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ARTICLE IN PRESS

Saudi Journal of Biological Sciences xxx (xxxx) xxx

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Identification of SARS-CoV-2 RNA-dependent RNA polymerase inhibitors from the major phytochemicals of *Nigella sativa*: An *in silico* approach

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ARTICLE INFO

Article history:
Received 17 January 2021
Revised 29 August 2021
Accepted 1 September 2021
Available online xxxx

Keywords:
Nigella Sativa
SARS-CoV-2
Docking
In silico
Phytochemical
Drug
RNA polymerase

ABSTRACT

The coronavirus disease 2019 (COVID-19), which emerged in December 2019, continues to be a serious health concern worldwide. There is an urgent need to develop effective drugs and vaccines to control the spread of this disease. In the current study, the main phytochemical compounds of *Nigella sativa* were screened for their binding affinity for the active site of the RNA-dependent RNA polymerase (RdRp) enzyme of the severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2). The binding affinity was investigated using molecular docking methods, and the interaction of phytochemicals with the RdRp active site was analyzed and visualized using suitable software. Out of the nine phytochemicals of *N. sativa* screened in this study, a significant docking score was observed for four compounds, namely α-hederin, dithymoquinone, nigellicine, and nigellidine. Based on the findings of our study, we report that α-hederin, which was found to possess the lowest binding energy (−8.6 kcal/mol) and hence the best binding affinity, is the best inhibitor of RdRp of SARS-CoV-2, among all the compounds screened here. Our results prove that the top four potential phytochemical molecules of *N. sativa*, especially α-hederin, could be considered for ongoing drug development strategies against SARS-CoV-2. However, further *in vitro* and *in vivo* testing are required to confirm the findings of this study.
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1. Introduction

The coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome-coronavirus (SARS-CoV)-2, has adversely affected the health of people across the globe (Mali et al., 2020). As of January 16, 2021, there are 92,262,621 confirmed cases of COVID-19 worldwide, including 1,995,037 deaths (https://www.who.int/emergencies/diseases/novel-coronavirus-2019). SARS-CoV-2 belongs to the *Coronaviridae* family and is closely related to the other members of the family, especially SARS-CoV and the Middle East respiratory syndrome coronavirus (MERS-CoV) (Hui et al., 2020; Mali et al., 2020). The positive stranded RNA genome of SARS-CoV-2 is surrounded by a lipid envelope which contains the spike proteins as well as the membrane proteins. The spike proteins of SARS-CoV-2 bind to the host

Abbreviations: COVID-19, coronavirus disease 2019; GUI, graphical user interface; MERS-CoV, Middle East respiratory syndrome coronavirus; RCSB PDB, Research Collaboratory for Structural Bioinformatics Protein Data Bank; RdRp, RNA-dependent RNA polymerase; RMSD, root mean square deviation; RMSF, root mean square fluctuations; SARS-CoV-2, severe acute respiratory syndrome-coronavirus-2.

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.09.002
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Please cite this article as: Shabir Ahmad Mir, A. Firoz, M. Alaidarous et al., Identification of SARS-CoV-2 RNA-dependent RNA polymerase inhibitors from the major phytochemicals of *Nigella sativa*: An *in silico* approach, Saudi Journal of Biological Sciences, https://doi.org/10.1016/j.sjbs.2021.09.002

Table 1
Chemical structures of the main phytochemical compounds of Nigella Sativa and remdesivir (control drug) along with their respective docking scores upon molecular docking with RNA-dependent RNA polymerase (RdRp) of SARS-CoV-2 (PDB ID: 6 M71).

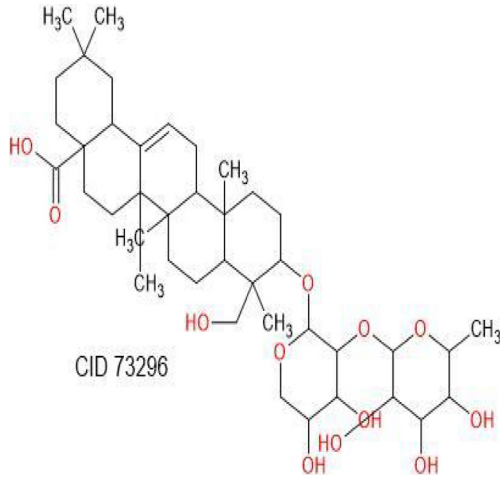
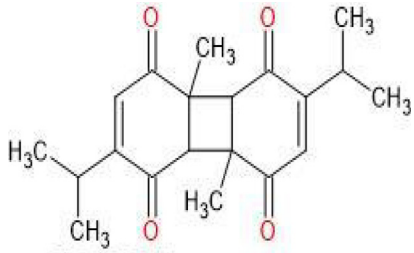
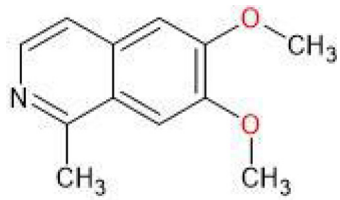
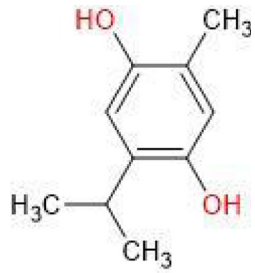
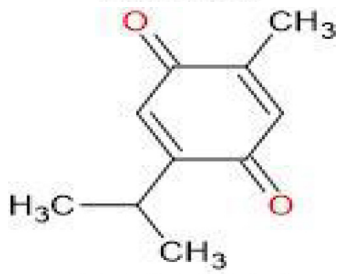
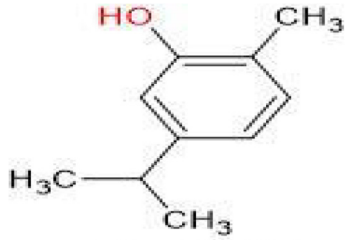
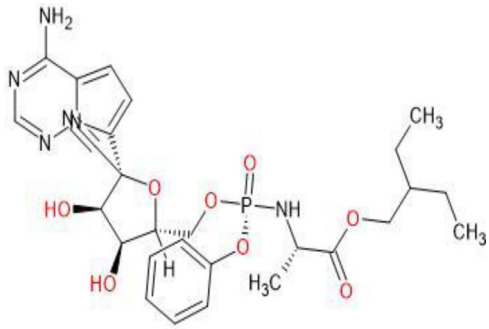
Phytochemical compounds/ligands	Molecular weight (g/mol)	Chemical structure/PubChem CID	Docking Score (kcal/mol)
α- Hederin	750.97	 CID 73296	−8.6
Dithymoquinone	328.41	 CID 398941	−6.1
Nigellicine	246.27	CID 11402337	−6.1
Nigellidine	294.35	CID 136828302	−6.0

Table 1 (*continued*)

Phytochemical compounds/ligands	Molecular weight (g/mol)	Chemical structure/PubChem CID	Docking Score (kcal/mol)
Nigellimine	203.24	 CID 20725	−5.1
Thymohydroquinone	166.22	 CID 95779	−4.6
Thymoquinone	164.20	 CID 10281	−4.6
Carvacrol	150.22	 CID 10364	−4.5
Thymol	150.22	CID 6989	−4.3
Remdesivir	602.6	 CID 121304016	−7.6

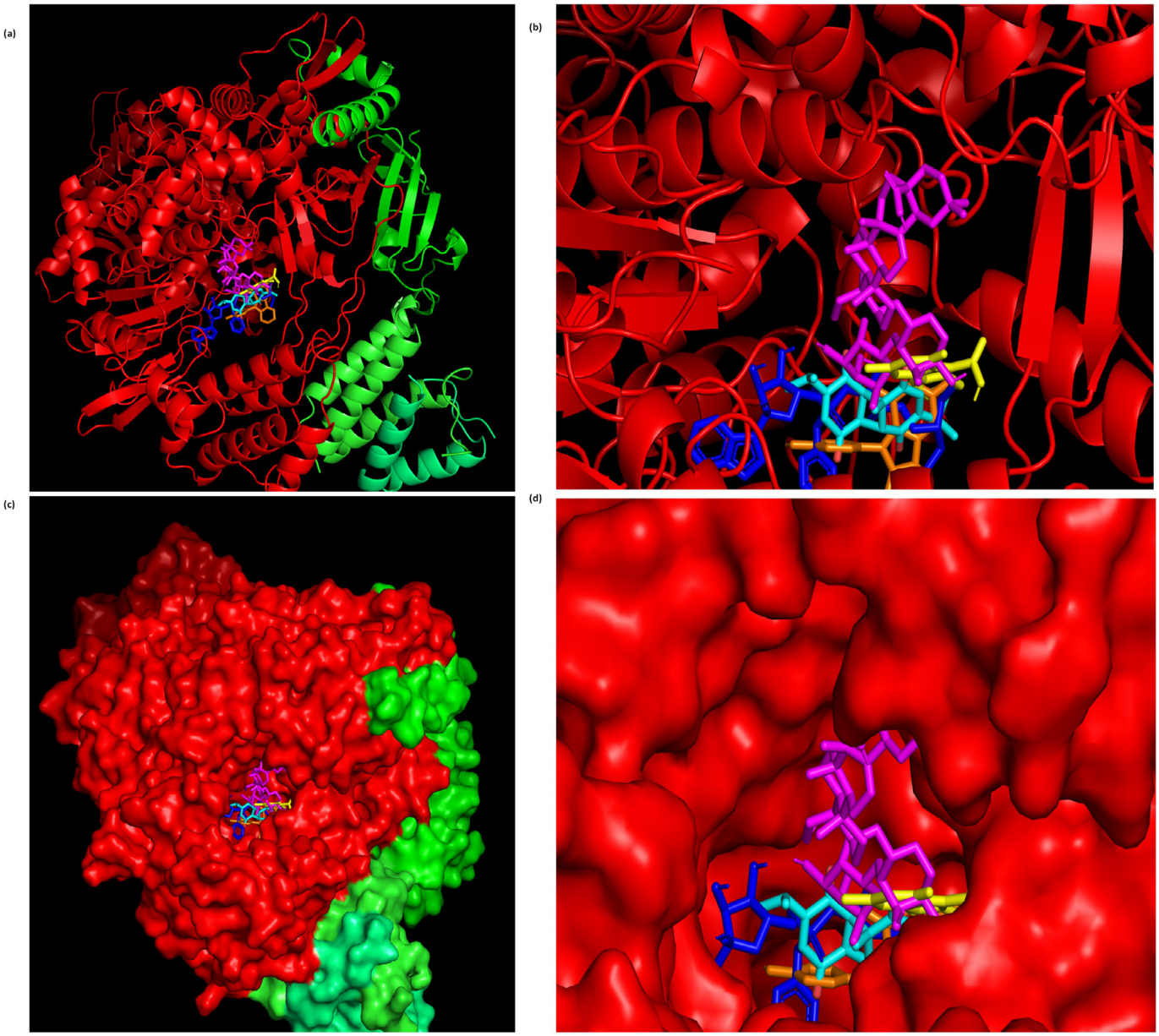


Fig. 1. Structure of RNA-dependent RNA polymerase (RdRp) (PDB ID: 6 M71) demonstrating the binding of the high affinity phytochemicals to its active site (binding pocket). Chains A, B, C, and D of RdRp are shown in red, green, tv-green, and lime-green color, respectively, whereas, the ligands, α-hederin, dithymoquinone, negillicine, negillidine, and remdesivir, are shown in magenta, cyan, yellow, orange, and blue color, respectively.(a) Cartoon representation of RdRp with the phytochemicals bound to its active site. (b) Magnified view (Cartoon representation) of the active site of RdRp occupied by the phytochemicals. (c) Surface representation of RdRp with the phytochemicals bound to its active site. (d) Magnified view (Surface representation) of the active site of RdRp occupied by the phytochemicals.

cell receptors and help the virus release the viral genome into the host cell where it is translated into two polyproteins and the structural proteins, and replication of the viral genome is initiated (V'kovski et al., 2020). The two-third of the RNA genome of SARS-CoV-2 encodes viral RNA-dependent RNA polymerase (RdRp), the associated accessory proteins, and the two large non-structural polyproteins. The remaining one-third of the genome codes for the four structural proteins (spike, envelope, membrane and nucleocapsid), and the other helper proteins (Luk et al., 2019). RdRp is very important for replication and transcription of the viral genome and is highly conserved among different RNA viruses. The core protein of RdRp consisting of a single chain of approximately 900 amino acid residues, shows minimal activity. However, enhanced activity is achieved by the attachment of additional key subunits (Ahn et al., 2012; Kirchdoerfer and Ward 2019; Subissi et al., 2014). Being a very essential enzyme in the life cycle of RNA viruses including SARS-CoV-2, the RdRp has already been targeted in various viral infections, including the hepatitis C virus, Zika virus and coronaviruses (Elfiky et al., 2013; Elfiky 2016; Elfiky 2017; Elfiky 2019; Elfiky and Elshemey 2016; Elfiky and Elshemey 2018; Elfiky and Ismail 2017; Elfiky and Ismail 2019; Ganesan and Barakat 2017). The active site of RdRp has has been reported to be highly conserved among the different corona viruses (Doublie and Ellenberger 1998; Elfiky 2020a; Elfiky and Ismail 2018). Therefore, RdRp is the main drug target for SARS-Cov-2 and other coronaviruses (Aftab et al., 2020; Elfiky 2021b; Elfiky 2020b; Yin et al., 2020).

The virus generally spreads from the infected person through close contact along with the droplets spilled during talking, coughing and sneezing (Chan et al., 2020). The lack of approved drugs or

vaccines for COVID-19 is the main concern of the ongoing pandemic. Therefore, developing a promising vaccine or drug intervention is of prime interest and importance in combating this viral disease (Vardhan and Sahoo 2020). Various efforts are being carried out by the researchers throughout the world for developing the effective treatment strategy for this infectious disease. As a result of these continued efforts various clinical interventions are on trial worldwide. In addition to drug repurposing and synthesis of new drug molecules, the herbal medicine system has gained global emphasis in providing favorable interventions to combat this pandemic viral disease (Patwardhan et al., 2020; Rastogi et al., 2020; Elfiky 2021a). One of the herbs, *Nigella sativa* (also known as black seed or Prophetic medicine) has been reported to possess several pharmacological properties including anti-microbial, anti-inflammatory and immunostimulatory activities (Ahmad et al., 2013; Molla et al., 2019). *N. sativa* belongs to the plant family 'Ranunculaceae' and its seeds in have been consumed to treat various diseases and infirmities (Mazaheri et al., 2019; Majeed et al., 2021; Yimer et al., 2019). Besides its use as a spice and a food preservative, it has been traditionally used as a protective and curative remedy for several disorders (Majeed et al., 2021). Moreover, the beneficial effects and safety of *N. sativa* seeds in different diseases is well established in the literature (Daryabeygi-Khotbehsara et al., 2017; He and Xu 2019; Koshak et al., 2017; Namazi et al., 2018; Sahebkar et al., 2016a; Sahebkar et al., 2016b; Tavakkoli et al., 2017) . Recently, various studies have highlighted the efficacy of *N. sativa* in the treatment of viral diseases (Ahmad et al., 2020; Barakat et al., 2013; Bouchentouf et al., 2020; Dorra et al., 2019; Elfiky, 2021a; Onifade et al., 2013a; Onifade et al., 2013b; Sekiou 2020). *N. sativa* has been reported to inhibit cytomegalovirus, herpes simplex virus, human immunodeficiency virus and hepatitis A virus infections (Salem and Hossain, 2000; Barakat et al., 2010). Some studies in literature have revealed that *N. sativa* inhibited growth of influenza virus H5N1 and replication of Hepatitis C virus (Oyero et al., 2016; Dorra et al., 2019), in addition to decreasing the coronavirus load in infected HeLa cells (Ulasli et al., 2014). Few studies have also focused on the *in silico* screening of its main phytochemicals against some drug targets of SARS-CoV-2 (Ahmad et al., 2020; Bouchentouf and Noureddine, 2020; Molla et al., 2019; Maiti et al., 2020). However, the inhibitory potential of these phytochemicals against the prominent drug target, the RNA-dependent RNA polymerase of SARS-CoV-2 has not yet been reported. Therefore, based on some previous studies in literature (Akram Khan and Afzal, 2016; Ahmad et al., 2020; Bouchentouf et al., 2020; Molla et al., 2019; Maiti et al., 2020), here we selected the major phytochemicals of *N. sativa* and screened them for their potential to inhibit the RdRp of SARS-CoV-2 using computer aided molecular docking methods.

2. Material and methods

2.1. Preparation of RdRp for molecular docking

The crystallographic three-dimensional (3D) structure of the RdRp (PDB ID: 6 M71) of SARS-CoV-2 was downloaded from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) (http://www.rscb.org) and saved as a PDB file (.pdb). AutoDock Tools 1.5.7 was used to prepare this target protein (PDB ID: 6 M71) for *in silico* molecular docking experiments (Goodsell and Olson 1990). The water molecules were removed, polar hydrogen atoms and the Kollman charges were added to the protein, and saved as a PDBQT file (.pdbqt). A grid box covering the active site pocket of 6 M71 was generated and the grid parameters were saved as a TXT file (.txt) for input during docking (Iheagwam and Rotimi 2020; Vardhan and Sahoo 2020).

2.2. Preparation of ligands

The 3D structures of the main chemical compounds of *N. sativa* as well as that of Remdesivir were downloaded as SDF files (.sdf) from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and converted to the PDB format (.pdb) using Open Babel graphical user interface (GUI) tool (O'Boyle et al., 2011). The PDB files of all the ligands (compounds of *N. sativa* and the positive control) were prepared for docking using AutoDock Tools 1.5.7. Ligand preparation included the addition of gasteiger charges, setting torsion roots and merging non-polar hydrogens. The prepared ligands were saved as PDBQT files (.pdbqt) for input during the docking procedure. We did not investigate Lipinski's physicochemical parameters of the ligands here because this information is already available in the literature (Ahmad et al., 2020; Bouchentouf and Noureddine 2020).

2.3. Molecular docking and visualization of the docking complex

The molecular docking of individual ligands into the target protein was performed using the AutoDock Vina software (Trott and Olson, 2009). The grid dimensions (Å) for active site- specific docking were searched from the available literature and fixed at: x = 28, y = 26, z = 32 (Iheagwam and Rotimi, 2020). The default exhaustiveness value of 8 was uniformly fixed for all ligands during the docking procedure. The known drug candidate, remdesivir was used as a positive control for docking the RdRp active site. AutoDock Vina results represent the docking scores as the Gibbs free energy of binding (ΔG (kcal/mol)). The Gibbs free energy of binding (ΔG) obtained as a result of molecular docking by AutoDock Vina and expressed in kcal/mol represents the efficacy of ligand binding to the active site of the selected receptor (Ardra et al., 2020). Further, the output files generated from docking experiments were converted to protein–ligand complexes using the PyMOL software (https://pymol.org/2/) (DeLano 2002), and the interaction of the ligands with the receptor residues was visualized and analyzed using the BioVia Discovery Studio Software (https://discover.3ds.com/discovery-studio-visualizer-download).

3. Results and discussion

3.1. Molecular docking of the phytochemical compounds of N. Sativa

RdRp is crucial for the replication of SARS-CoV-2 and is therefore considered a key target for the development of antiviral drugs (Aftab et al., 2020; Elfiky 2021b; Elfiky 2020b; Yin et al., 2020). Recently, several studies have reported the *in silico* screening of phytochemicals from *N. sativa* against different drug targets of SARS-CoV-2 (Ahmad et al., 2020; Bouchentouf and Noureddine 2020; Maiti et al., 2020; Rajapaksa et al., 2020; Sampangiramaiah 2020; Silva et al., 2020), however, RdRp, an important drug target has not been included in these studies. Therefore, to fill the gap, the same strategy has been applied in the current study to screen the *N. sativa* phytochemicals for their potential to inhibit RdRp of SARS-CoV-2.

Our results revealed that out of the total nine compounds of *N. sativa* that we screened by *in silico* analysis, four (α-hederin, dithymoquinone, nigellicine and nigellidine) had significant binding affinity towards the active site of RdRp as indicated by their considerably lower binding energies (ΔG values less than the cut-off value of −6 kcal/mol) (Shityakov and Foerster, 2014). α-hederin possessed the lowest binding energy (ΔG = −8.6 Kcal/mol) in complex with 6 M71, followed by dithymoquinone (ΔG = −6.1 kcal/m

ol), nigellicine (ΔG = −6.1 kcal/mol) and nigellidine (ΔG = −6.0 kc al/mol). The binding affinity (ΔG = −8.6 Kcal/mol) of α-hederin was found to be higher than that of remdesivir (−7.6 kcal/mol), the already known antiviral compound used as a positive control in this study. The binding energies of the other three compounds were lower, but close to that of remdesivir (Table 1).

3.2. In silico analysis of the docking complexes and visualization of the enzyme-ligand interactions

In the current study we have used the recently determined X-Ray crystal structure of RdRp (PDB ID: 6 M71) for screening the selected phytochemicals. According to the available literature, the key residues of RdRp involved in the interaction include; Y618, C622, N691, N695, M755, I756, L757, L758, S759, D760, D761, A762, V763, E811, F812, C813 and S814 (numbering for PDB ID: 6 M71). The key residues in the active site are D761 and D762. These are involved in the actual reaction of the RdRp enzyme (Elfiky 2021b; Elfiky 2020b;Yin et al., 2020). We used the RdRp holoenzyme for the initial screening of the selected phytochemicals. The structure of RdRp (PDB ID: 6 M71) and the binding of high-affinity phytochemicals/ligands to its active site is shown in Fig. 1. The 3D and 2D visualization of the interaction of the top four phytochemicals of *N. sativa* with the active site residues of RdRp is shown in Fig. 2. The phytochemicals α-hederin and negillicine interacted with the active site residues through four and three conventional hydrogen bonds, respectively, whereas the other two phytochemicals, dithymoquinone and negillidine interacted with the active site residues through two and one hydrogen bonds, respectively. There are also some non-bonded interactions, such as van der Waals forces, pi-alkyl, pi-cation, etc., between the phytochemicals and active site amino acid residues of RdRp as depicted in the 2D illustration (Fig. 2). The control drug, remdesivir is a nucleotide analog that binds to RdRp in a similar manner as a nucleotide does, thereby inhibiting viral RdRp activity through RNA chain termination. The interaction of various active site amino acid residues of RdRp with remdesivir is available in the literature (Elfiky 2020b; Yin et al., 2020). In the present study, we observed that upon *in silico* screening of the selected phytochemicals, the top four compounds (α-hederin, dithymoquinone, negillicine and negillidine), especially α-hederin efficiently bound to the binding pocket of RdRp and interacted with one or more interacting residues present in the RNA binding tunnel of RdRP (Fig. 2) which might lead to the inhibition of its activity. Our results support those of earlier studies and suggest that *N. sativa* and its phytochemicals are worth studying further and could be recommended as an antiviral herbal supplement against COVID-19.

Based on its lowest docking energy on docking with RdRp (PDB ID: 6 M71) and its efficient interaction with the active site residues of 6 M71, we report here α-hederin as the most potent inhibitor of SARS-CoV-2 RdRp, among the nine compounds screened in this study. For further confirmation, we docked the top-ranked phytochemicals (α-hederin, dithymoquinone, negillicine and negillidine) with a single chain of RdRp (Chain A, the core enzyme). We observed that α-hederin, dithymoquinone, negillidine and negilli-

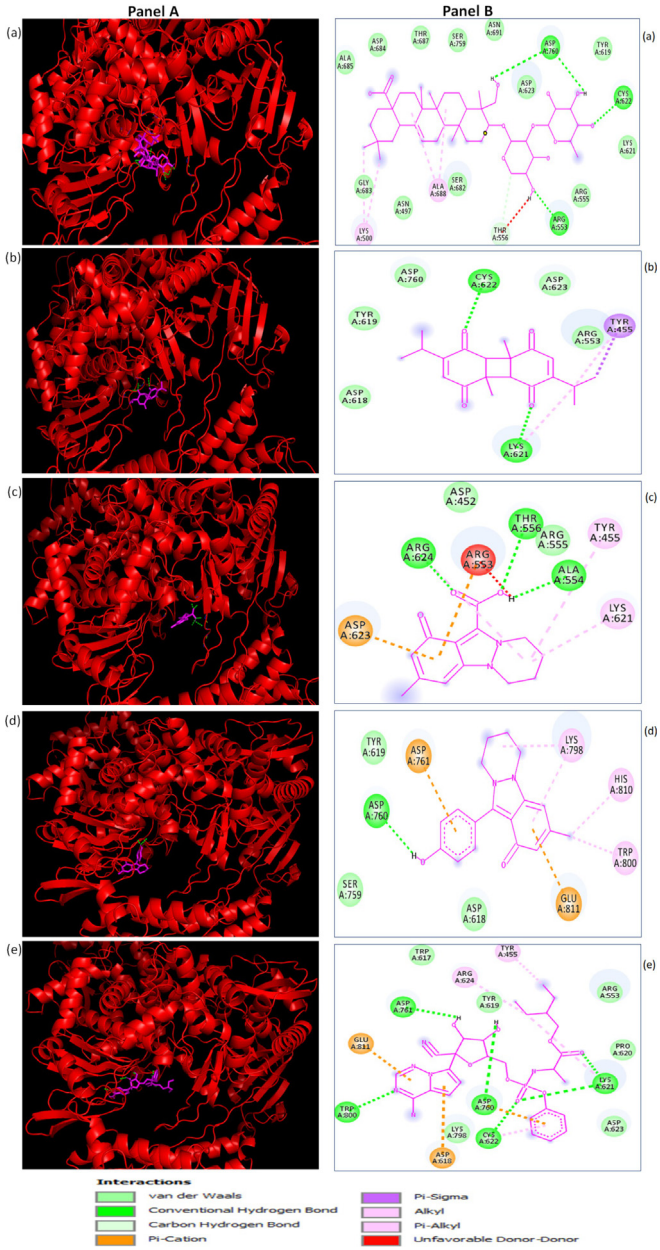


Fig. 2. Molecular docking analysis revealing the binding positions of the selected top 4 compounds of *Nigella Sativa* [α-hederin (a), dithymoquinone (b), nigellicine (c), nigellidine (d)] and remdesivir (e) to the severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) RdRp (PDB ID: 6 M71; shown as a ribbon structure in red). Panel A shows the three-dimensional illustration of the interaction of ligands to the 6 M71 structure and Panel B shows the two-dimensional diagrams displaying the interactions with specific amino acid residues in the active site. All the ligands (α-hederin, dithymoquinone, nigellicine, nigellidine, and remdesivir) are shown in magenta color.

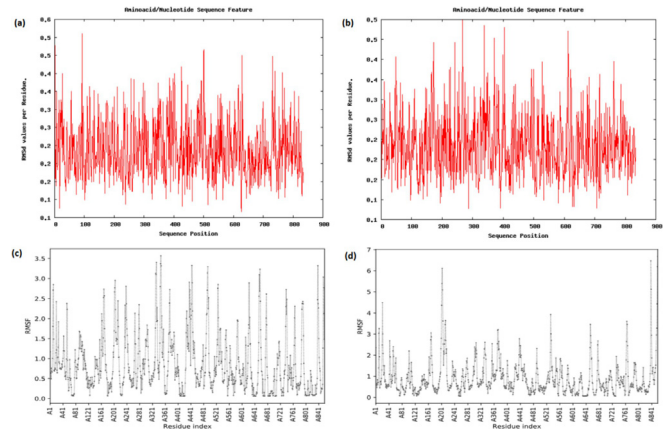


Fig. 3. Molecular dynamic simulations of the RdRp/α-hederin docking complex. (a) Root mean square deviation (RMSD) profile of the RdRp chain A alone. (b) RMSD profile of the RdRp chain A/α-hederin complex. (c) Root mean square fluctuations (RMSF) profile of the RdRp chain A alone (d) RMSF profile of the RdRp chain A/α-hederin complex.

cine bind into the active site of RdRp (Chain A) with a docking score of −8.6, −6.7, −6.6 and −6.1 kcal/mol, respectively. The stability of the docking complex of the top-ranked compound (α-hederin) was confirmed by molecular dynamic simulation using the Molecular Dynamics on Web (MDWEB) server (https://mmb.irb-barcelona.org/MDWeb/) and CABS flex server (http://212.87.3.12/CABSflex2/index) as shown in Fig. 3.

4. Conclusion

In this *in silico* study, we identified four phytochemicals from *N. sativa* that have the potential to inhibit the RdRp of SARS-CoV-2. Of the four compounds, α-hederin, dithymoquinone, negillicine, and negillidine, α-hederin had the highest binding affinity towards the active site residues of RdRp. Our docking results prove that the top four potential phytochemical molecules of *N. sativa*, especially α-hederin, could be considered for ongoing drug development strategies against SARS-CoV-2. However, further *in vitro*, *in vivo*, and clinical studies are warranted to establish the comprehensive pharmacological roles of these phytochemicals. Our study also supports the available literature regarding the traditional use of *N. sativa* to prevent viral diseases.

Funding information

This study was financially supported by the Deanship of Scientific Research (DSR) at Majmaah University, Al Majma'ah, Saudi Arabia [Grant no. RGP-2019–30].

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors are highly thankful to the Deanship of Scientific Research (DSR) at Majmaah University for financially supporting this study under project number RGP-2019-30 and for their support in proofreading the manuscript through professional editors at Editage, a division of Cactus Communications.

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