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*Current Medicinal Chemistry*, 2022, *29*, 152-162

RESEARCH ARTICLE

# Cordycepin as a Promising Inhibitor of SARS-CoV-2 RNA Dependent RNA Polymerase (RdRp)

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**Abstract:** ***Background*:** SARS-CoV-2, which emerged in Wuhan, China, is a new global threat that has killed millions of people and continues to do so. This pandemic has not only threatened human life but has also triggered economic downturns across the world. Researchers have made significant strides in discovering molecular insights into SARS-CoV-2 pathogenesis and developing vaccines, but there is still no successful cure for SARS-CoV-2 infected patients.

***Objective*:** The present study has proposed a drug-repositioning pipeline for the design and discovery of an effective fungal-derived bioactive metabolite as a drug candidate against SARS-CoV-2.

***Methods*:** Fungal derivative "Cordycepin" was selected for this study to investigate the inhibitory properties against RNA-dependent RNA polymerase (RdRp) (PDB ID: 6M71) of SARS-CoV-2. The pharmacological profile, intermolecular interactions, binding energy, and stability of the compound were determined utilizing cheminformatic approaches. Subsequently, molecular dynamic simulation was performed to better understand the binding mechanism of cordycepin to RdRp.

***Results*:** The pharmacological data and retrieved molecular dynamics simulations trajectories suggest excellent drug-likeliness and greater structural stability of cordycepin, while the catalytic residues (Asp760, Asp761), as well as other active site residues (Trp617, Asp618, Tyr619, Trp800, Glu811) of RdRp, showed better stability during the overall simulation span.

***Conclusion*:** Promising results of pharmacological investigation along with molecular simulations revealed that cordycepin exhibited strong inhibitory potential against SARS-CoV-2 polymerase enzyme (RdRp). Hence, cordycepin should be highly recommended to test in a laboratory to confirm its inhibitory potential against the SARS-CoV-2 polymerase enzyme (RdRp).

ARTICLE HISTORY

Received: April 01, 2021
Revised: June 21, 2021
Accepted: June 28, 2021

*DOI:*
*10.2174/0929867328666210820114025*

**Keywords:** Cordycepin, bioactive metabolite, drug repurposing, SARS-CoV-2, COVID-19, molecular dynamic simulation.

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Current Medicinal Chemistry

## 1. INTRODUCTION

SARS-CoV-2 (severe acute respiratory syndrome coronavirus)/COVID-19 infection was first diagnosed in Wuhan, a grand city of China, in late December 2019, and it was announced as a causative pathogen by January 2020 [1, 2]. WHO (World Health Organization) announced the state of strict emergency around the globe by 30th January, 2020 [3]. Fever, cough, dyspnea, difficulty in breathing, fatigue, myalgia, decreased leukocyte count, and lymphopenia are the primary concerns associated with SARS-CoV-2 infections that need to be controlled initially. If prolonged, then it may cause severe pneumonia, cardiac problems, respiratory failure, and finally death [4-6]. Even though more than one year has passed, it has still been observed that a majority of the population from developed, as well as developing countries, are suffering from SARS-CoV-2 infection. In the United States alone, there are nearly 30 million cases of coronavirus infections and over 540,000 deaths as of 25 March, 2021 [7]. Based on the latest report of WHO, a total number of 124,215,843 confirmed cases has been reported, 2,734,374 patients died due to SARS-CoV-2 infection, and 431,895,992 people have been vaccinated until 25 March, 2021 [8]. Fig. (**1**) shows the severity of this infection around the globe. The statistical information is retrieved from the coronavirus online portal [9]. Many vaccines are designed and tested in several countries, but it is still challenging to manage this devastating disease around the world, and hence, there is an urge for novel and highly efficacious drugs which should be cheap and more frequently available for SARS-CoV-2 affected patients around the world [10].

Coronavirus is a 26-32 kb positive-sense single-stranded RNA virus that encodes 9860 amino acids with 29,891 nucleotides [11]. The virus was discovered to be a member of the Betacoronaviruses (β-CoV) family [12-14]. The SARS-CoV-2 genome has approximately 80% match to the SARS-CoV genome and about more than 90% similarity to the RaTG13 (bat coronavirus) [15-17]. As per its genome, it has 14 ORFs that encode 27 proteins [18-20]. Morphologically, the virus has two types of proteins, such as structural proteins, which includes S (spike), M (membrane), E (envelope), and N (nucleocapsid) proteins, while nsp1 to nsp16 are non-structural proteins, that include RdRp (RNA dependent RNA polymerase), also known as nsp12 [18, 21]. RdRp plays a significant role in the SARS-CoV-2 infection cycle and pathogenesis, involving viral replication and transcriptional processes [22-24]. The palm, thumb, and fingers make up the RdRp domain architecture, which represents a slightly cupped "right hand" grip (Fig. **2**) [25]. In the palm region, the conserved polymerase motif (A-G) forms the SARS-CoV-2 RdRp active site, which is positioned analogous to other RNA polymerases [22, 26, 27].

RdRp enzyme has been targeted previously in various viral infections due to similar conserved active binding domain residues such as HCV [28], Zika virus [29], and CoV [30]. The main catalytic amino acids at 759, 760, and 761 positions (Ser-Asp-Asp) in the palm domain is the binding cleft for RNA; this, along with Asp618 (divalent cation-binding residue), is required for replication [25, 31-35]. Therefore, the protein RdRp offers a potential opportunity as the target for the chosen bioactive metabolite.

Cordycepin is selected for this study as it is found in several fungal species, especially Cordyceps, the entomopathogenic fungus, and it presents multiple biological activities [34, 35]. Cordycepin is structurally similar to adenosine (Fig. **3**), although its ribose moiety lacks a 3' hydroxyl group [36-38]. As a result, it acts as an inhibitor of poly(A) polymerase, causing premature protein synthesis to stop [39, 40]. Furthermore, active RNAs from the genome of SARS-CoV-2 are considered to be extremely 3'-polyadenylated, contributing to all viral proteins' synthesis [41, 42]. Hence, cordycepin may inhibit the polyadenylation mechanism in SARS-CoV-2 RNAs, and this could be a significant achievement considering viral replication inhibition within the host body. Cordycepin has presented antiviral potential against several human viruses, including EBV [43, 44], murine leukemia virus [45], HIV [46], influenza virus [47], as well as different plant viruses [48, 49]. The anti-SARS-CoV-2 potential of cordycepin has also been reported against receptor binding domain and main protease in previous studies [50, 51]. Cordycepin is currently being investigated in a clinical setting (NCT00709215) [52]. Therefore, cordycepin should be repurposed to assess efficacy and protection for COVID-19 treatment.

Many barriers stand in the way of rational strategies for the development of viable therapeutics [53, 54]. In this context, drug repurposing is the pandemic's most-required approach until the FDA-approved medicine becomes available in the market for SARS-CoV-2 infections. Several researchers are using this approach by computer-aided drug design schemes to design and discover novel inhibitors as SARS-CoV-2 drugs [55-58]. Using state-of-the-art cheminformatic techniques, the current study investigated the delicate molecular mechanisms by which cordycepin's antiviral potential is

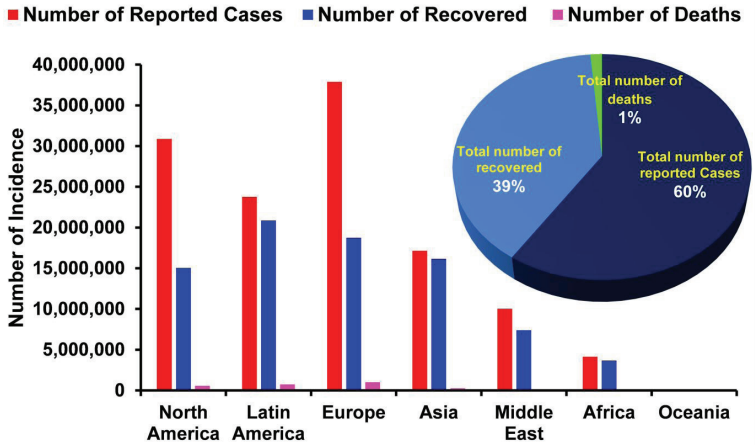


**Fig. (1).** Current statistics of COVID-19 incidence around the globe till 24th March 2021 [9]. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

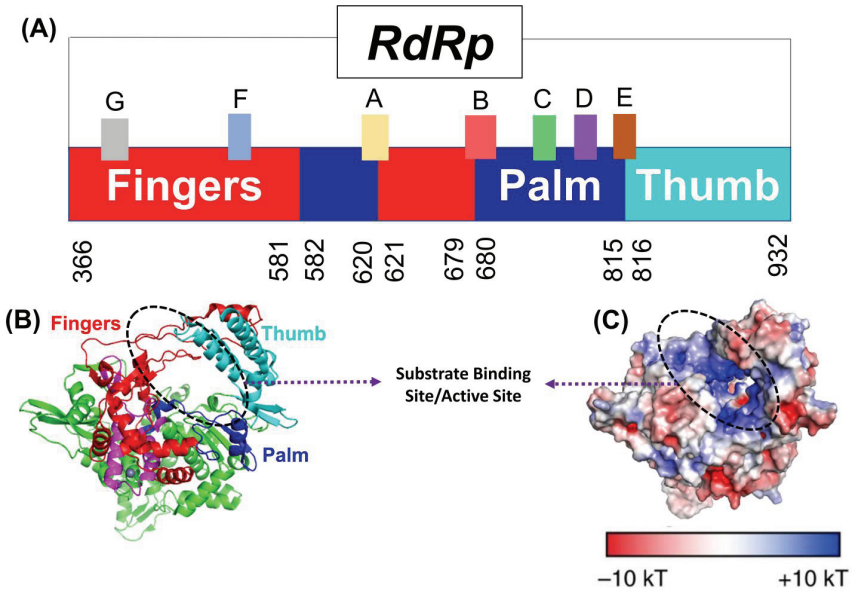


**Fig. (2).** Structure of the SARS-CoV-2 RdRp protein. (**A**) Diagram of the RdRp protein domains. The seven conserved motifs (A-G) in the catalytic site are illustrated in the colors indicated. (**B**) Structural architecture of RdRp protein (PDB ID: 6M71) [thumb (blue), palm (blue), fingers (red)]. (**C**) Surface electrostatics of RdRp protein. The substrate-binding site is charged positively, whereas the rest of the protein is charged negatively. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

mediated against SARS-CoV-2 RdRp. The pharmacological profile, intermolecular interactions, binding energy, and stability of the compound were determined using both molecular dynamics (MD) simulations as well as docking protocol for a deeper understanding of the binding mechanism of cordycepin to RdRp, which have never been explored before. It is evident from the promising results of this computational study that fungal metabolite "cordycepin" could be a potential drug against SARS-CoV-2 infections. Therefore, the investigation lays the groundwork for future research into the feasibility of repurposing cordycepin to treat COVID-19.

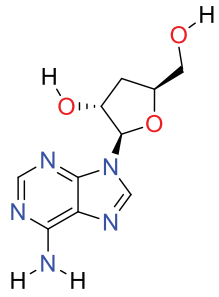

**Fig. (3).** Chemical structure of Cordycepin. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

## 2. MATERIAL AND METHODS

### 2.1. Retrieval and Preparation of Receptor-ligand Structure

The crystal structure of the RdRp protein of SARS-CoV-2 was retrieved from the RCSB-PDB database (PDB ID: 6M71) [22, 59]. Following retrieval, the protein was prepared with the software package Swiss-PDB viewer (v4.1.0) upon energy minimization. Subsequently, the water molecules and other hetero-atoms available in the structure were erased utilizing the PyMol software package (v1.1) [60]. The highly important secondary metabolite found in fungal species, cordycepin, was chosen for the study. The 3D structure of cordycepin was collected from the PubChem database [61]. The Open Babel software package (v2.3.1) was then utilized to prepare and generate the PDB format of the ligand [62].

### 2.2. Pharmacokinetics and Toxicity Prediction

The drug-likeliness and pharmacokinetic characteristics of cordycepin were evaluated with the SwissADME server [63]. The optimized canonical SMILES format of the ligand was used to register the pharmacokinetic profiles. Likewise, utilizing the ProTox-II server, we ran prediction models for different toxicity levels of the molecule [64].

### 2.3. Molecular Docking

Molecular docking approach was deployed to get insights on probable conformations and binding mode of cordycepin at RdRp's substrate recognition site. AutoDock Vina docking algorithm was utilized to determine the binding energy and the interactions between cordycepin and RdRp [65]. For docking, the grid box dimensions were X: 31.08, Y: 29.76, Z: 23.81; with the grid center being set X= 145.85, Y= 141.66, Z= 156.30. Post-docking analysis, including three-dimensional visualization and two-dimensional schematic diagrams of the selected ligand cordycepin within the respective binding site of RdRp showing hydrophobic residues and hydrogen bonds within distance range of 4Å were generated by Ligplot+ [65].

### 2.4. Molecular Dynamics (MD) Simulation

Stability and intermolecular interactions of 6M71 (RdRp)-Cordycepin complex was elucidated by performing the 50 nanoseconds (ns) molecular dynamic (MD) simulation implemented in Desmond software [66]. The simulation was performed through the Desmond package having a solvent system with OPLS3 force-field [67]. The molecular system was solvated with TIP3P water molecules and Na/Cl ions were used to neutralize the system electrically. System minimized its energy by the heating process before MD simulation. A complex of cordycepin-RdRp was also minimized using the minimization method that includes the steepest descent steps-based protocol. Moreover, the system was brought in equilibrium condition at 1000 steepest descent steps and the system was updated. Finally, within an orthorhombic box with dimensions set at 10 Å distances, simulation was run at 50ns at 300k temperature and 1 atm pressure using the NPT-ensembles [68]. PRODIGY server was utilized on MD snapshots retrieved during MD simulations for intermolecular contacts-based binding energy calculations [69, 70].

## 3. RESULTS AND DISCUSSION

### 3.1. Pharmacokinetic Study of Cordycepin

It is important to assess the pharmacokinetic features (i.e., bioavailability, safety, drug-likeliness) of cordycepin to determine the possible biological activity and to get an idea about its potential as a candidate drug molecule for COVID-19. In this context, *in-silico* approaches were utilized to predict the drug-likeliness, physicochemical, and ADMET properties of cordycepin. The summary of predicted pharmacokinetic characteristics of cordycepin is listed in Table **1**. The Log P value of cordycepin was reported to be -0.80, which is well within the considerable limit (-1.36 to 3.78). The results of the SwissADME server demonstrate that hydrogen acceptors, hydrogen donors, and the molecular weight of cordycepin are 6, 3, and 251.24, respectively, with no violation recorded for Lipinski's rules. Cordycepin's Log P value is less than 5, molecular weight is less than 500, the number of hydrogen bond acceptors is less than 10, and some hydrogen bond donors are less than 5. Hence, Lipinski's rule of 5 was deemed valid for cordycepin. Cordycepin also adheres to Veber's law with rotatable bonds count (2) and the TPSA value (119.31) is shown to be within the accepted limit for oral administration. The bioavailability (0.55) of the compound was found to be at an optimum level. Besides, we anticipate cordycepin to be exceedingly soluble in water. BBB permeability, GI absorption, carcinogenicity, and toxicity help assess the distribution, absorption, as well as safety profiles of a compound. Based on these parameters, cordycepin suggests high gastrointestinal absorption, no blood-brain-barrier permeability, and no carcinogenicity and toxicity. Overall, cordycepin appears to be a safe and excellent drug-like molecule, according to the conducted pharmacokinetic investigation (Table **1**).

**Table 1. Summary of predicted pharmacokinetic characteristics of cordycepin.**

| Physicochemical Features | |
|---|---|
| Log P | -0.80 |
| Molecular Weight (g/mol) | 251.24 |
| Hydrogen Donors | 3 |
| Hydrogen Acceptors | 6 |
| No. of Violations | 0 |
| Total polar surface area (TPSA) ($Å^2$) | 119.31 |
| No. of Rotatable Bonds | 2 |
| **Drug-likeliness** | |
| Bioavailability Score | 0.55 |
| Lead Likeness | Yes |
| **ADMET Properties** | |
| Water Solubility Class | Very Soluble |
| GI Absorption | High |
| BBB Permeant | No |
| Carcinogens | Non-carcinogen |
| Toxicity | No |

### 3.2. Molecular Docking Simulation

RdRp is the viral polymerase enzyme, which is a key facilitator of the viral infection cycle. Hence, RdRp inhibition will halt the viral replication event [19, 71]. The catalytic triad of RdRp consists of amino acid (AA) residues Ser759, Asp760, Asp761, and ligand binding with these AA residues is critical for disrupting the activity of the enzyme [72]. Molecular docking simulations for cordycepin with RdRp were initially executed to better understand the binding mechanism. The docking score of -6.6 kcal/mol was recorded for cordycepin. The nature of their intermolecular interactions with the surrounding AA residues located in RdRp's active site is reflected in the docking score (Fig. **4**). Cordycepin showed strong interactions, including both hydrophobic and hydrogen bond mediated interactions with the catalytic residues Asp760, Asp761 (Table **2**). In addition, neighboring amino acid residues Trp617, Asp618, Trp800, and Glu811 in RdRp's active site are being reported to interact with cordycepin via non-bonding interactions (Table **2**). Based on the molecular docking simulation study, cordycepin exhibits strong inhibition potential against SARS-CoV-2 polymerase enzyme (RdRp), and the stability of the compound was further validated with MD simulation protocol.

### 3.3. MD Simulation of Cordycepin-RdRp Complex

The docking study provides an initial insight into the compatibility of cordycepin into RdRp's substrate recognition site by implementing a rigid body simulation of the compound into the binding pocket of the enzyme. MD simulation helps researchers to accurately determine the interactions and stability of the ligand-receptor complex. Hence, the compound cordycepin was subjected to 50 ns MD simulation. Fig. (**5**) shows how the cordycepin-RdRp complexes' RMSD (root mean square deviation) and RMSF (root mean square fluctuation) trajectories changed throughout the MD production runs. The RMSD calculates the atom's convergence from a reference state, while the RMSF of a protein provides an idea of the protein's dynamic nature that contributed to the system's overall versatility. The RMSD values of the molecule cordycepin persist mostly inside 2.5 Å (average 2.48 ± 0.48 Å), whereas the protein's average RMSD was observed to be relatively higher (average 2.77 ± 0.30 Å) (Fig. **5A**). The RMSD trajectories of cordycepin-RdRp are indicative of their higher structural stability. Fig. (**5B**) depicts the local residue flexibility of RdRp. Higher fluctuations were reported in the (19-53), (858-925), and (1039-1082) regions. The catalytic residues (Ser759, Asp760, Asp761) as well as other active site residues (Trp617, Asp618, Tyr619, Trp800, Glu811) of RdRp showed better stability during the overall simulation span and thus suggested that the cordycepin-RdRp complex was stable throughout the simulation time. Ligand RMSF was also analyzed corresponding to the proteins binding region residues and ligand first frame, and it was found to be stable in both cases during the simulation (Fig. **5C**). Structural superposition procedure was employed after retrieving the docking and MD simulation structure of the cordycepin-RdRp complex (Fig. **6**). This approach provides important insights into the binding mode and conformational variation of ligand-protein of a particular complex. The cordycepin molecule fluctuates very little and is considered natural. Moreover, the superimposed configuration of the cordycepin-RdRp complex derived from docking and MD simulation shows little variance, indicating that the cordycepin is extremely stable when bound to the protein RdRp.

### 3.4. Intermolecular Interactions of Cordycepin-RdRp Complex

Intermolecular interactions of the cordycepin-RdRp complex during 50 ns time span of molecular dynamics simulation has been explained in Fig. (**7**). Fig. (**7A**) demonstrates the histogram to explain different types of

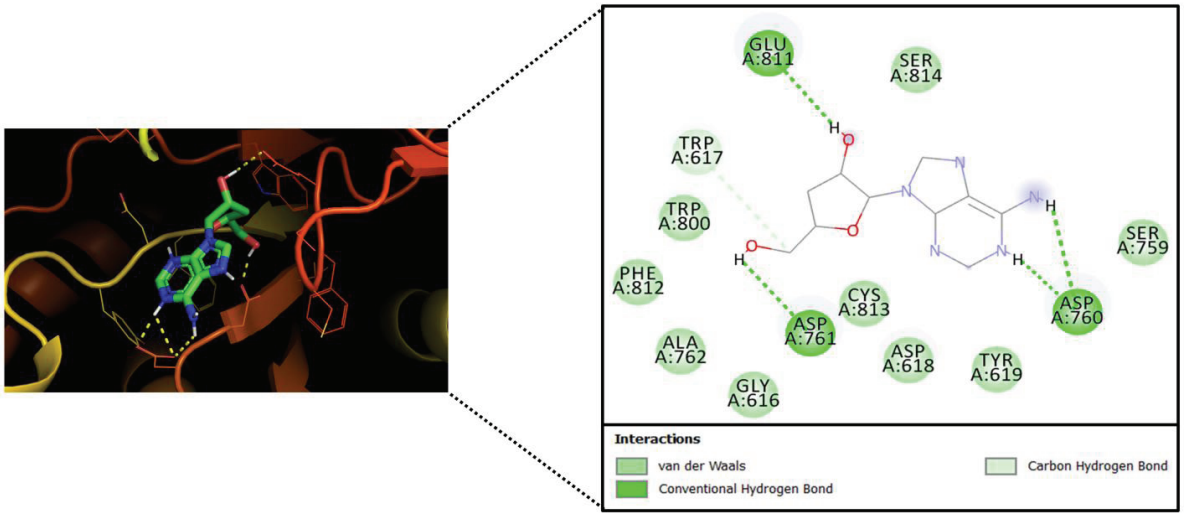


**Fig. (4).** Binding mode of cordycepin to RdRp of SARS-CoV-2. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

**Table 2. Binding affinity and non-bonding interactions of the cordycepin-RdRp complex from molecular docking and MD simulation.**

| Binding affinity (kcal/mol) | Molecular interactions before simulation | | Molecular interactions after 50ns simulation | |
|---|---|---|---|---|
| | Hydrophobic interacting residues | Hydrogen bonding residues | Hydrophobic interacting residues | Hydrogen bonding residues |
| -6.6 | Trp617, Asp618, **Asp760**, **Asp761**, Trp800, Glu811 | **Asp760**, **Asp761**, Glu811 | **Asp760**, **Asp761**, Trp617, Trp800 | **Asp761**, Trp617, Tyr619, Trp800, Glu811 |

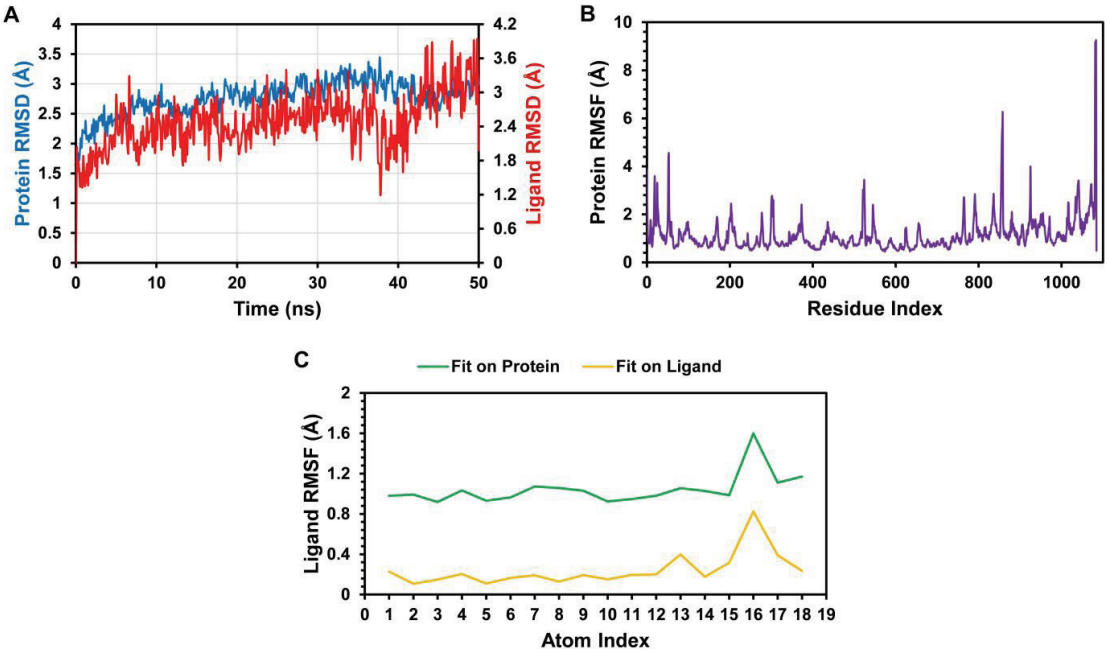


**Fig. (5).** (**A**) RMSD profile of protein (RdRp) and ligand (cordycepin), (**B**) RMSF profile of protein (RdRp) residues, (**C**) ligand RMSF profile of cordycepin for RdRp-cordycepin complex during 50 ns molecular dynamics simulation. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

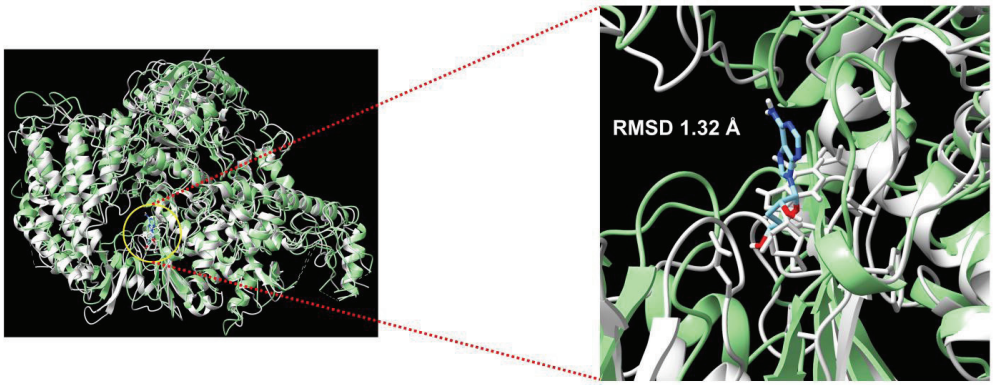


**Fig. (6).** Superimposed view of the cordycepin-RdRp complex from molecular docking (light gray one) and MD simulations (colorful one). *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

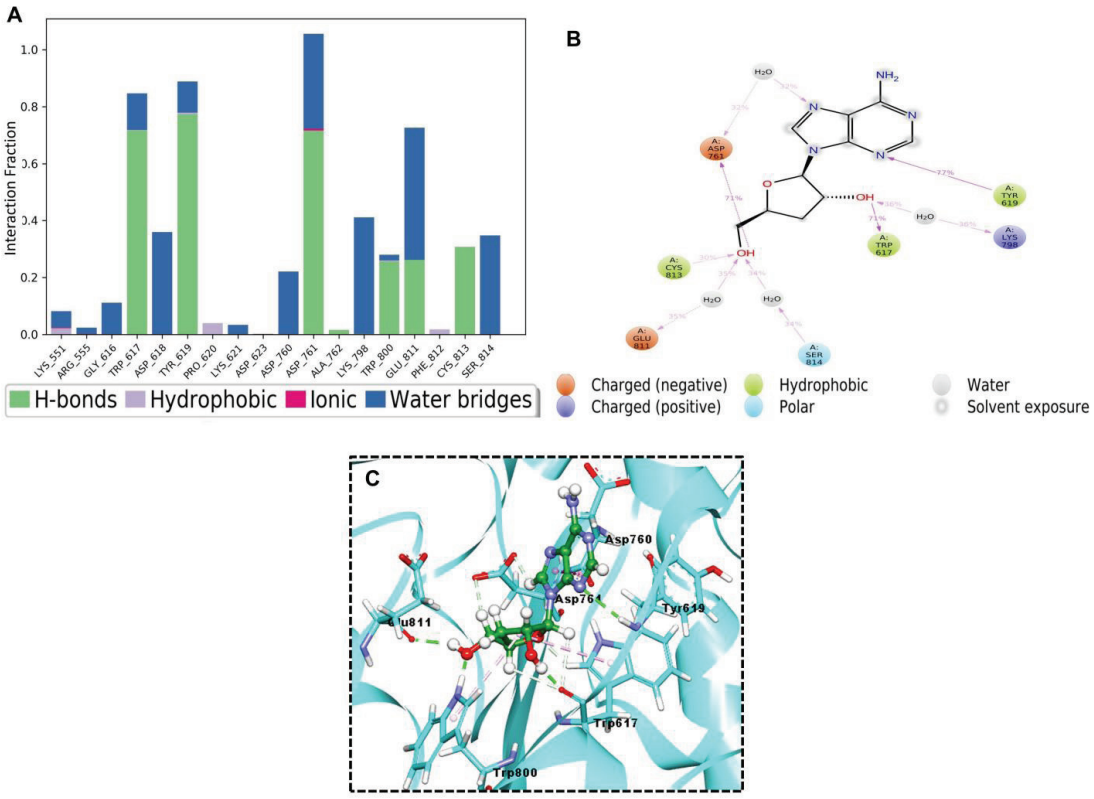


**Fig. (7).** Intermolecular interactions of the cordycepin-RdRp complex during 50 ns molecular dynamics simulation. (**A**) Histogram showing different types of interactions, (**B**) 2D view of the interactions, (**C**) Interactions in the active site of RdRp. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

interactions, while (Fig. **7B**) depict the two-dimensional demonstration of macromolecular - RdRp inhibitor interactions. Fig. (**7C**) shows the intermolecular interactions between cordycepin and RdRp's active site residues after 50 ns MD simulation. In accordance with the docking result (Fig. **4**), the MD simulation studies also elucidate that cordycepin exhibited strong binding interactions with both catalytic and active site residues of RdRp (Fig. **7C**). The MD simulation studies revealed that cordycepin had good binding interactions with both the catalytic and active site residues of RdRp (Fig. **7C**), which is consistent with the docking result (Fig. **4**). Cordycepin interacted with RdRp residues Trp617, Tyr619, Asp761, Glu8111 to form hydrogen-bond-water interactions, as well as Gly616, Asp618, Asp760, Lys798, Ser814 to form water bridge interactions, and Pro620, Phe812 to form hydrophobic interactions (Fig. **7A** and **7B**). The interactions in the

cordycepin-RdRp complex were characterized and found to be very strong. Cordycepin is highly stable and forms the predicted interactions with the important amino acids Asp760, Asp761, Asp618, Trp617, Tyr619, and Glu811 of RdRp's active site. The calculated value of the binding energy of cordycepin with RdRp is -7.7 ± 0.43 kcal/mol. To sum up, the MD simulation outcomes endorse the docking data, suggesting that cordycepin interacts actively with RdRp.

## CONCLUSION

SARS-CoV-2 has posed a serious threat to countless countries and organizations around the world since its discovery in Wuhan in 2019. The virus, which has more diverse pathogenesis and modes of transmission than SARS-CoV-1 and MERS, caused a sharp rise in morbidity and mortality rates in many countries, quickly spreading across the planet's six continents. Given the pandemic's urgent needs, it is required to develop an efficacious drug that should be cheap and more frequently available for COVID-19 patients. In this study, the bioactive metabolite was computationally examined against SARS-CoV-2 RdRp protein to inhibit the replication of the pathogen and disrupt viral pathogenesis. Promising results of pharmacological and toxicity estimation along with molecular docking simulations revealed that fungal metabolite cordycepin exhibits strong inhibition potential against SARS-CoV-2 polymerase enzyme (RdRp). The strong molecular stability of cordycepin was obtained from molecular dynamic simulations. Significant results of this *in silico* study could be helpful to develop an anti-SARS-CoV-2 drug. Hence it is highly recommended to confirm the activity of cordycepin as a SARS-CoV-2 polymerase enzyme (RdRp) inhibitor in laboratory settings.

There are some drawbacks to this research. The capacity to simulate multiple-ligand interactions in the protein cavity is limited by current *in-silico* approaches. In the binding cavity of the target protein RdRp, just one ligand was used, whereas several ligands can access the same binding regions in the biological system and display synergistic actions. On that basis, the findings of the computational investigation can be used as a reliable framework for future *in vitro* and *in vivo* testing, and more experimental validation is needed to assess how the projected benefits will apply to complex biological systems.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

## HUMAN AND ANIMAL RIGHTS

No animals/humans were used for the studies that are the basis of this research.

## CONSENT FOR PUBLICATION

Not applicable.

## AVAILABILITY OF DATA AND MATERIALS

Not applicable.

## FUNDING

This work was funded by the National Natural Science Foundation of China (31870017), the Department of Science and Technology of Yunnan Province (2018FY001(-006)).

## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

## ACKNOWLEDGEMENTS

The authors would like to thank Yunnan Herbal laboratory, College of Ecology and Environmental Science, Yunnan University, Kunming 650091, Yunnan, China.

The authors would also like to thank Myra Janet from the department of Information and Technology, Victoria University, Sydney campus, Australia, for editing and revising this manuscript.

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