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Carvacrol: A PL^{pro} Inhibitor of SARS-CoV-2 Is a Natural Weapon for COVID-19 †

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Abstract: The outbreak of SARS-CoV-2 creates the biggest crisis to *human health* and adversely affects the economic growth worldwide. Recently, several vaccines have been emerged by the manufacturers to combat COVID-19. Unfortunately, no therapeutic medication has yet been approved by FDA for the treatment of this disease. In this aspect, several research groups have paid attention to find out the potential SARS-CoV-2 protein inhibitors from the bio-molecules available in medicinal plants, spices, and vegetables. In this paper, we have performed the structure-based virtual screening (VS) of 120 compounds derived from *Nigella sativa* (NS) against M^{pro}, PL^{pro}and Spike proteins of SARS-CoV-2. Strong binding interactions of M^{pro} occurred with hits NS-40, and NS-84 whereas hits NS-72D and NS-95D showed strong binding interaction with Spike protein. Interestingly, four promising hits namely NS-21, NS-40, carvacrol (NS_08) and menthol exhibited good binding interactions with both M^{pro} and Spike proteins. It was observed that carvacrol, a monoterpenoid phenol having several biological activities, shows favourable binding affinity towards papain-like protease of SARS-CoV-2. This small molecule may be used as a natural weapon to combat the COVID-19.

Keywords: carvacrol; black seed; SARS-CoV-2; COVID-19; natural product

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1. Introduction

The present pandemic situation due to COVID-19 caused by SARS-CoV-2 [1–4] is a burning issue to the research community, health workers, and government officials worldwide. The COVID-19 is the biggest crisis to *human health* and adversely affects the economic growth all over the world [5]. This pandemic situation affects all sector of the society including education [6–8]. It forcibly effects the base line of world economy, and as a result, millions of people become joblessness. Thus, it become a big challenge to the research community and health workers to combat the COVID-19 pandemic. Recently, several manufacturers such as Pfizer Biotech, AstraZeneca University of Oxford, Serum Institute of India Pvt. Ltd., Moderna Biotech, Sinopharm/BIBP have launched vaccines in the market to combat COVID-19. Unfortunately, no therapeutic medication has yet been approved by FDA for the treatment of this disease.

Natural products provide a wealth of biologically active molecules with antiviral activity. From ancient times, in the Indian traditional healthcare system, several plant extracts have been used for the treatment of viral infections [9–11]. Many bio-products have been used for the treatment of the Dengue virus, Coronavirus, Enterovirus, Hepatitis B, Influenza virus, and HIV. Recently, several research groups [12–15] have paid attention

to finding out the potential SARS-CoV-2 protein inhibitors from the bio-molecules available in medicinal plants, spices, and vegetables by applying in silico drug design approach. In this aspect, baicalin, quercetin and its derivatives, hesperidin, and catechins are the most studied flavonoids. Khaerunnisa et al. (2020) observed that many secondary metabolites of plants such as quercetin, kaempferol, luteolinglucoside, apigeninglucoside, curcumin, demethoxycurcumin, naringenin, catechin, oleuropein, and epicatechin-gallate have the potential to inhibit main protease (M^{pro}) of SARS-CoV-2 [16]. Traditionally, several natural products such as neem, tulsi, tea, garlic, ginger, onion, black seed, turmeric, etc. have been used for the remedy of flu-like diseases or common cold. Among these, *Nigella sativa* (NS) or black seed is mostly studied one because of its antiviral and other pharmacological activities including antiviral, anti-inflammatory, antimicrobial, and immunostimulatory activities.[17]

Nigella sativa or black seed is an important plant, used as medicine around the globe [18]. The oil of black-seed has anti-viral properties and is commonly used to treat cold, acute as well as chronic asthma and bronchitis. The conventional therapeutic use of *Nigella sativa* has been reported by several research group to treat various diseases including hypertension, diabetes, influenza, inflammation, eczema, headache, cough, bronchitis, and fever [19]. It was reported [20] that oil extracted from black seed was used effectively in the treatment of viral infection caused by "Murine cytomegalovirus (MCMV). *Niglea saltiva* also showed strong antiviral properties against the Laryngotrachietis Virus (ILTV) infection [21]. Due to the potential therapeutic activities of *N. sativa* compounds, recently, Ahmad et al. [19] carried out an *in silico* investigation of the chemical constitutions of black seed for the development of potent natural antiviral against SARS-CoV-2. The authors observed that dithymoquinone, a compound of *Nigella sativa* inhibits SARS CoV-2 spike by binding at SARS-CoV-2:ACE2 interface. Considering the immunomodulatory, immunotherapeutic and antiviral properties of black seed the present study is designed to search the potentiality of in-house built database of 120 secondary metabolites of *Nigella sativa* against the M^{pro}, PL^{Pro} and Spike proteins of SARS-CoV-2 by molecular docking analysis. We found that carvacrol, a monoterpenoid phenol have favourable binding affinity towards papain-like protease of SARS-CoV-2.

2. Materials and Methods

2.1. Data Collection

The compounds of *Nigella sativa* were collected from literature [22–26]. The compound numbers and with their sources are listed in Table 1. The X-ray crystal structures of SARS-CoV-2 main protease (PDB ID: 6LU7, 2.16 Å), papain-like protease proteins (PDB ID: 6WX4, 1.66 Å) and spike receptor (PDB ID: 6M0J, 2.45 Å) was retrieved from RCSB protein data bank.

Table 1. Binding score of ligands with M^{pro}, Spike protein and PL^{pro}.

Binding Affinity with M^{pro}		**Binding Affinity with Spike**		**Binding Affinity with PL^{pro}**	
Compound	**Autodock Vina Binding Affinity (kcal/mol)**	**Compound**	**AutodockVina Binding Affinity (kcal/mol)**	**Compound**	**AutodockVina Binding Affinity (kcal/mol)**
NS_21	−6.7	NS_21	−7.4	NS_79	−6.4
NS_40	−7.5	NS_40	−6.1	NS_70	−5.7
NS_66	−6.5	NS_72D	−7.7	NS_93	−5.7
NS_84	−7.9	NS_95D	−7.5	NS_102	−5.5
Carvacrol (NS_08)	−5.3	Carvacrol (NS_08)	−5.1	Carvacrol (NS_08)	−5.8
Menthol	−5.0	Menthol	−5.2	Menthol	−5.6

2.2. Ligand Preparation

The structures of the compounds of *N. sativa* were drawn using Chem Draw Professional 15.1 and saved as sdf format. The compounds were then imported into Discovery Studio visualise 2020 (https://discover.3ds.com/discovery-studio-visualizer-download (accessed on)), and exported as a single sdf file. The single sdf file containing ligands were converted to pdbqt file format followed by energy reduction.

2.3. Protein Preparation and Receptor Grid Generation

The crystal structures of SARS-CoV-2 main protease (PDB ID: 6LU7, 2.16 Å), papain-like protease proteins (PDB ID: 6WX4, 1.66 Å) and spike receptor (PDB ID: 6M0J, 2.45 Å) were retrieved from Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB). All protein was imported independently into Autodock Tools 4, the water molecules and hetero atoms, if any, were removed, polar hydrogens were added, followed by adding Gasteiger and Kollman charges. The protein structures were finally saved in pdbqt format. The grid dimensions for M^{pro} and spike receptor were used from our previous work [15]. The active site amino acid residues of the PL^{pro} in complex with peptide like inhibitors were CYS-111, LEU-162, GLY-163, ASP-164, PRO-248, TYR-264, CYS-270, GLU-271, and TYR-286. The residue CYS-111 was covalently bonded with co-ligand [27]. The grid center of papain-like protease center is x = −15.503, y = 9.428, z = 14.080 and grid dimension: x = 60, y = 64, z = 62. The spike receptor centeris x = –32.00, y = 11.00, z = 28.000 and grid dimension: x = 52, size_y = 52, size_z = 54.

2.4. Molecular Docking Study

The compounds from *N. sativa* were docked with SARS-CoV-2 Mpro and PLpro using AMDock [28] containing of Autodock Vina [29]. The docking were analyzed using the ProteinPlus [30] and PyMOL 2.5.2 [The PyMOL Molecular Graphics System, Version 2.0].

2.5. In-Silico Prediction of Absorption, Distribution, Metabolism, and Excretion (ADME)

One of the important factors in drug discovery is ADME properties. The ADME provides range of values to compare particular molecular properties of compounds with 95% of known drugs. The ADME (absorption, distribution, metabolism, and excretion) parameters, pharmacokinetic properties and drug-like nature of the compounds of *N. sativa* were predicted by Swiss ADME (www.SwissADME.ch (accessed on)) [31]. Due to poor ADME qualities, about 40% of drug candidates fail in clinical trials. By anticipating ADME characteristics, poor drug candidates can be identified early on in the development process, avoiding last-stage failures. This forecast may lead to reductions in terms of money, time, and resources [32].

3. Result and Discussion

We know that the lower value of binding score i.e., the higher negative value indicates the higher binding affinity of the ligand towards receptor. From the Table 1, it is clear that two compounds NS_40 and NS_84 showed highest binding affinity towards SARS-CoV-2 M^{pro}. The binding affinity of these compounds NS_40 and NS_84 with M^{pro} were -7.5 kcal/mol and −7.9 kcal/mol, respectively. On the other hand, compounds NS_72D and NS_95D exhibited highest binding affinity with SARS-CoV-2 spike protein and showed binding score of −7.7 kcal/mol and −7.5 kcal/mol, respectively towards SARS-CoV-2 spike protein. The binding affinity of SARS-CoV-2 PL^{pro} of NS_79 and NS_08 were −6.4 kcal/mol and -5.8 kcal/mole, respectively. The result showed that selected hits exhibited good binding affinity towards the active site of the proteins.

The SARS-CoV-2 spike receptor's active site's 3D surface topology revealed that the majority of hits bind outside of the active region. The amino acid residues CYS-145 and HIS-41 in the active site of SARS-CoV-2 M^{pro} constitute a catalytic dyad that serves as a

general acid-base and a nucleophile, respectively [33,34]. Yoshino et al. identified that HIS-41, GLY-143, and GLU-166 are the three often interacting amino acid residues and therefore important target for SARS-CoV-2 M^{pro} inhibition [35]. It was observed that both the selected hits NS_84 and NS_40 interacts with HIS-41 and NS_84 also binds with GLY-143 (Figure 1). Both of the hits were deeply embedded into the active site of M^{pro}, as evidenced by a 3D surface topology image (Figure 2). Therefore, both the hits exhibited decent docking score and interaction with crucial amino acid residues. The hit NS_08 interacts with the active site amino acid residues TYR-264 and TYR-268 of PL^{pro} and NS_79 interacts with GLY-163 and TYR-273. Both of the hits that were entered into the PL^{pro} active site are shown in Figure 2. The 2D ligand interaction diagram of the best scored hits and binding postures of selective hits in the 3D surface topology in the active site of SARS-CoV-2 M^{pro} and PL^{pro} are shown in Figure 2.

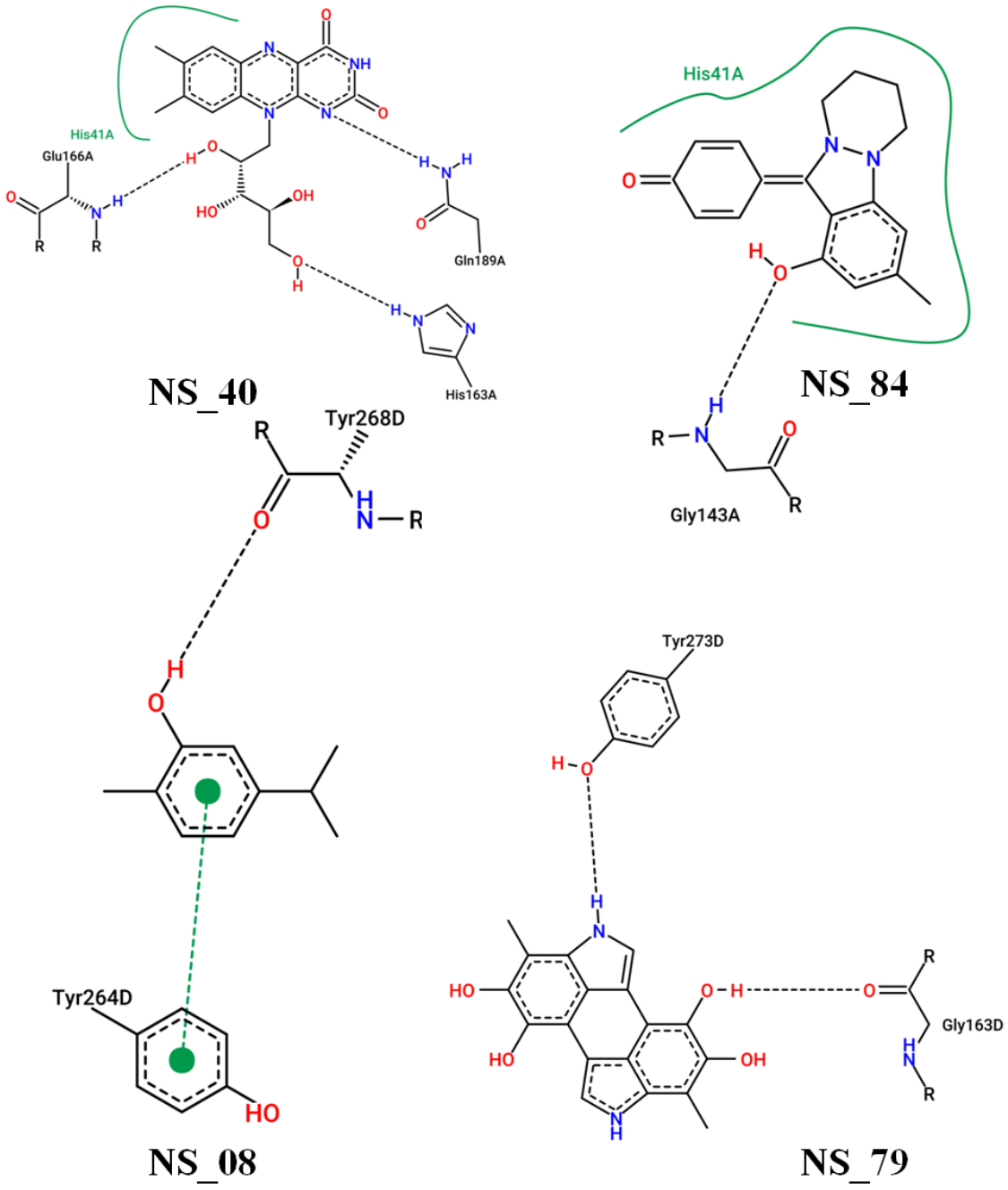

Figure 1. The 2D ligand interaction diagram displays the best hits (NS_40, NS_84) in the SARS-CoV-2 Mpro active site and the best hits (NS_08, NS_79) in the PLpro active site.

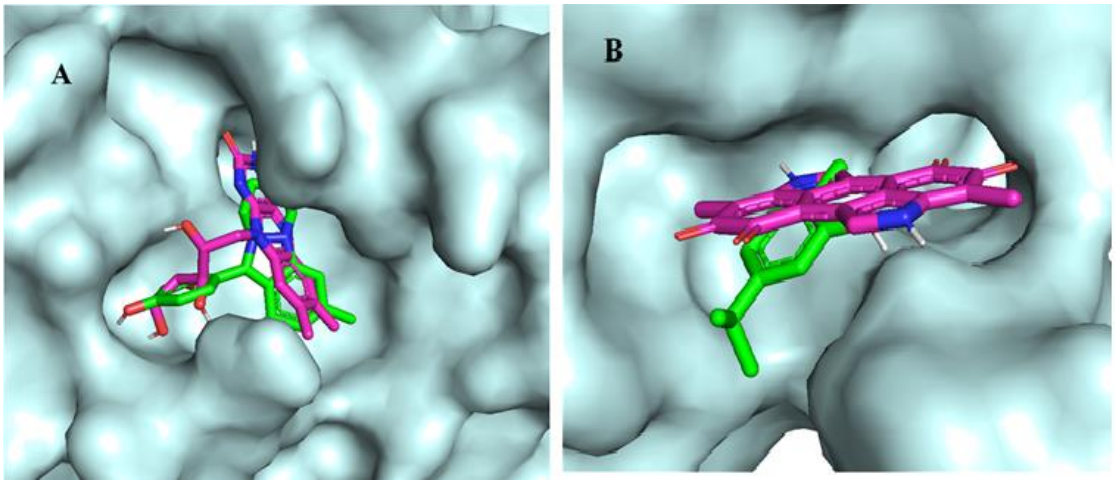

Figure 2. Binding postures of selective hits (NS_40, NS_84) in the 3D surface topology in the active site of SARS-CoV-2 M^{pro} (**A**): M^{pro} active site, Green: NS_84, Purple: NS_40) and hits (NS_08, NS_79) in the active site of PL^{pro} (**B**): PL^{pro} active site, Green: NS_08, Purple: NS_79).

The ADME profiles of SARS-CoV-2 M^{pro} and PL^{pro} inhibitors were predicted using the online server SwissADME (http://www.swissadme.ch/ (accessed on)) [36]. The predicted ADME properties are listed in Table 2. The molecular weights of each of the selected hits were less than or equal to 376.36 g/mol. The polar surface area of hits NS 08, NS 40, NS 79, and NS 84 were 20.23, 161.56, 99.86, and 49.27, respectively, which is less than 130Å^2. Polar surface area may be used to describe the transport qualities of a medication. The hits were all expected to be highly absorbed in the intestine because to their high projected gastro-intestinal absorption properties. The compounds NS_40 and NS_79 are not able to cross the blood-brain barrier, however, substances NS_08 and NS_84 could penetrate. Drug-likeness qualities are assessed using Lipinski's Rule of Five. The chosen hits all adhered to Lipinski's Rule of Five, indicating that they are all drug-like. The anticipated ADME results showed that the majority of these hits' attributes fall within the acceptable range, meaning that the ADME values for the most of the hits were suitable. The structure of the hits are shown in Figure 3.

Table 2. Swiss ADME predicted the physiochemical properties of SARS-CoV-2 M^{pro} and PL^{pro} inhibitors.

Parameters	NS_08	NS_40	NS_79	NS_84
MW	150.22	376.36	318.28	295.36
NHA	11	27	24	22
NAHA	6	14	18	15
NRB	1	5	0	1
NHBA	1	8	4	2
NHBD	2	5	2	2
MR	40.01	96.99	93.74	88.27
TPSA (Å^2)	20.23	161.56	99.86	49.27
iLOGp	2.24	0.91	0.97	−0.57
Log S (ESOL)	−3.31		−1.59	−4.60
MLOGP	2.76	−0.54	−0.49	2.73
GI	High	Low	High	High
BBBP	Yes	No	No	Yes
vLROF	0	0	0	0
vGR	1	0	0	0
vVR	0	0	0	0

BS	0.55	0.55	0.55	0.55
SA	1.00	3.84	2.00	2.85

MW: Molecular weight; NHA: Num. heavy atoms; NAHA: Num. arom. heavy atoms; NRB: Num. rotatable bonds; NHBA: Num. H-bond acceptors; NHBA: Num. H-bond donors; MR: Molar Refractivity; TPSA: Topological Polar Surface Area; Log S: Solubility class; SC: Solubility class: 1.34e-02 mg/mL; 3.27e-05 mol/l Class; GI: Gastrointestinal absorption; BBBP: Blood Brain Barrier Penetration; vLROF: Violation of Lipinski's rule of five; vGR: Violation of Ghose rule; vVR: Violation of Veber rule; BS: Bioavailability Score; SA: Synthetic accessibility.

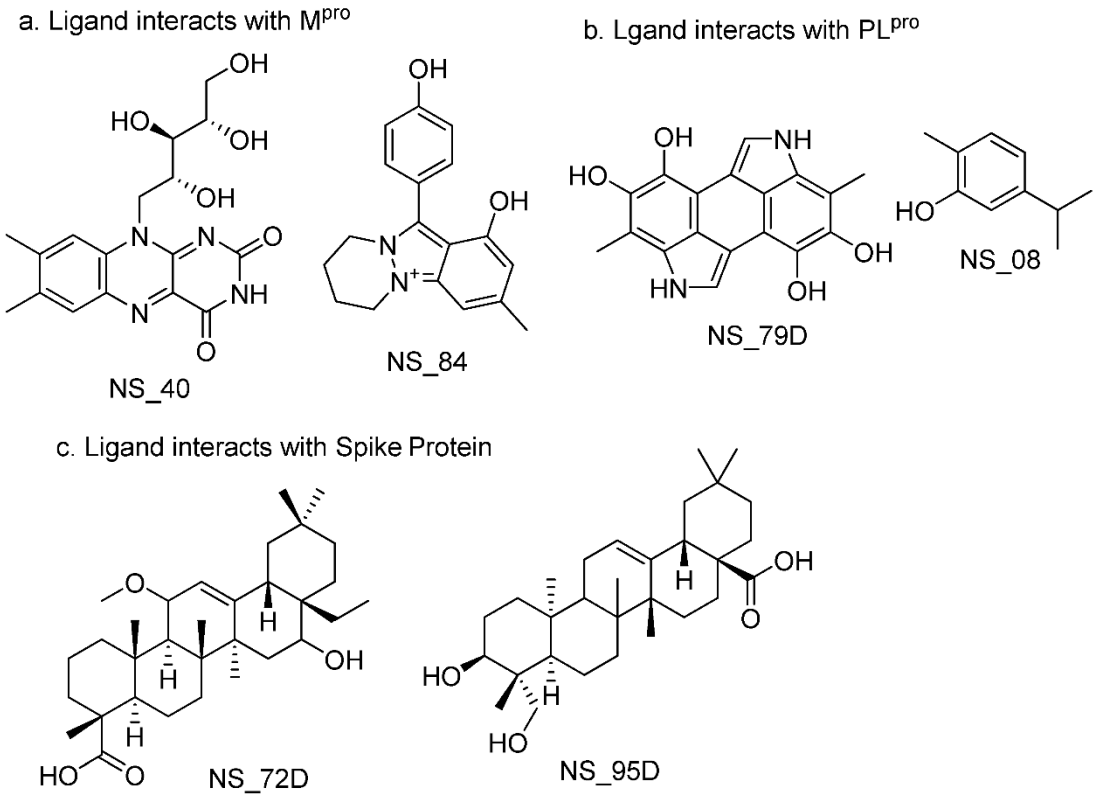

Figure 3. Structure of selected hits.

4. Conclusions

From the binding interaction it was concluded the black seed compounds namely NS-40, NS_84, NS_72D, NS_95D and carvacrol may be active against SARS-CoV-2 proteins. Considering the binding affinity of carvacrol with SARS-CoV-2 PL^{pro} and antiviral/anti-microbial property of carvacrol, molecular weight, cellular permeability, suitability of administration demand it's in vitro investigation against SARS-CoV-2. Carvacrol may be used as SARS-CoV-2 replication inhibitor as well as the preventive therapeutic to secondary infection of COVID-19 patient. Thus, the carvacrol which is available in the commercial market as various form of oregano oil or in black cumin oil can be used for frontline fighter of COVID-19 to prevent their infection, which will reduce the spread of disease.

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Conflicts of Interest: The authors declare that there are no conflict of interest.

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