Identification of *Nigella Sativa* Constituents As Putative SARS-CoV-2 Target Inhibitors

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Abstract- *Nigella sativa (N. sativa) is a widely used medicinal plant all over the world. It belongs to family Ranunlaceae. It is used in various form of treatment like Siddha, Ayurveda and Unani. Seeds and oil have history of models in system of medicine and foods. The seeds of Nigella Sativa have been widely used in the treatment of different diseases and ailments. It is the greatest form of healing in Islamic literature. Recommended usage in Tibb-e-Nabwi. It is also known as prophetic medicine. It has various properties like anti-hypertensive , Liver tonics, diuretics, digestive, anti-diarrheal, appetite stimulant, Analgesics, anti-bacterial and in skin disorders. Extensive studies have been carried out on N. sativa by various researchers and a wide spectrum of its pharmacological actions have been explored which has properties like anti-diabetic, anti-cancer, immunomodulator, analgesic, antimicrobial, anti-inflammatory, spasmolytic , bronchodilator, hepato-protective, renal protective, gastro-protective, antioxidant properties,etc. Due to its extrculous power of healing, N. sativa has got the place among the top ranked evidence on herbal medicines. Most of the therapeutic properties of this plant are due to the presence of thymoquinone which is major bioactive component of the essential oil. Also useful for treating bloating, flatulenas, asthma, diabetes and canbe used to provide relief from painful menstrual cycles. Bioactive component thymoquinone (TQ), have been manifested to have ability to persuade oxidative stress and inflammation, and to promote immunity, cell survival, and energy metabolism, which under lie diverse health benefits, including protection against metabolic, cardiovascular, digestive, hepatic, renal, respiratory, reproductive, and neurological disorders, cancer, and so on. Plants matured in one year and are between one and two feet tall (60–70 cm). Its leaves are gray–green, its flowers are colored white to blue, its fruits are capsules containing numerous black aromatic seeds, and the seeds contained up to 40%fixed oil and about 1.4% essential oil. Black seed oil have been consumed as remedy for viral infections which is obtained from seed of Nigella sativa .*

Keywords- Nigella sativa, Miracle herb, Ranunlaceae, Tibb-e-Nabwi,Thymoquinone, Black seeds, Anti-diabetic, Antioxidant, Essential oil

I. INTRODUCTION

For many centuries, various indigenous medical systems and folk remedies have employed medicinal plants to treat illnesses. Further more , since they are thought to be safer than contemporary allopathic medications, medicinal plants are also utilized in the creation of herbal remedies. Since only a small number of plant species have been fully studied for their potential, safety assessment, mechanism of action, and toxicological studies, many researchers are concentrating on medicinal plants.

The seed of this plant is generally known as in southern Asia, sauda in the Middle East, black cumin in English.

Recent research in ethno pharmacology revealed that Nigella species are frequently employed in conventional and traditional medicine. Of these, Nigella sativa is arguably the most well-known species in the genus, having been utilized as a natural medicine in many regions of the world.

Narcissus sativa has been traditionally associated with several medicinal properties, including analgesic, liver tonic, diuretic, appetite stimulant, and digestive.

A number of studies have demonstrated the oil numerous properties, including those of an antioxidant ,antitumor , antibacterial, anti-inflammatory , and many more

The majority of this plant' medicinal qualities are a result of thymoquinone (TQ), a significant active ingredient in the essential oil. Because one of the Prophetic hadiths states that black seed is the cure for all ailments save death, Muslims view it as one of the best types of medicinal treatment available.

It is typically obtained by cold pressing seeds.

The ability of N. sativa oil to shield against oxidative stress damage brought on by reactive species and free radicals is one of its most researched qualities .

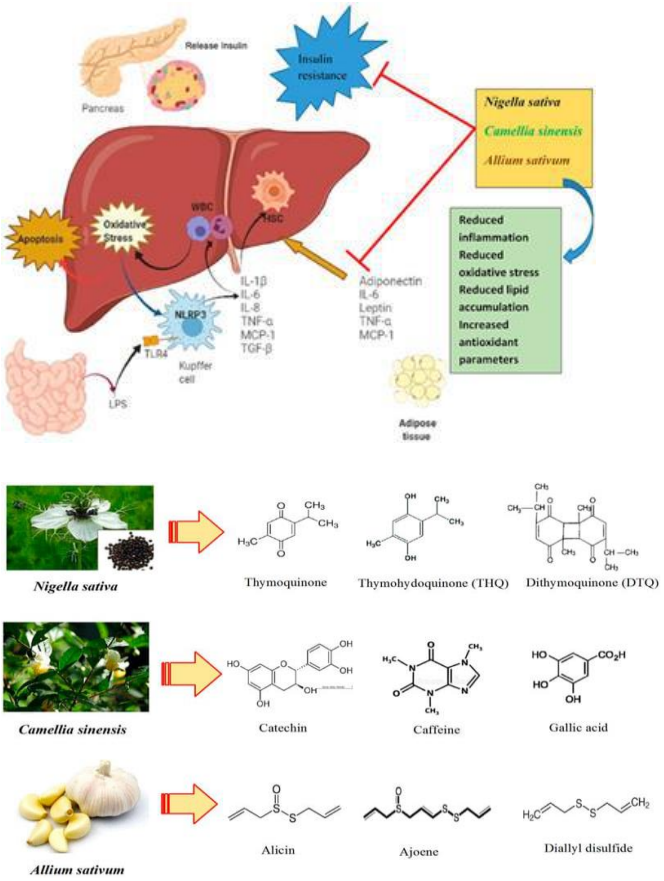
According to Siti et al. (2015), antioxidant qualities are frequently linked to the protection of degenerative conditions and diseases like cancer, cardiovascular diseases, and disorders of the central nervous system like Parkinson and Alzheimer's disease .

An illness of the lungs that impedes breathing (Chronic Obstructive Pulmonary Disease, or COPD) is cured when black seed oil helps taken orally to enhance breathe easier.

Conditions in a man that prevent him from getting a woman pregnant within a year of trying to conceive (male infertility). Taking black seed oil by mouth increases sperm count and how quickly sperm can move. It' not clearif it improves pregnancy rates.

Marketed as a food item as well as a natural medicine, the oil is generally recognized as safe (GRAS) by the US Food and Drug Administration.

Nigella sativa, also known as N. sativa (Family Ranunlaceae), is gaining popularity among medicinal plants due to its wide range of pharmacological potential and rich religious and historical background.



METHODS:-

Sample collection and preparation

Mature N. sativa seeds were collected in October 2020 (average temperature 29 °C) near the end of the rainy season from a fallow farmland in Northern Nigeria (N10° 9′ 32.2524", E8° 8′ 1.8816"). No licenses or permissions were needed for the collection or study. Proficient F.N. Mbagwu, a taxonomist from the Department of Plant Science and Biotechnology at Imo State University in Owerri, Nigeria, identified them. The voucher specimen was then placed in the university's herbarium and given the herbarium number IMSUH-468.

After being cleaned with tap water, they were allowed to air dry for a week on laminated paper. An electric blender was used to further reduce the particle size after the dried seeds had first been ground with a mortar and pestle. After being transferred, the seed powder was kept in a refrigerator at 4 °C in an airtight plastic container.

EXTRACTION OF OIL:-

According to Duru (2020), a Soxhlet device set up on a heating mantle was used to extract oil from the ground seed powder. A fresh, white cotton cloth that had been cleaned with hexane (≥98.5%, Sigma-Aldrich, Darmstadt, Germany) was used to encase the 100 g of seed powder. The extractor's thimble-holder contained the sample, which was progressively filled with condensed, redistilled hexane from a distillation task set on a heating mantle. The extracted oil is transferred by siphon back into the distillation process when the solvent reaches the overflow level in the thimble-holder. Up until the point of total extraction, the process was repeated. Next, using rotary evaporation, the extracted oil was separated from the solvent.

CHARACTERZATION OF OIL:-

Gas chromatography mass spectrometer (GC–MS) equipment (Model: 7890 GC and 5977B MSD, Agilent Technologies, USA) was used to characterize the oil sample. A standard nonpolar column L×I.D. of 30 m×0.25 mm with a film thickness of 0.25 μm was employed in an HP-5 MS capillary. The mobile phase (carrier gas: He) flow rate was calibrated to be 1.0 mL/min. The temperature program (oven temperature) in the gas chromatography section was set at 40 °C and increased to 250 °C at a rate of 5 °C per minute with an injection volume of 1μL. The oil sample was fully scanned at a range of 40–650 m/z after being dissolved in methanol (≥99.8%, Sigma-Aldrich, Darmstadt, Germany). The NIST

mass spectral library search program was used to compare the results .

IDENTIFICATION AND PREPARATION OF LIGAND:-

The compounds present in the crude oil sample were identified and their 3D structure-data files (SDF) downloaded from the PubChem database. They were reduced in size using the Universal Force Field at 200 steps in the PyRx virtual screening tool. After that, they were transformed into Auto Dock ligands (pdbqt) and applied to the docking study.

IDENTIFICATION AND PREPARATION OF MOLECULAR TARGET:-

The literature (Konkolova et al. 2020; Tan et al. 2020; Michalska et al. 2020; Jin et al. 2020; Gao et al. 2020; Wang et al. 2020) was consulted in order to identify the five SARS-CoV-2 molecular targets (Fig. 1), which are Replicas polyprotein 1a (6YHU), RNA binding protein of NSP9 (6W4B), ADP ribose phosphatase of NSP3 (6VXS), RNA-dependent RNA polymerase RDRP (7BTF) and one protein of the host, ACE2-Angiotensin-converting enzyme (6LZG).

After removing the co - crystallized ligand and interfering crystallographic water molecules, UCSF Chimera 1.14 was used to minimize the protein's energy. At the 300 steepest descent steps at 0.02 Å, the protein was minimized. With 10 update intervals and 10 steps at 0.02 Å, the conjugate gradient was obtained. Dock Prep was used to add Gustier charges in order to achieve a good structure conformation.

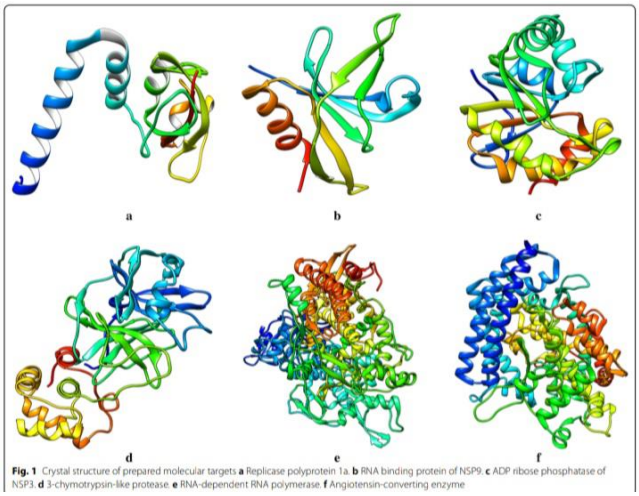


Fig. 1 Crystal structure of prepared molecular targets **a** Replicase polyprotein 1a. **b** RNA binding protein of NSP9. **c** ADP ribose phosphatase of NSP3. **d** 3-chymotrypsin-like protease. **e** RNA-dependent RNA polymerase. **f** Angiotensin-converting enzyme

DOCKING PROCEDURE AND ANALYSIS OF RESULTS:-

Docking the phytochemical compounds from the oil on specific binding pockets of SARS-CoV-2 proteins allowed for their ranking according to binding energies, which was used for screening. Using Auto dock Vina in PyRx software, the ligands and proteins were multiple docked . The Excel spreadsheet containing the molecular docking results was arranged, and the Conditional Formatting feature was utilized to view the data's Heat Map.

(ADMET) ANALYSIS ABSORPTION, DISTRIBUTION, METABOLISM, ELIMINATION AND TOXICITY:-

According to Yang et al. (2019), the compounds that exhibited the lowest binding energy for every protein were chosen and subsequently subjected to the ADMET sar 2 server for the analysis of their drug-like characteristics, pharmacokinetics, and pharmacodynamics parameters.

ANALYSIS OF PROTEIN-LIGAND INTERACTION:-

Biovia Discovery studio client 20.1 (BIOVIA 2020) and UCSF Chimera software were used to visualize the interactions between the most potent compounds in the oil and the amino acid residues of the proteins.

Table 2 - Displays the compounds' binding affinities to the SARS-CoV-2 receptors from the oil extract.

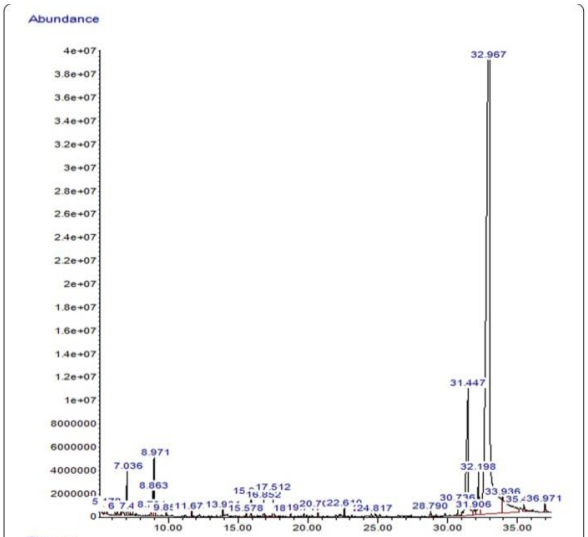


Fig. 2 Chromatogram of phytochemicals in the hexane extract of *N. sativa* seed oil

Table 1 Phytochemical compounds identifed in N. sativa seed oil :

PEAK	% wt	Name of compound	PUBMED CID	THERAPEUTIC USES
1	0.22	1,2-dimethylcyclopentan-1-ol	551296	Still unknown
2	0.1	α – thujane	17868	Anti-

	4			inflammatory,anti-arthritic, antimicrobial
3	0.19	α – pinene	6654	Anti-inflammatory, antioxidant, anticancer, antimicrobial
4	0.13	1,3-dimethylcyclohexanol	558957	Still unknown
5	1.30	(Z)hept-2-enal	5362616	Still unknown
6	0.23	β – pinene	14896	Antimicrobial
7	0.17	1-cynaopyrrolidine	73737	Still unknown
8	0.63	m-cymene	10812	Anti-inflammatory
9	1.61	D-Limonene	440917	Anti-inflammatory, Anticancer, Antimicrobial
10	0.14	γ – Terpinene	7461	Anti-inflammatory, Antioxidant, Antimicrobial
11	0.18	Trans-4-methoxy thujane	-	Antimicrobial
12	0.15	Terpineol	17100	Antioxident, Anticancer, Anticonvulsant, Antiulcer, Antihypertensive, Anti-nociceptive
13	0.11	Thymoquinone	10281	Anti-inflammatory, hepatoprotective, Antioxident,Anti cancer, cytotoxic
14	0.52	2-decenai,(E)-	5283345	Antinematicidal
15	1.39	2,4-Decadienal,(E,E)-	5283349	Antinematicidal
16	0.08	Geranyl-2-methylbutyrate	6437648	Still unknown
17	0.09	Tetradecane	12389	Antimicrobial
18	0.20	α – bergamotene	86608	Antimicrobial
19	0.20	β – bisabolene	10104370	Antimicrobial, Antiulcer
20	0.12	Caryophyllene oxide	1742210	Anti-inflammatory, Antimicrobial, Antiparasitic, Analgesic
21	0.06	Hexadecane	11006	Antibacterial
22	0.22	Myristic acid	11005	Antimycomicrobial
23	0.25	Methyl linoleate	8181	Anti-inflammatory
24	10.00	palmitic acid	985	Antioxident, Antibacterial, Anti-inflammatory, Antitumor
25	1.62	Methyl linoleate	5284421	Antioxident
26	79.61	Linoleic acid	5280450	Antidiabetic
27	0.44	9,17-octadecadienal,(Z)-	5365667	Anti-inflammatory, Antioxident

Table 2 - Binding affinities of compounds in the oil of N. sativa seed for SARS-CoV-2 proteins

Table 2 Binding affinities of compounds in the oil of *N. sativa* seed for SARS-CoV-2 proteins

Compound	ΔG Energy (Kcal/mol)					
	3CLpro	ACE2	NSP3	NSP9	RDRP	RP1A
1,2-dimethylcyclopentan-1-ol	-4.2	-5.0	-3.9	-4.2	-4.5	-3.5
α-thujene	-4.7	-6.6	-4.8	-5.0	-5.8	-4.4
α-pinene	-4.7	-5.2	-4.8	-4.7	-5.8	-4.4
1,3-dimethylcyclohexanol	-4.5	-5.0	-4.4	-4.4	-5.1	-3.8
(Z)-hept-2-enal	-3.8	-5.0	-3.8	-4.0	-4.3	-3.4
β-pinene	-4.8	-5.2	-4.8	-4.7	-5.7	-4.4
1-cynaopyrrolidine	-3.8	-4.7	-3.4	-3.4	-4.3	-3.0
m-cymene	-5.0	-6.9	-4.9	-5.4	-5.8	-4.8
D-Limonene	-5.0	-6.8	-4.8	-5.1	-5.6	-4.6
γ-Terpinene	-5.0	-7.0	-4.7	-5.2	-5.7	-4.9
Trans-4-methoxy thujane	-5.0	-5.4	-4.9	-5.0	-5.3	-4.4
Terpineol	-5.3	-6.8	-5.1	-5.3	-6.2	-4.7
Thymoquinone	-4.9	-7.0	-5.2	-5	-5.7	-5.0
2-decenal, (E)-	-3.7	-5.6	-4.2	-4.2	-4.5	-4.0
2,4-decadienal, (E,E)-	-4.2	-5.7	-4.5	-4.8	-5.0	-4.2
Geranyl-2-methylbutyrate	-5.1	-6.4	-5.7	-5.3	-5.6	-5.1
Tetradecane	-3.8	-5.7	-4.2	-4.1	-4.2	-4.3
α-bergamotene	-5.4	-6.2	-6.1	-5.7	-6.2	-5.7
β-bisabolene	-5.5	-8.0	-5.7	-6.2	-6.0	-5.1
Caryophyllene oxide	-6.0	-5.9	-6.3	-6.3	-6.9	-5.5
Hexadecane	-3.9	-4.7	-4.4	-4.0	-4.6	-3.8
Myristic acid	-4.6	-4.9	-4.6	-4.5	-5.1	-4.2
Methyl palmitate	-3.8	-5.1	-4.9	-5.0	-4.9	-4.6
Palmitic acid	-4.4	-5.2	-4.7	-4.4	-5.1	-4.2
Methyl linoleate	-4.2	-4.9	-4.7	-5.4	-5.3	-4.8
Linoleic acid	-5.0	-5.7	-5.2	-4.9	-5.5	-5.1
9,17-Octadecadienal, (Z)-	-4.2	-6.0	-4.6	-4.3	-4.8	-4.2
Remdesivir (Control drug)	-7.8	-8.1	-7.2	-6.8	-8.0	-6.0

Key: Good Fair Poor

Table 3 ADMET properties of a α-bergamotene, β-bisabolene and caryophyllene oxide relative to Remdesivir

ADMET properties	α - bergamotene	β – bisabolene	Caryophyllene oxide	Remdesivir
Molecular weight	204.36	204.36	220.36	602.56
WlogP	4.7	5.0	3.9	2.2
H-bond acceptor	0	0	1	13
H-bond donor	0	0	0	4
Rotatable bonds	3	3	0	13
Blood brain barrier	+	+	+	+
Carcinogenicity (binary)	-	-	-	-
Human intestinal absorption	+	+	+	+
Acute oral toxicity	2.32	2.05	2.24	3.43
Water solubility	-4.97	-4.89	-3.45	-4.12

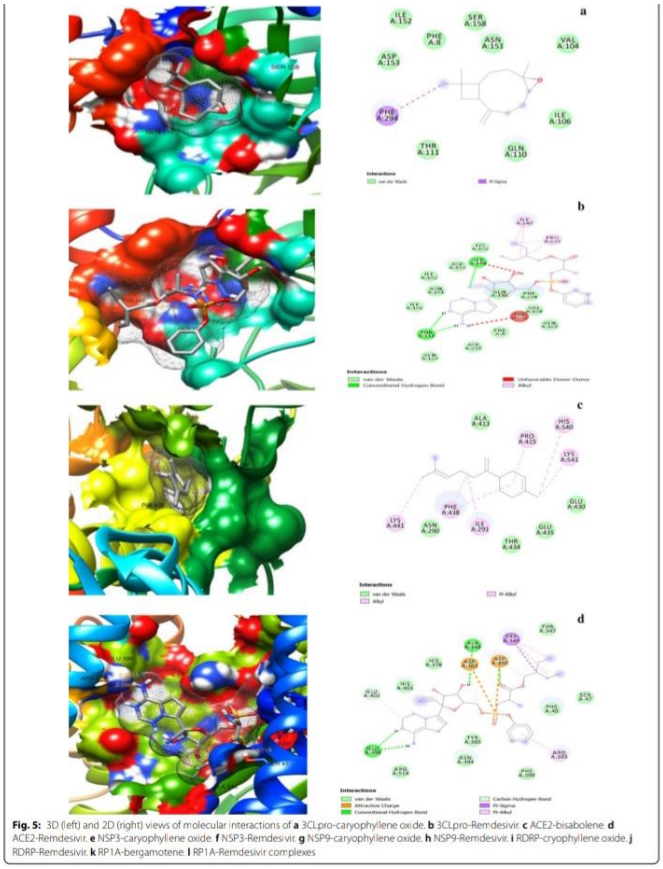
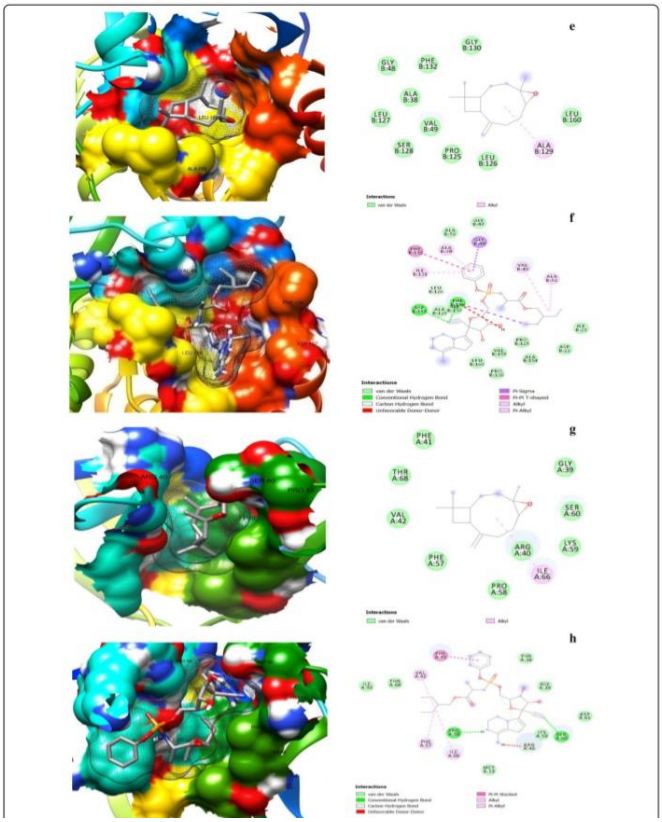


Fig. 5: 3D (left) and 2D (right) views of molecular interactions of **a** 3CLpro-caryophyllene oxide. **b** 3CLpro-Remdesivir. **c** ACE2-bisabolene. **d** ACE2-Remdesivir. **e** NSP3-caryophyllene oxide. **f** NSP3-Remdesivir. **g** NSP9-caryophyllene oxide. **h** NSP9-Remdesivir. **i** RDRP-cryophyllene oxide. **j** RDRP-Remdesivir. **k** RP1A-bergamotene. **l** RP1A-Remdesivir complexes

II. CONCLUSIONS

Investigated were the phytochemical composition and binding affinities of compounds in the Nigella sativa seed oil extract on SARS-CoV-2 molecular targets. The oil's GC-MS analysis revealed that it contained a large number of compounds with established biological and medicinal properties.

According to the docking studies conducted on the SARS-CoV-2, caryophyllene oxide, α-bergamotene, and β-bisabolene showed promise as inhibitors of these proteins' activities. Additionally, their ADMET results suggested that they would have favorable pharmacological and pharmacodynamics characteristics in the human body. Thus, more in vivo, in vitro, and clinical trials are required to confirm these compounds' potential for the coronavirus pandemic eradication.



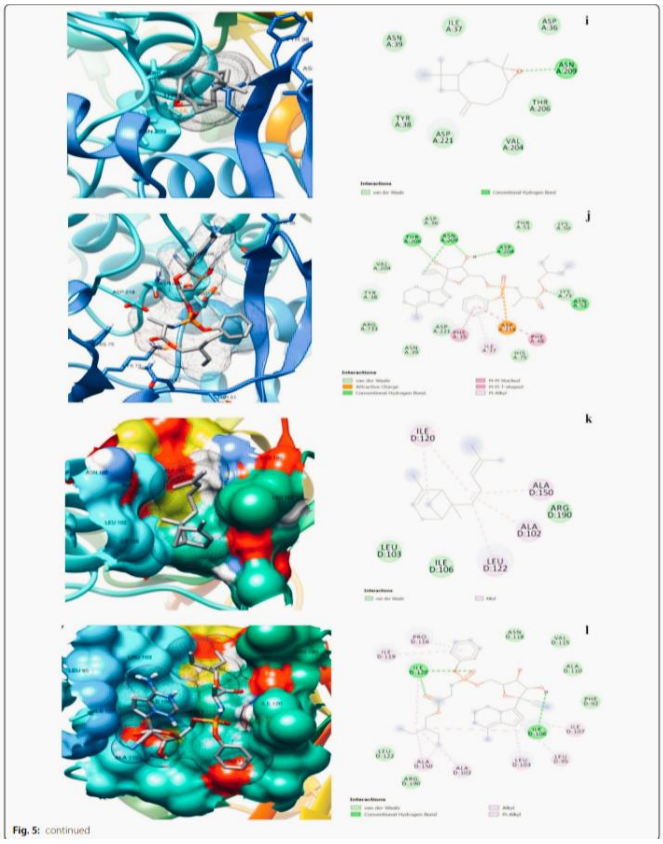


Table 4 - Protein residue interactions with the compounds and Remdesivir

Protein-ligand complex	Interacting residues						
	Hydrogen bond	Unfavorable Donor-donor	Pi-sigma	Pi-alkyl	alkyl	Carbon hydrogen	Attractive charge
3CLpro-Caryopyelleneoxide	-	-	PHE294	-	-	-	-
3CLpro-Remde-Sivir	SER158; THR111	SER158; THR292	-	-	1LE249; PRO293	-	-
ACE2-β-bisabolene	-	-	-	HIS450; LYS541;LYS441	HIS540; PRO415; PHE438; ILE291	-	-
ACE2-Remdesivir	GLU398; ALA348; ASP350	-	TRP349	TRP349; ARG393	-	GLU402; ALA348; ASP382	ASP38; ASP350
NSP3-Cryophyllene oxide	-	-	-	-	ALA129	-	-
NSP3-Remdesivir	ASP157; PHE156	PHE156	GLY48; PHE156	ALA38;ILE131	VAL49; ALA52	LEU126	-
NSP9-Cryophyllene oxide	-	-	-	-	ILE68	-	-
NSP9-Remdesivir	PRO58; SER6	ARG40	-	VAL42; PHE56	ILE66	ARG40	-

	0						
RDRP-Cryophyllene oxide	ASN209	-	-	-	-	-	-
RDRP-Remdesivir	ASN52; THR206; ASN209; ASP208	-	-	ILE37	-	-	ASP218
RP1A-α-bergamotene	-	-	-	-	ILE120;ALA150;ALA102;LEU122	-	-
RP1A-Remdesivir	ILE120; ILE106	-	-	PRO116;ILE119; ILE107; LEU95; LEU103	ILE120;ILE106;ALA150;ALA102	-	-

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