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Original Article

Computational Prediction of *Nigella sativa* Compounds as Potential Drug Agents for Targeting Spike Protein of SARS-CoV-2

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ABSTRACT

SARS-CoV-2 was first identified in Wuhan, China in December 2019 and has rapidly devastated worldwide. The lack of approved therapeutic drugs has intensified the global situation, so researchers are seeking potential treatments using regular drug agents and traditional herbs as well. **Objectives:** To identify new therapeutic agents from *Nigella sativa* against spike protein (PDB ID: 7BZ5) of SARS-CoV-2. **Methods:** The 46 compounds from *N. sativa* were docked with spike protein using Molecular Operating Environment (MOE) software and compared with commercially available anti-viral drugs e.g., Arbidol, Favipiravir, Remdesivir, Nelfinavir, Chloroquine, Hydroxychloroquine. The Molecular Dynamic Simulation (MDS) analysis was also applied to determine ligand-protein complex stability. Furthermore, the pharmacological properties of compounds were also analyzed using AdmetSAR and SwissADME. **Results:** Out of its total 46 ligands, 8 compounds i.e., Methyl stearate, Eicosadienoic acid, Oleic acid, Stearic acid, Linoleic acid, Myristoleic acid, Palmitic acid, and Farnesol were selected for further analysis based on their minimum binding energy ranges from -7.45 to -7.07 kcal/mol. The docking scores of *N. sativa* phytocompounds were similar to drugs taken as control. Moreover, post simulation analysis of Methyl stearate complex predicted the most stable conformer. **Conclusions:** Further, *in-vivo* experiments are suggested to validate the medicinal use of Methyl stearate as potential inhibitors against spike protein of SARS-CoV-2.

INTRODUCTION

COVID-19 pandemic is caused by the novel coronavirus SARS-CoV-2, which is a member of the Coronaviridae family in the Nidovirales order [1]. The virus was first identified in Wuhan, China in late 2019 and has since spread globally, leading to widespread illness and death [2]. Coronaviruses are responsible for a range of diseases, including respiratory, digestive, enteric, and neurological disorders [3]. The highly transmissible nature of the virus has resulted in its spread to 216 countries worldwide [4]. As of the latest reported figures, there have been 759,408,703 confirmed cases of COVID-19 and 6,866,434 deaths attributed to the virus on a global scale according to WHO. The name coronavirus was derived from the crown-like appearance of spike protein on the surface of viral envelope [5]. The genome contains positive sense single-stranded RNA of 26-32 kilobases in length with the size ranges from 65-125 nanometers in diameter [6, 7]. Spike protein of SARS-CoV-2 facilitates the entry in host cell by interacting its glutamine residue at 394 positions in RBD domain with the lysine 31 residue on the human ACE2 receptor [8]. Spike protein is type 1 transmembrane fusion protein and is highly glycosylated [9, 10]. S1 (N-terminal)

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subunit of Spike protein makes circular head of S protein, and second functional subunit is S2 (C-terminal) subunit that shapes the end of the protein and is embedded in viral envelope [11]. In viral-host cell interaction primarily S1 subunit identify and attach to the receptor present on the host cell and then S2 subunit, a highly well-conserved part assist in virus attachment to the host cellular membrane [12]. Due to the severity of this critical situation and the absence of a specific treatment, the scientific community and researchers are trying to find potential therapeutic agents that could be more effective in curing COVID-19. In this effort, scientists are also reviewing a large number of herbal plant species to identify active drug compounds [13]. The identification and efficacy of medicinal components in these plants could pave the way for combating COVID-19. The medicinal properties of *N. sativa*, a widely known herb, has potential in treating COVID-19 [14]. In the present study, in-silico method was used to screen *N. sativa* to identify its potential therapeutic compounds that can inhibit the SARS-CoV-2 infectious cycle. The identified compounds were then compared with clinically proposed drugs for COVID-19, including Arbidol, Favipiravir, Remdesivir, Nelfinavir, Chloroquine, and Hydroxychloroquine [15]. The study was performed using molecular docking and dynamic simulation. MOE is a drug discovery software that involves visualization, modelling, simulations, and methodology development. The physicochemical and drug likeness properties of the ligands were determined using various tools, including SwissADME, AdmetSAR, and Pfizer's rule of five.

METHODS

Dataset preparation

The two datasets of compounds from *N. sativa* having antiviral properties and commercially available assisting drug against COVID-19 taken as control was prepared. The viral spike protein against which the in-silico therapy has to propose was selected based on its primary role in onset of viral attachment and infection. The library was prepared by retrieving structures from PubChem and PDB (ID: 7BZ5) and perform preparatory changes to make ligand/protein suitable for docking analysis. The missing hydrogen bonds, charges were adjusted along with removal of repeated chains, heteroatoms, water molecules and already attached ligands. The energy minimization of ligands and protein were done by universal force field (UFF) with conjugate gradient algorithm of 500 iteration and Chimera using AMBER forcefield (AMBER ff14SB) respectively. The chemical compounds of *N. sativa* are given in Supplementary Table S1, while properties of protein are given in table 1.

Table 1: Crystallographic Properties of Spike Protein of SARS-CoV-2

Protein	PDB code	Classification	Organism	Expression system	Resolution	Method	Total structure Weight (DA)	Chain
Spike (S1)	7BZ5	Viral protein	SARS-CoV-2	Homo sapiens	1.84A	X-RAY Diffraction	73.36kDa	A

Virtual library screening of *N. sativa* against SARS-CoV-2 spike protein

The selected datasets were virtually screened for minimum binding energy with viral protein by docking with MOE. The ligands are allowed to interact with protein by creating a grid box with dimension of 20×20×20 Å around the active site of protein. The active site of spike protein was retrieved from previously published literature. MOE compute binding energy by calculating difference between the sum of energy in free state of ligand and protein and sum of energy in protein-ligand complex using Merck Molecular Force Field (MMFF). The binding energy (ΔG) of protein-ligand interactions was calculated using following empirical equation [16]. $\Delta G = (V^{L\text{-}L}_{bound} - V^{L\text{-}L}_{unbound}) + (V^{P\text{-}P}_{bound} - V^{P\text{-}P}_{unbound}) + (V^{P\text{-}L}_{bound} - V^{P\text{-}L}_{unbound} + \Delta S_{conf})$ where *P* refers to the protein, *L* refers to the ligand, $V^{L\text{-}L}_{bound}$ energy in bounded state of ligand, $V^{L\text{-}L}_{unbound}$ energy in unbounded state of ligand, $V^{P\text{-}P}_{bound}$ energy in bounded state of protein, $V^{P\text{-}P}_{unbound}$ energy in unbounded state of protein, $V^{P\text{-}L}_{bound}$ energy in bounded state of protein and ligand, $V^{P\text{-}L}_{unbound}$ energy in unbounded state of protein and ligand, ΔS_{conf} denotes the loss of conformational entropy upon binding.

Calculation of properties using ADMET analysis

AdmetSAR and Swiss ADME were used to analyzed drug-likeness of selected *N. sativa* compounds by computing pharmacokinetic and pharmacological properties.

Molecular dynamic simulations of the top-scoring ligand-protein complex

The Nanoscale Molecular Dynamics (NAMD) was used to perform molecular dynamic (MD) simulation to examine protein stability, conformational changes of ligand-protein complex, kinetics and free binding energy changes by allowing to interact in virtual environment similar to in-vivo condition. Ligands that were scrutinized through docking and drug likeliness analysis were selected for further MD simulation. The protein-ligand complex was prepared in same orientation with maximum score. The topologies of ligand and protein were made by Visual Molecular Dynamics (VMD) to define bonds/angles, number of molecules and atom types. The simulation inputs of ligand and protein were built from CHARMM-GUI web server with CHARMM36 forcefield and protein structure format generator of VMD respectively. Solvation box was made around a complex to provide medium and energy minimization was performed using conjugate gradient method. The periodic boundary conditions were adjusted with 310K temperature and 1 atm

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pressure for simulation procedure. The MD simulation was executed for 1ns (500000 steps) using NAMD software after adjusting all parameters. Afterwards, the results were analyzed by plotting histogram of RMSD, hydrogen bond and heat map.

RESULTS

Molecular docking scores with clinical drugs

Clinical drugs used against COVID-19 were docked with spike protein and taken their results as control to compare with the compounds of *N. sativa*. Remedsivir, Nelfinavir and Chloroquine gave the lowest binding scores of -7.9, -7.2 and -7.2 Kcal/mol respectively. The docking scores of clinical drugs are listed in table 2.

Table 2: Docking scores of commercially available drugs against spike protein

Ligands	Docking Score (Kcal/mol)
Arbidol	-7.2468
Favipiravir	-4.3916
Remdesivir	-7.9584
Nelfinavir	-7.2471
Chloroquine	-6.4304
Hydroxychloroquine	-7.0793

Docking scores of *N. sativa* compounds

Molecular docking was performed between Spike Protein (ID: 7BZ5) of SARS-CoV-2 and Forty-six compounds of *N. sativa* separately for estimation of possible interactions between ligand and protein. The more negative binding energy indicates a stronger binding interaction. The binding energies of *N. sativa* compounds are given in Supplementary Table S2 while compounds with highest docking scores are listed in Table 3 with Methyl stearate binding energy of -7.4Kcal/mol.

Table 3: The best docked compounds of *N sativa* with spike protein

Ligands	Docking Score (Kcal/mol)
Methyl stearate	-7.4506
Eicosadienoic acid	-7.4234
Oleic acid	-7.1822
Stearic acid	-7.0729
Linoleic acid	-6.9182
Myristoleic acid	-6.4648
Palmitic acid	-6.3564
Farnesol	-6.1679

2D/3D interactions of best docked complexes having lowest binding energies are shown in Figure 1. It displays the spatial orientation of ligand in the binding pocket of protein with interaction to its surrounding amino acids.

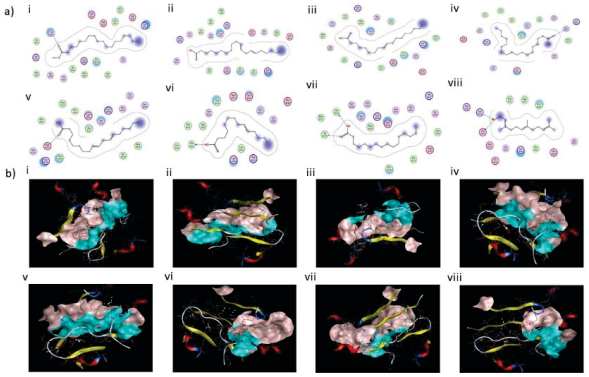

Figure 1: 2D/3D interaction between ligands of N sativa and spike protein.

a) 2D-interaction of complexes i) Methyl stearate with amino acid SER129 as hydrogen acceptor, distance/energy of 2.95Å/-1.3kcal/mol ii) Eicosadienoic acid with electrostatics interaction iii) Oleic acid with amino acid ASP123 as hydrogen donor, distance/energy of 2.81Å/-5.2kcal/mol iv) Stearic acid with amino acid SER209 as hydrogen acceptor, distance/energy of 3.11Å/-2.0kcal/mol v) Linoleic acid with amino acid ASP123 as hydrogen acceptor, distance/energy of 2.93Å/-4.2kcal/mol vi) Myristoleic acid with amino acid PRO125 as hydrogen donor, distance/energy of 2.83Å/-3.8kcal/mol vii) Palmitic acid with amino acid VAL116/ILE118 as hydrogen donor/acceptor, distance 3.35Å/3.18Å and energy -0.8/-1.1kcal/mol viii) Farnesol with amino acid GLU124/LYS211 as hydrogen donor/acceptor, distance 3.13Å/3.09Å and energy -1.3/-4.4kcal/mol b) 3D-interaction of complexes i) Methyl stearate ii) Eicosadienoic acid iii) Oleic acid iv) Stearic acid v) Linoleic acid vi) Myristoleic acid vii) Palmitic acid viii) Farnesol

Drug likeliness analysis

Compounds with highest scores were further analyzed for pharmacological properties by using AdmetSAR and Swiss ADME. This analysis predicted that all compounds follow Lipinski rule of five with only one violation which is acceptable for drug likeliness. The properties of blood-brain permeability and percentage of oral absorption in humans were calculated and found to be within the acceptable limits required for human usage (Table. 4).

Table 4: Lipinski's physiochemical guidelines for *N. sativa* compounds

Ligands	Molecular weight (g/mol)	H-Donor	H-Acceptor	Log p	Log S	TPSA (Å²)	Follow Lipinski's/ Violations
Methyl stearate	298.51	0	2	6.42	-3.399	26.30	Yes/1
Eicosadienoic acid	308.51	1	1	6.66	-4.04	37.30	Yes/1
Oleic acid	282.47	1	1	6.11	-4.04	37.30	Yes/1
Stearic acid	284.48	1	1	6.33	-3.502	37.30	Yes/1
Linoleic acid	280.45	1	1	5.88	-4.04	37.30	Yes/1
Myristoleic acid	226.36	1	1	4.55	-3.791	37.30	Yes/0
Palmitic acid	256.43	1	1	5.55	-3.502	37.30	Yes/1
Farnesol	222.37	1	1	4.40	-2.472	20.23	Yes/0

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Molecular dynamic simulation analysis

Methyl stearate that gave the best docking score and have pharmacological properties was selected for MD simulation to investigate its time-dependent binding ability and conformational stability in spike protein binding pocket. Although molecular docking determines the three-dimensional orientation of ligand within the receptor pocket with minimum energy further conformational stability is necessary to assess the inhibitory strength of compounds against spike protein. Histogram of root mean square deviation (RMSD), hydrogen bonds and heat map are plotted to analyze the results.

RMSD analysis

Root mean square deviation (RMSD) was calculated to determine the average distance between atom groups. The RMSD plot indicated that the Methyl stearate-spike protein complex remained stable for almost whole time of molecular dynamic simulation with high stability shown at 1.5 Å from 60 to 125 frames. Afterwards, the RMSD value slightly increased to 1.7 Å from 125 to 200 frames (Figure 2).

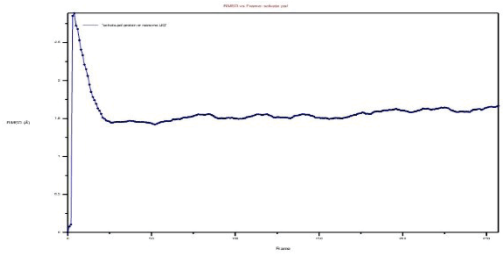

Figure 2: The RMSD graph of Methyl stearate complex with spike protein

Analysis of hydrogen bonds

The stability of complexes was investigated by hydrogen bond analysis as formation of strong hydrogen bond reduces the gap between residues and therefore increases the stability of complex. Hydrogen bond analysis showed that the major bond is formed between SegHP1-GLY44 (donor) and SegLIG-LIG1 (acceptor) with an occupancy rate of 26.74%. The H-bond graphs of Methyl stearate is shown in Figure 3.

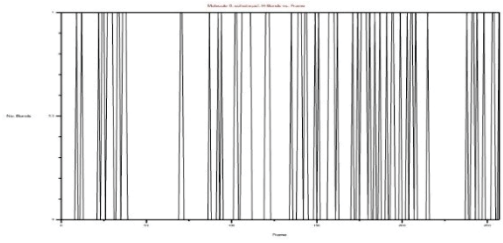

Figure 3: The histogram of H-bond analysis of Methyl stearate complex with spike protein

Complex analysis using heat maps

Heat map plots display how a specific characteristic, such as potential energy, temperature, pressure, or density, of a simulated system is spread out over time. The heat signature of Methyl stearate-spike protein complex showed quite stability and given in Figure 4.

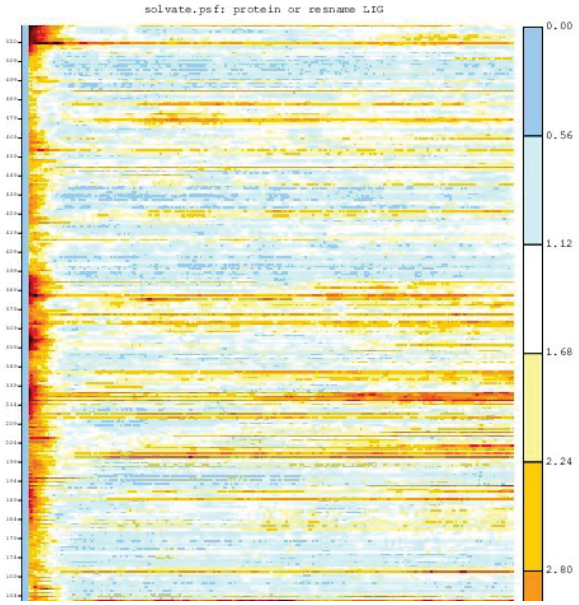

Figure 4: The heatmap graph of Methyl stearate complex with spike protein

DISCUSSION

The global health crisis caused by the COVID-19 pandemic had drastic effects on individuals around the world and is widely recognized as a major threat to public health. Despite the absence of an approved drug, efforts are underway in various areas of medicine, such as allopathic and homeopathic to find a solution of the problem. The high mutation rate of the RNA genome is a significant obstacle in drug discovery, as it can lead to reduced effectiveness of drugs [17]. This is a major limitation that researchers must overcome in order to develop more effective treatments for diseases caused by RNA viruses such as COVID-19. The study aimed to evaluate the potential of therapeutic drug agents derived from bioactive compounds of *Nigella sativa* using molecular docking and dynamic simulation techniques against the spike protein of SARS-CoV-2, which is considered a potential target for drug development and effective treatments for COVID-19. The structures of total 46 *N. sativa* compounds were identified and retrieved from literature and databases respectively. Their binding energies with the target protein were calculated through docking using MOE. The stability and drug likeliness of ligands which presented lowest binding energies with viral protein was further evaluated through MDS and ADMET analysis. The *in-silico* analysis predicted Methyl stearate as the potential inhibitor. Similar studies by Saif *et al.*, have been reported in the past in predicting the potential inhibitor of this virus. Recent studies predicted the

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promising inhibitor against main protease and spike protein from the compounds of *Olea europaea*, *Curcuma longa* and *Carica papaya* respectively [18, 19]. The present study was limited to computational analysis, as molecular dynamics simulations require significant computational power for extended periods of time. Therefore, more accurate assessments of the stability of these complexes over longer time frames would require higher computational system [20].

CONCLUSIONS

Current computer-aided drug designing (CADD) investigated Methyl stearate from *N. sativa* compounds as potential inhibitor of SARS-CoV-2 by demonstrating lowest binding energy and forming stable complex with viral spike protein. Further wet-experimental research is needed to validate its inhibitory effect on SARS-CoV-2 before clinical trials.

Conflicts of Interest

The authors declare no conflict of interest.

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