 | 4.2 |
| Nimbiol | ASN 628 | 2.5 |
| | PRO 461 | 3.7 |
| | ARG 349 | 3.6 |
| | PRO 677 | 3.6 |
| Nimbocinol | ASN 359 | 3.5 |
| | PRO 323 | 3.6 |
| | ARG 349 | 3.0 |
| | LEU 460 | 3.4 |
| | VAL 675 | 3.4 |
| Sage | SER 255 | 2.8 |
| | THR 252 | 3.0 |
| | PRO 461 | 3.1 |
| | LYS 267 | 3.4 |

Several drugs have been analysed to determine the potential of synthetic analogues to target SARS-CoV-2. Wang *et al.* and Yao *et al.* evaluated the potency of remdesivir and chloroquine to inhibit SARS-CoV-2 on the basis of their ability to target SARS-CoV and MERS-CoV [20,21], but neither of these passed the clinical trials against the disease [22]. A study by Masui *et al.* in 2017 and Ganjhu *et al.* in 2015 have also emphasized the potential role of herbal remedies in targeting several viral diseases including influenza, rabies and enterovirus [23,24]. The present study was designed to investigate the role of phytochemicals in targeting SARS-CoV-2 replication. We screened

**TABLE 4. Physiochemical properties (Log S, Log P and Log D) of ligands exhibiting highest binding affinity with RdRp of SARS-CoV-2**

| Ligands | Log S (solubility) | Log D (distribution coefficient D at pH 7.4) | Log P (distribution coefficient P) |
|---|---|---|---|
| Aloe | −3.93 | −0.637 | 1.365 |
| Azadirachtin | −2.916 | 1.314 | −0.203 |
| Cirsilineol | 3.573 | 0.943 | 2.897 |
| Columbin | −3.899 | 0.945 | 2.533 |
| Nimbiol | −4.526 | 1.473 | 4.371 |
| Nimbocinol | −5.0178 | 3.529 | 4.847 |
| Sage | −5.178 | 3.529 | 4.344 |

**TABLE 5.** ADMET evaluation of the ligands to analyse their biological activities

| Ligands | Absorption | | | Distribution | | | Metabolism | | Excretion | | Toxicity | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Log Papp $CaCO_2$ | Human intestinal absorption | F30% availability | Plasma protein binding | Blood–brain barrier | Volume distribution | CYP450 1A2 inhibitor | CYP450 3A4 inhibitor | $T_{1/2}$ (half-life) | Clearance | Human hepatotoxicity | Mutagenicity | $LD_{50}$ (mg/kg) | Skin sensitization |
| Aloe | 5.126 | 0.449 | 0.554 | 74.04 | 0.455 | 0.879 | 0.715 | 0.248 | 0.94 | 1.468 | 0.866 | 0.928 | 2.78 | 0.327 |
| Azadirachtin | 5.714 | 0.45 | 0.334 | 50.32 | 0.106 | 0.113 | 0.086 | 0.775 | 2.045 | 1.369 | 0.032 | 0.274 | 3.73 | 0.26 |
| Cirsilineol | 5.068 | 0.608 | 0.463 | 84.73 | 0.494 | 0.443 | 0.787 | 0.703 | 1.769 | 1.851 | 0.676 | 0.27 | 2.81 | 0.21 |
| Columbin | 4.771 | 0.616 | 0.476 | 74.83 | 0.744 | 0.208 | 0.093 | 0.318 | 1.296 | 1.778 | 0.592 | 0.248 | 3.19 | 0.304 |
| Nimbiol | 4.601 | 0.848 | 0.574 | 85.75 | 0.965 | 0.567 | 0.175 | 0.316 | 1.78 | 1.817 | 0.448 | 0.074 | 2.41 | 0.486 |
| Nimbocinol | 4.885 | 0.774 | 0.476 | 88.1 | 0.921 | 0.004 | 0.065 | 0.5 | 1.696 | 1.872 | 0.514 | 0.14 | 3.57 | 0.39 |
| Sage | 4.846 | 0.784 | 0.376 | 80.91 | 0.83 | 0.001 | 0.099 | 0.206 | 2.118 | 1.402 | 0.544 | 0.3 | 3.21 | 0.381 |

cost-effective moieties belonging to plants which can be easily grown even in wild conditions, and furthermore the processing of these medicinal plants does not require highly equipped technology for their extraction and purification. These medicinal plants have remained an integral part of our dietary routine for centuries and no adverse effects have been reported so far with their usage. Hence, an *in silico* study was carried out to hinder the virus replication by disrupting the domain of RdRp with potential inhibitors like aloe, azadirachtin, columbin, cirsilineol, nimbiol, nimbocinol and sage belonging to *Aloe vera*, *Azadirachta indica*, *Ocimum sanctum*, *Tinospora cordifolia* and *Salvia officinalis*. A molecular docking study was carried out at different domains of RdRp to analyse their inhibitory potential. Seven potential ligands were screened from long lists of phytochemicals on the basis of their binding affinity. Disruption of RdRp at 71 × 53 × 25 Å grid points by the above-mentioned ligands revealed that LEU460, ASN459, TYR456, PRO461 and PRO677 could form the potential active site that needs to be targeted to control the virus proliferation. The inhibitory potential of these molecules was more profound than chloroquine, lamivudine, favipiravir and remdesivir. Apart from determining their inhibitory potential, our effort of simultaneous exploration of their structural and functional characteristic features produced encouraging results. Docked conformers were observed to be structurally stable with respect to their native pose on the basis of their RMSD values. Our proposed drug-like molecules were observed to form hydrogen bonds in addition to hydrophobic bonds with crucial amino acid residues of RdRp, thereby hindering their function, which is essential for virus replication. Moreover, physiochemical and biological properties of these phyoto-ligands have validated their potency to be acclaimed as potential inhibitors of RdRp. The set of compounds screened in this study, could potentially act as mono-therapeutic or as a combination therapeutic approach against SARS-CoV-2.

Computational analysis on the basis of a docking algorithm revealed that these seven phytochemicals may disrupt the replication domain of SARS-CoV-2 to target the virus attack. An upsurge of these phytochemicals will act as structural and functional templates for *de novo* synthesis of drugs as an efficient possible treatment strategy against COVID-19.

## Conclusion

Computational interpretation of the present study revealed that the conserved domain of RdRp can be efficiently targeted by phytochemicals belonging to *Aloe vera*, *Azadirachta indica*, *Ocimum sanctum*, *Tinospora cordifolia* and *Salvia officinalis* to overcome the current COVID-19 pandemic. Medicinal plants

have been used for centuries to treat viral infection and the present study has validated those facts in the case of COVID-19. On the basis of our computational study we can conclude that phytochemicals can significantly hinder the replication domain of SARS-CoV-2 through its ability to exhibit efficient binding ability, structural stability and drug-like physiochemical and biological properties even better than the synthetic analogues currently in use against the disease. We believe that insights gained by *in silico* analysis in the current study will prove valuable in further exploring the targeted site of SARS-CoV-2 and potential inhibitors.

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## Authors' contribution

VP and RM have framed the entire work under the expert guidance of AB.

## Conflict of interest

The authors have declared that there are no conflicts of interest.

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