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Deciphering underlying mechanism of Sars-CoV-2 infection in humans and revealing the therapeutic potential of bioactive constituents from *Nigella sativa* to combat COVID19: *in-silico* study

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ABSTRACT

COVID-19, emerged at the end of 2019 have dramatically threatened the health, economy, and social mobility of people around the world and till date no medication is available for its treatment. An amazing herb, *Nigella sativa*, having antiviral, antihypertensive, anti- diarrhoeal, analgesics, and anti-bacterial properties, needs to be explored for its efficacy against SARS-CoV-2, the causative agent of COVID-19. *In-silico* studies were carried out to understand the role of its bioactive constituents in COVID-19 treatment and prevention. Firstly, the disease network was prepared by using ACE2 (Angiotensin-II receptor), as it is the entry site for virus. It was used to decipher the mechanism of SARS-COV-2 infection in humans. Second, the target receptors for *N. sativa* were predicted and protein interaction studies were conducted. Further, docking studies were also performed to analyse it for treatment purpose as well. This study concludes that pathways undertaken by *N. sativa* bioactive constituents were similar to the pathways followed in SARS-COV-2 pathology, like renin-angiotensin system, kidney functions, regulation of blood circulation, blood vessel diameter, *etc*. Also, in docking studies, the constituents of *N. sativa*, α-hederin, Thymohydroquinone and Thymoquinone were observed to be efficiently binding to ACE2. Also, the bioactive phytoconstituents are involved in molecular pathways like HIF1, VEGF, IL-17, AGE-RAGE, chemokine and calcium signaling pathways which can be majorly helpful in combating hypoxia and inflammation caused due to compromised immune system and oxidative stress. Therefore, *N. sativa* standardized extract having the above phyto-constituents could be useful in COVID-19 and hence opens a new treatment line.

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SARS-COV-2; Angiotensin-II receptor (ACE2); *Nigella sativa*; α-hederin; protein interaction network

Introduction

Three different strains of CoV (Coronavirus) have crossed the species obstruction to cause fatal pneumonia in humans since the start of the 21st century: SARS-CoV (severe acute respiratory syndrome) (Drosten et al., 2003), MERS-CoV (Middle-East respiratory syndrome) (Zaki et al., 2012) and SARS-CoV-2 (causative agent of COVID-19) (Huang et al., 2020), first emerged in the Wuhan city, December 2019, in the province of Hubei, China, was isolated and sequenced in January 2020. An ongoing eruption of COVID-19 (atypical pneumonia) has affected more than 36, 471, 856 individuals and claimed 1,061,788 lives in 213 countries as of October 8, 2020. It became a super- hot spreading virus, such that the World health organization declared it as a Public Health emergency of International concern on January 30, 2020 (Zhou et al., 2020). MERS-CoV virus was reported to be originated from bats, where camels acted as a reservoir host (Haagmans et al., 2014). SARS-CoV and SARS-CoV-2 are genetically closely related and believed to be originated from bats, whose intermediate reservoir host is unknown. The persistent spillovers of CoVs in humans besides the recognition of numerous coronavirus strains in bats, including SARS-CoVs, imply the future continues zoonotic transmission events (Anthony et al., 2017; Ge et al., 2013; Zhou et al., 2020). In addition to an extremely pathogenic strains of CoV; MERS-CoV, SARS-CoV and SARS-CoV-2, belonging to same genus (b-coronavirus), four other low-pathogenic endemic strains in humans are also reported: HCoV-229E, HCoVHKU1, HCoV-NL63 and HCoV-OC43.Till date, no vaccines or therapeutics are available against any human infecting strain of CoVs.

CoV enters into the host cell via transmembrane spike (S) made up of glycoproteins which helps in forming homo-trimers protruding from its surfaces (Tortorici & Veesler, 2019). S is comprised of two functional subunits (S1 and S2) which permit its efficient binding to the host cell surface receptor and also, helps in viral and cellular membranes fusion (Burkard et al., 2014; Kirchdoerfer et al., 2018; Millet & Whittaker, 2014). ACE2 (Angiotensin converting enzyme 2) has been reported to be a central component mediating the viral entry into the host cell. ACE2 is expressed in gastro-intestinal tract, endocrine tissues, kidneys, liver/gall bladder, testis and to smaller extents in lungs. The Receptor Binding

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Domain of spike proteins induces conformational changes in ACE2 receptors, which further dissociates the S1 subunit of the spike and hence initiates host cell membrane fusion. Therefore, ACE2 might play a vital role in drug designing. Amidst all these few names came into the picture mainly hydroxychloroquine, arbidol, remdesivir, and favipiravir etc. these drugs are taken as potential candidates according to National Health Commission (NHC) of the People's Republic of China. But none proved to be successful in severe respiratory distress or each one works differently according to an individual's immune system.

Traditional and herbal medicines are the part of Indian medicines since ages. The full potential and capabilities of Indian system of medicine is still unmapped. Exploring herbs and medicinal plants are one of the hopeful platforms to combat the disease and decrease the acute side effects of chemical agents (Khare, 2004). One such Indian plant which comes under the category of spices is *Nigella sativa*. It belongs to the family Ranunculaceae. The herb is available in the Indian households by the name of Black caraway or Kalonji. It is considered as curative herb and in Islam it is one of the miracle plants which is described as universal healer (Goreja, 2003). *N. sativa* has proven antiviral, antihypertensive, liver tonics, diuretics, digestive, anti-Diarrhoeal, appetite stimulant, analgesics, anti-bacterial properties (Abdel-Sater, 2009; Abdel-Zaher et al., 2011; Abel-Salam, 2012; Assayed, 2010; Boskabady et al., 2010; Salem, 2005). The more attributed role of Black caraway includes antidiabetic, anticancer, immunomodulator, analgesic, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, hepato-protective, renal protective, gastro-protective and antioxidant nature. The main constituents include thymoquinone, thymol, carvacrol, γ-terpinene and p-cymene *etc.* (Sahak et al., 2016; Ahmad et al., 2013).

In the current study, we have deciphered the mechanism of action of *N. sativa* for its usefulness against SARS-CoV-2. Since ACE2 is a target site for SARS-CoV-2 therefore a network was generated around this protein to depict a disease network and *N. sativa* constituents was used as a treatment network. This study uncovers the targets of phytochemicals and their mode of action and responsible protein targets for their efficacy. The study revolves around the pathways and processes which needs to be targeted in COVID-19. *N. sativa* is a new hope and can be used as therapeutics for combating the disease.

Methods

ACE2 interaction network and hypothesis

ACE2 is the proposed attachment site for SARS-CoV-2 in humans. Protein interaction studies were conducted around this protein to understand how the attachment of virus with ACE2 affects the pathology in human system. Computational protein-interaction analysis provides the knowledge about the indirect and probable interactions which might be occurring with the query protein. This is a very useful approach for predicting the new interactions of proteins in the in-vivo system. Cytoscape v3.7.2 (Shannon et al., 2003) was used to design the interaction network. BiNGO plugin was used for the analysis and prediction of overrepresented pathways in master network (Maere et al., 2005). Overrepresented pathways are the set of pathways which are collectively taken-up by the set of proteins under study. This helps in understanding the final fate of protein-interactions.

Pharmaco-networking of N. sativa constituents

Literature mining was done to find the different constituents of *N. sativa*. Their PubChem IDs (Kim, et al.) were retrieved, and constituents (ligands) were downloaded in .SDF format. QikProp v6.3 (rel 13) was used to perform computational ADME analysis (Schrödinger Release 2020-1: QikProp, Schrödinger, LLC, New York, NY, 2020). ADME stands for Absorption, Distribution, Metabolism and Excretion. It was performed to predict the druggability of the compounds. Further, receptors were predicted for constituents of *N. sativa* in human system by using Swiss target prediction tool (Gfeller et al., 2014). These receptors were additionally used to analyse their mode of action via interaction analysis.

Docking studies

ACE2 was downloaded from PDB database in Maestro v12.3 environment (Schrödinger Release 2020-1: Maestro, Schrödinger, LLC, New York, NY, 2020) and prepared for docking analysis. All the ligands of *N. sativa* were processed by using LigPrep (Schrödinger Release 2020-1: LigPrep, Schrödinger, LLC, New York, NY, 2020) to generate all the possible stereoisomers. Extra-Precision (XP) docking was performed using Glide (Schrödinger Release 2020-1: Glide, Schrödinger, LLC, New York, NY, 2020). Docking analysis was performed to analyse the best binding ligand from N. sativa constituents to ACE2 receptor.

Results

Cytoscape v3.7.2 was used to create master network, which comprised of total 2693 nodes and 3736 edges. The master network had clustering coefficient of 0.562 and was divided into clusters using k-means clustering and the most tightly knit cluster around ACE2 was chosen (Figure 1(a)). Network Analysis revealed various hub nodes in the network. Hub nodes are those proteins which control the disperse of signaling in the network (Figure 1(a)). Major hub nodes obtained from the protein-protein interaction network was ACE2, INS, AKT1, VEGFA, IL-6, NOS3, JUN, IL-10, HIF1A etc. (Table 1).

ACE2 alone has various functions related to blood-vessel dilation, controlling blood pressure, cytokine production, inflammatory response, and protein transport. The initial symptoms of breathlessness, dysregulated blood pressure and inflammation are the ones which are reported initially in COVID-19 diagnosis. The next receptors downstream in this signaling event are IL6, AKT1, VEGFA, INS, IGF1, etc. (Figure 1(b)).

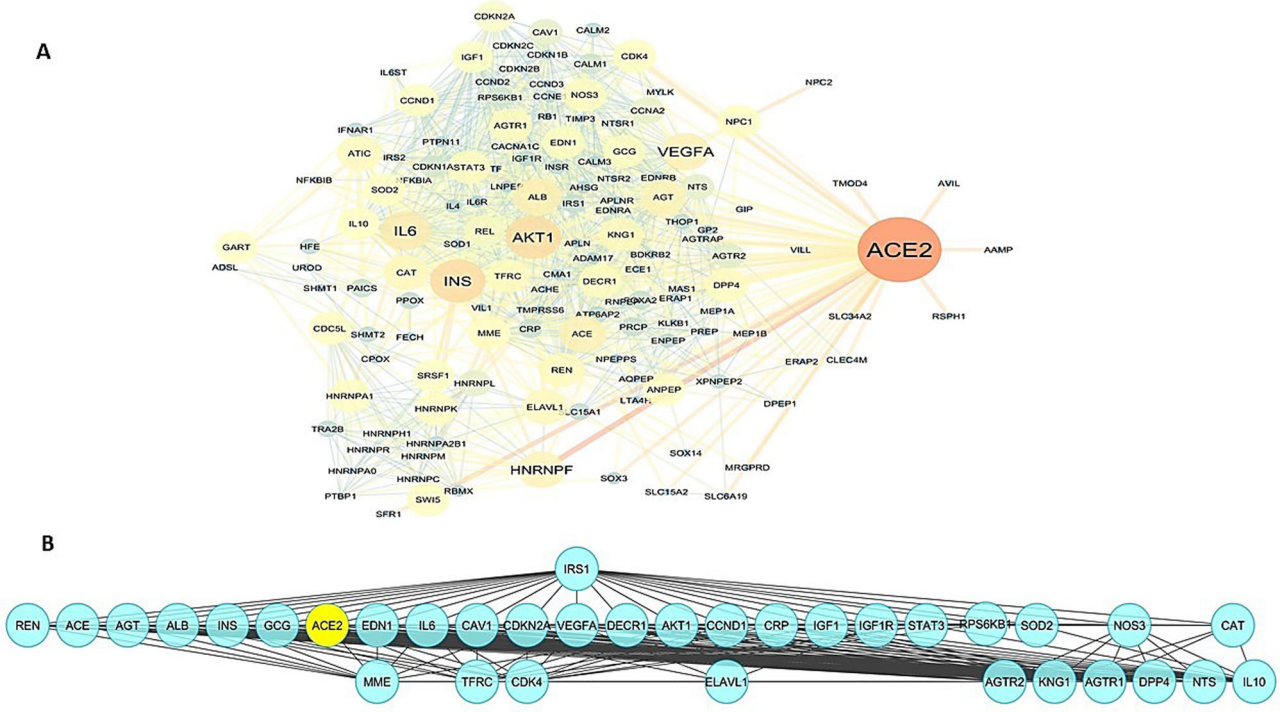

Figure 1. (a) Hub nodes in ACE2 protein related protein interaction network (b) Second line receptors to be used for targeting SARS-CoV2.

IL-6, IL-8, IL-10 and TNF-α were present in similar concentrations in SARS infected patients and may potentially cause SARS-associated ARDS. Similarly, VEGFA is the key regulator of vascular permeability, hence called as "vascular permeability factor". High VEGF expression allows the pulmonary vascular albumin extravasation which leads to the vascular permeability, causing pulmonary oedema. Furthermore, Nitric Oxide (NO) (NOS3) has been observed to inhibit the viral replication during early stages which reduces the chances of spread of virus giving ample time to the immune system for recovery. KNG has a role in contact activation of blood coagulation. The histidine rich domain of high molecular weight KNG (HMW-KNG) releases bradykinin which helps in the release of anti-microbial peptides.

Therefore, these protein receptors are interconnected and have a cascade effect in overall pathology of COVID-19. It is hypothesized that combination of medications for VEGFA (Bevacizumab), IL6 (Tocilizumab), AKT1 (BAY 1125976), IGF1 (Increlex), *etc*. might be useful to manage the disease. Therefore, when SARS-CoV-2 binds to human ACE2 receptor, the above-mentioned protein receptors and their cascading interactions to other proteins will be affected. This interaction is further seen as pathology in human system.

We have investigated the potential of *N. sativa* as a phytotherapy against COVID-19. Therefore, computational studies were carried out to further add to this data and validate whether *N. sativa* bioactive constituents can be effective for management of COVID-19 symptoms. For this, the structures of constituents of *N. sativa* were retrieved from PubChem (Figure 2).

A total of 12 bioactive constituents were retrieved namely thymoquinone, thymohydroquinone, dithymoquinone, p-cymene, carvacrol, 4-terpineol, t-anethol, longifolene, α-pinene, thymol, nigellidine, nigellicine and α-hederin (Figure 3). The computational ADME of *N. sativa* bioactive constituents is shown in Table 2. Along with these compounds Remdesivir was also retrieved as it is the upcoming medication in COVID-19 and is in clinical trials and it was used in this study to compare its efficacy to *N. sativa* bioactive constitutents in human system.

Receptor prediction was carried out for all the constituents and then receptors were utilized to find out the biological pathways and processes in which these constituents will be useful. Separate networks were created for each constituent and hub nodes were identified. Hub nodes provide the information about the genes which will be majorly targeted by these constituents and the type of biological processes involved. Remdesivir was formulated for RNA-dependent RNA polymerase (RdRp) of SARS-CoV-2 virus but in current study we have proposed its probable targets in human system as well (Table 3) so that we can compare its therapeutic capability to *N. sativa* bioactive constituents.

Protein interaction network created for remdesivir was analysed and it was observed to be targeting certain families of proteins and pathways such as Cancer, Relaxin signaling pathway, PI3K-Akt signaling pathway, IL-17, chemokine, HIF-1 signaling pathway, AGE-RAGE, VEGF pathway, Foxo pathway etc. On the other end, *N. sativa* constituents collectively were also targeting these as well other families such as Nuclear receptors, Cytochrome P450, Oxidoreductases, Erasers, Lyases, Enzymes, Family A G protein coupled receptors, calcium signaling pathways, Circadian pathways, Foxo pathway etc. These results reveal that there is clearly a lot of protection which can be contributed by this wonder herb (Figure 5).

Table 1. Top 10 hub nodes in ACE2 network with their affected pathways.

Human Readable Labels	Degree	Betweenness Centrality	Closeness Centrality	Average Shortest Path Length	Affected Pathways (Predicted by BiNGO)
ACE2 (Angiotensin converting enzyme II)	70	0.245142	0.666667	1.5	Angiotensin maturation, active regulation of amino acid transport, active regulation of cardiac muscle contraction, positive regulation of reactive oxygen species metabolic process, regulation of blood vessel diameter, regulation of cytokine production, regulation of inflammatory response and regulation of systemic arterial blood pressure by renin angiotensin
INS (Insulin)	67	0.085781	0.651163	1.535714	Protein kinase B activity, T cell activation, endoplasmic reticulum to Golgi vesicle-mediated transport, insulin receptor signaling pathway, glucose homeostasis, regulation of protein secretion and regulation of synaptic plasticity
AKT1(RACalpha serine/threonine-protein kinase)	65	0.08418	0.614035	1.628571	Activation-induced cell death of T cells, activation of protein kinase B activity, cellular response to hypoxia, cellular response to insulin stimulus, cellular response to tumour necrosis factor, cellular response to vascular endothelial growth factor stimulus, I-kappaB kinase/NF-kappaBsignaling, interleukin-18-mediated signaling pathway, regulation of apoptotic process
VEGFA (Vascular endothelial growth factor A)	57	0.043122	0.598291	1.671429	Activation of protein kinase activity, cellular response to hypoxia, cytokine-mediated signaling pathway, dopaminergic neuron differentiation, lung development, response to hypoxia
IL6 (Interleukin-6)	56	0.055244	0.619469	1.614286	Acute-phase response, cellular response to hydrogen peroxide, cellular response to lipopolysaccharide, cytokine-mediated signaling pathway, defence response to Gram-negative bacterium, defence response to Gram-positive bacterium, defence response to virus, glucagon secretion, humoral immune response, inflammatory response
ALB(Serum albumin)	56	0.039049	0.598291	1.671429	Cellular protein metabolic process, cellular response to starvation, high-density lipoprotein particle remodeling, maintenance of mitochondrion location negative regulation of apoptotic process, negative regulation of programmed cell death, platelet degranulation, post-translational protein modification, receptor-mediated endocytosis
ACE (Angiotensin converting enzyme)	50	0.037446	0.557769	1.792857	angiotensin maturation, antigen processing and presentation of peptide antigen via MHC class I, kidney development, positive regulation of blood pressure, regulation of angiotensin metabolic process, regulation of blood pressure, regulation of renal output by angiotensin
AGT (Angiotensinogen)	45	0.031026	0.585774	1.707143	Activation of MAPK activity, activation of phospholipase C activity, aging, angiotensin-activated signaling pathway, cell-cell signaling, cell growth involved in cardiac muscle cell development, cell surface receptor signaling pathway, cytokine secretion, ERK1 and ERK2 cascade, phospholipase C-activating G protein-coupled receptor signaling pathway, stress-activated MAPK cascade, renin-angiotensin regulation of aldosterone production
KNG1 (Kininogen-1)	43	0.019695	0.56	1.785714	Antimicrobial humoral immune response mediated by antimicrobial peptide, blood coagulation, intrinsic pathway, cellular protein metabolic process, G protein-coupled receptor signaling pathway, inflammatory response, killing of cells of other organism, platelet degranulation, positive regulation of apoptotic process, positive regulation of cytosolic calcium ion concentration, post-translational protein modification, vasodilation
REN (Renin)	42	0.021562	0.56	1.785714	angiotensin maturation, hormone-mediated signaling pathway, kidney development, regulation of blood pressure, regulation of MAPK cascade, renin-angiotensin regulation of aldosterone production, response to cAMP, response to cGMP

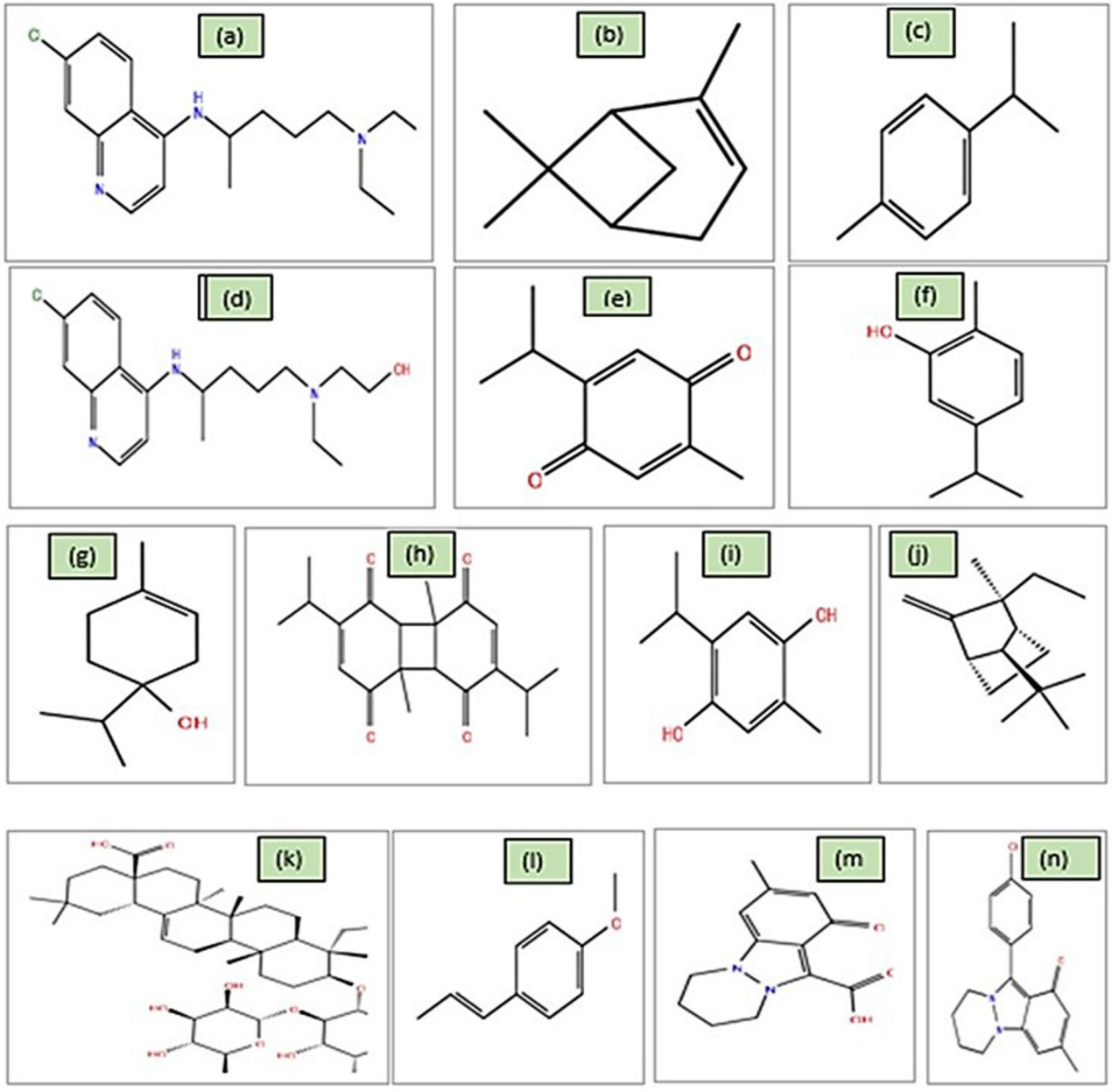

Figure 2. 2 D structures of the constituents of *N. sativa* obtained from PubChem. (a) 2719 (Chloroquine), (b) 6654 (Alpha-pinene), (c) 7463 (P-cymene), (d) 3652 (Hydroxychloroquine), (e) 10281 (Thymoquinone), (f) 10364 (Carvacrol), (g) 11230 (Terpinen-4-ol), (h) 398941 (Dithymoquinone), (i) 95779 (Thymohydroquinone), (j) 1796220 (Longifolene), (k) 73296 (alpha-Hederin), (l) 637563 (trans-Anethole), (m) 11402337 (Nigellicine), (n) 136828302(Nigellidine)

Glide Standard-Precision (SP) docking was performed and the best binding ligand to ACE2 was observed to be α-hederin (-6.265 kcal/mol), Thymohydroquinone (-5.466 kcal/mol) and Thymoquinone (-5.048 kcal/mol) (Table 4). Their binding energies were good and therefore, these compounds can be looked further to use them as future therapeutics. The docking poses of best binding compounds are displayed in Figure 3(a–c).

Remdesivir, which is the current most suited therapeutic drug for COVID-19 along with α-hederin, Thymohydroquinone and Thymoquinone were further explored more for their mode of action (Figure 4(a–c)). Since the initial diagnosis of COVID-19 is compromised immunity and it is always associated with inflammation and oxidative stress therefore it is suggested that *N. sativa* bioactive constituents can be very useful for boosting our immunity and helping patients to overcome the symptoms of COVID-19. While Remdesivir was observed to be targeting proteins involved in growth and proliferation (Figure 4(a)), α-hederin was observed to be engaged in regulation of blood pressure, regulation of cell communication, regulation of vascular processes, negative regulation of cell death, response to stress and immune effector processes. The genes identified for regulating immune response via α-hederin are ACE2, F2, SRC etc (Figure 4(b)). Thymohydroquinone was observed to be targeting proteins involved in response to oxidative stress, negative regulation of cell death, regulation of blood pressure, positive regulation of kinase activity, regulation of immune response etc. Regulation of immune response by thymohydroquinone was through genes like HSP90AA1,

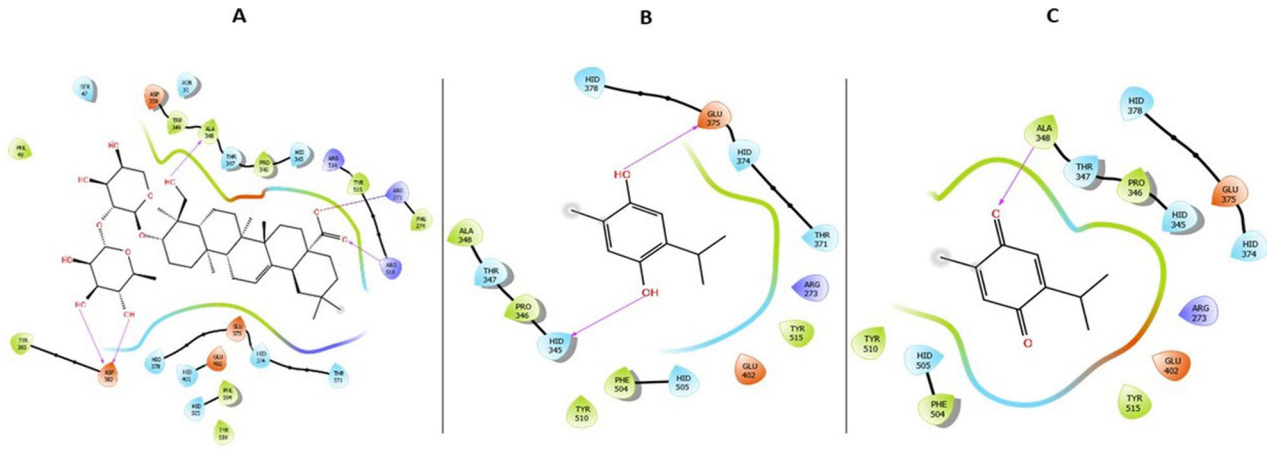

Figure 3. (a) α-hederin(b) Thymohydroquinone(c) Thymoquinonein in ACE2 receptor binding site.

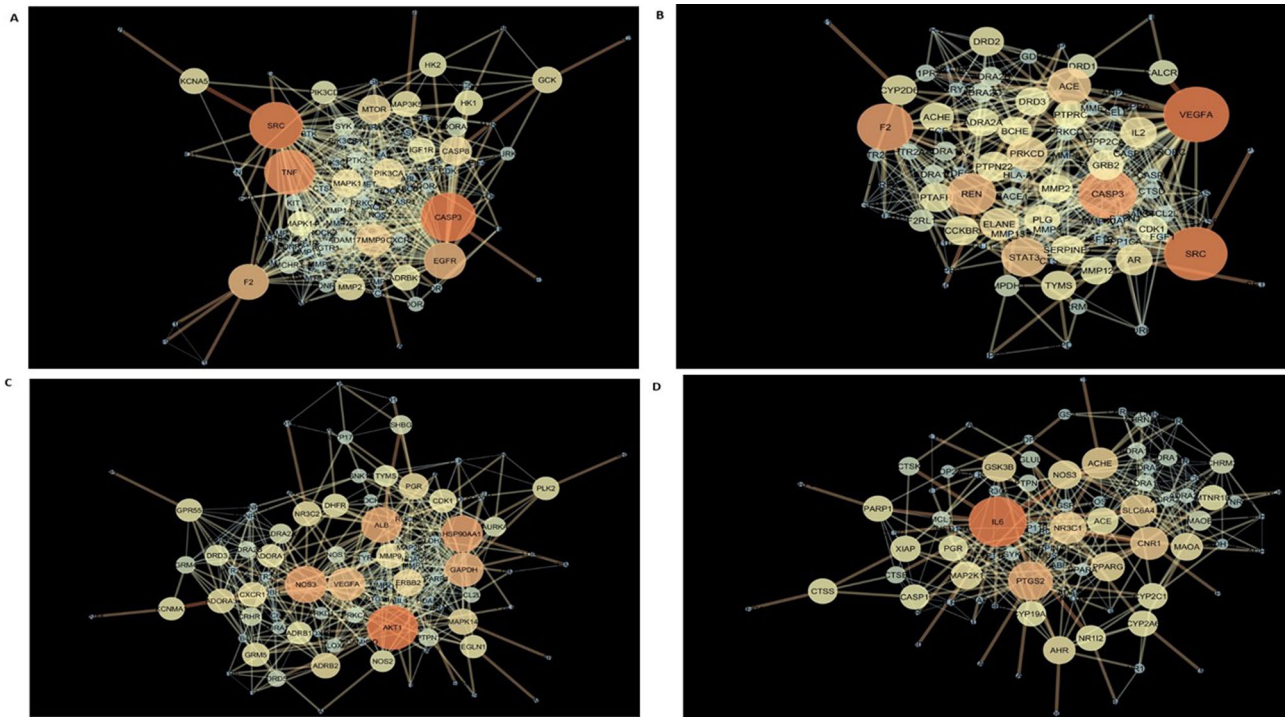

Figure 4. Mechanism of action (a) Remdesivir (b) α-hederin (c) Thymohydroquinone (d) Thymoquinone.

PRKCA, VEGFA, ADORA1 *etc.* (Figure 4(c)) and Thymoquinone was seen to be targeting kinases, heat shock proteins and oxidoreductases (Figure 4(d)).

Both α-hederin and thymoquinone have ACE as their predicted receptor in human system and therefore can be explored further for targeted therapies. The plant extract of *N. sativa* with the above-mentioned bioactive constituents is proposed to excel and outweigh any other treatment proposed so far for COVID-19 (Figure 5).

The best docking compound among all the *N. sativa* constituents is α-hederin and it is a saponin which possesses multiple properties like antioxidant, anti-inflammatory, antitumor and is also known to be affective against fungi and parasites. Many studies have been conducted at cell lines and in-vivo models and it was shown to impressively working in conditions like cancer, asthma, and inflammation (51). We need more wet-lab studies to explore its effectiveness in viral infections.

Numerous ACE2 medications are already tried on COVID-19 patients but they are also not exhibiting the good results. This might be because chemical-based compounds are targeted to single receptor and therefore have a limited mode of action to work in the body, other than their accompanied side effects. On the contrary, natural compounds derived from herbs and medicinal plants have proven to work in body in an all-round manner.

Discussion

In order to come up with the medications to manage SARS-CoV-2 infections, it is important to understand the virus-receptor recognition mechanisms. WHO has declared COVID-

Table 2. Computational ADME analysis of *N. sativa* constituents by QikProp v3.1.

Compound Name and PubChem ID	SMW	D	QPlogP	LogKhsa	Log BB	PM	Caco	Rule of 5	OA	Similar compounds and similarity index (%)
Alpha-Pinene (6654)	136.236	0.048	3.618	0.343	0.865	3	9906	0	100	Pempidine 78.80 Clomethiazole 76.61 Tranylcypromine 74.79 Levamisole 72.26 Mecamylamine 70.18
P-CYMENE (7463)	134.221	0.063	3.657	0.344	0.699	2	9906	0	100	Clomethiazole 89.85 Phenylpropanol 84.91 Isaxonine 84.31 Mephentermine 82.16 Pempidine 81.71
Thymoquinone (10281)	164.204	0.146	0.754	−0.635	−0.322	2	1091	0	86	Ethadione 91.24 Paramethadione 91.07 Acipimox 90.18 Metacetamol 90.04 Menadione 88.99
CARVACROL (10364)	150.220	1.514	3.298	0.056	0.073	3	3697	0	100	Mephentermine 91.54 Phenylpropanol 91.35 Isaxonine 90.81 Paroxypropione 88.74 Etilamfetamine 88.69
Terpinen-4-ol (11230)	154.252	1.902	2.950	0.101	0.224	4	5373	0	100	Sobrerol 88.09 Aptrol 85.79 Mephentermine 85.24 Pempidine 84.79 Phenylpropanol 84.79
alpha-Hederin (73296)	750.965	13.055	2.210	−0.287	−3.032	8	7M	3	16M	Azithromycin 68.93 Flurithromycin 67.56 Metildigoxin 66.53 Clarithromycin 64.48 beta 61.13
Thymohydroquinone (95779)	166.219	2.985	1.919	−0.171	−0.357	4	1396	0	94	Aptrol 95.09 Sobrerol 93.88 Prothionamide 93.02 Bethanidine 91.64 Mexiletine 91.28
Dithymoquinone (398941)	328.407	0.713	1.531	−0.527	−0.598	4	714	0	87	Nitisinone 81.63 Trimetozine 80.19 Fropenem 78.15 Carboquone 78.13 Emorfazone 77.37
trans-Anethole (637563)	148.204	1.354	3.167	0.120	0.284	2	9906	0	100	Clomethiazole 91.29 Mephentermine 89.60 Phenylpropanol 89.55 Isaxonine 89.01 Fenipentol 87.70
LONGIFOLENE (1796220)	204.355	0.434	4.846	0.857	1.028	1	9906	0	100	Chlornaphazine 79.84 Levamisole 78.13 Medazepam 78.10 Pyrantel 77.74 Methsuximide 77.61
Nigellidine (136828302)	294.352	7.916	3.728	0.666	−0.379	3	1219	0	100	Mesterolone 90.40 Ondansetron 89.21 Mazindol 88.92 Metandienone 88.84 Methandrostenolone 88.77
Nigellicine (11402337)	246.265	0.873	2.748	−0.597	−0.053	2	1794	0	88	Flumequine 91.32 Ketorolac 88.64 Oxolinic 86.54 Anagrelide 85.98 Glutethimide 85.82

SMW-Solute Molecular weight, D- Solute dipole moment, QPlog P- QP log P for octanol/water, QPlog S - QP log S for aqueous solubility, LogKhsa -QP log K hsa Serum Protein Binding, Log BB - QP log BB for brain/blood, PM: No of primary metabolites, Caco- Apparent Caco-2 Permeability (nm/sec), Rule of 5 - Lipinski Rule of 5 Violations, Rule of 3- Jorgensen Rule of 3 Violations, OA- % Human Oral Absorption in GI.

Table 3. Pathways targeted by Remdesivir and constituents of *N. sativa*.

PubChem ID	Compound Name	Hub Nodes	Pathways targeted
121304016	Remdesivir	CDK2, SRC, AURKA, ABL1, CDK1, MAPK1, MAPK14, TOP1, MTOR, CASP8, AURKB, CCNB1, PRKCA, PTK2, CDK5, SYK, MET, IGF1R, CASP3, PDGFRB, EIF4A1, PIK3CA, MAP3K5, CDK2, SRC	Pathways in cancer, Relaxin signaling pathway, PI3K-Akt signaling pathway, Endocrine resistance, Phospholipase D signaling pathway, AGE-RAGE signaling pathway in diabetic complications, HIF-1 signaling pathway, IL-17 signaling pathway, VEGF signaling pathway, MAPK pathway, Platelet activation, Chemokine signaling pathway, mTOR signaling pathway, FoxO signaling pathway, EGFR tyrosine kinase inhibitor resistance.
6654	Alpha-pinene	ESR1, AR, MAPK1, PTPN11, MAPK14, PTPN1, RARA, RXRA, NR3C1, PPARG, PTPN6, MAPK3, TOP1, ESR2, CDC25A, CD81, NCOA1, NOS2, TERT	Th17 cell differentiation, VEGF signaling pathway, cAMP signaling pathway, Type 2 diabetes, Fat digestion and absorption, Neuroactive ligand-receptor interaction, Calcium signaling pathway, TNF signaling pathway, Toll-like receptor signaling pathway, HIF-1 signaling pathway, IL-17 signaling pathway, Ras signaling pathway, Pathways in cancer
7463	P-Cymene	ESR1, HDAC1, AR, KDM1A, GSK3B, ESR2, ALB, KIF11, IGF1R, HTR3A, HTR2C, ALOX5, SHBG, CHRM3, AHR, PPARA	Amphetamine addiction, Neuroactive ligand-receptor interaction, Calcium signaling pathway, Cocaine addiction, Alcoholism, Regulation of lipolysis in adipocytes, AGE-RAGE signaling pathway in diabetic complications, Inflammatory mediator regulation of TRP channels, c-GMP PkGsignaling pathway, NF-Kbsignaling pathway, IL-17 signaling pathway, cAMP signaling pathway
10281	Thymoquinone	GSK3B, PLK1, PARP1, PABPC1, NR3C1, MTNR1A, PTPN1, SOAT1, MTNR1B, XIAP, BRD4, PPARG, TOP2A, SYK, MAP2K1, IMPDH2, PTPN2, PGR, GLI1, MCL1, ALOX5	Neuroactive ligand-receptor interaction, Calcium signaling pathway, c-GMP PkGsignaling pathway, Alcoholism, Apoptosis, cAMP signaling pathway, lysosome, Influenza A, PI3K-AkT TNF signaling pathway, Pathways in cancer, NF-Kbsignaling pathway
10364	Carvacrol	ESR1, CDK2, HDAC1, AR, AURKA, AKT1, HDAC6, HDAC3, ADRB2, GAPDH, ESR2, ALB, MAPK8, CLK1,LDHA, PRKCA, LDHB, HDAC11, CCNA2, MAPK9, IGF1R	Neuroactive ligand-receptor interaction, MAPK signaling pathway, Calcium signaling pathway, Relaxinsignaling pathway, Pathways in cancer, Alcoholism, PI3K-AkT TNF signaling pathway, Ras signaling pathway, Th17 cell differentiation, Influenza, Regulation of lipolysis in adipocytes, Endocrine and other factor- regulated calcium reabsorption, Adrenergic signaling in cardiomyocytes, Amphetamine addiction, VEGF signaling pathway, TNF signaling pathway, Toll-like receptor signaling pathway, Apoptosis, c-GMP PkGsignaling pathway, FOXO signaling pathway, Cocaine addiction
11230	Terpinen-4-ol	MAPK8, AR, JAK1, ESR1, JAK2, PTPN6, NR3C1, MAPK10, PPARA, PPARG, PTPN1, CHRM2, HTR2C, ESR2, CHRM4, HTR2A, DRD2, OPRK1, OPRD1, PGR, OPRM1, PARP1, MTNR1A	Neuroactive ligand-receptor interaction, Calcium signaling pathway, Inflammatory mediator regulation of TRP channels, Th17 cell differentiation, Pathways in cancer, AGE-RAGE signaling pathway in diabetic complications, cAMP signaling pathway, Tuberculosis, AMPK signaling pathway, Influenza, c-GMP PkGsignaling pathway, JAK-STAT pathway, Circadian rhythm, Relaxinsignaling pathway, Apoptosis- multiple species
73296	alpha-Hederin	GRB2, ITGA4, PPP1CA, SRC, AR, AURKA, PPP2CA, STAT3, CDK1, LGALS3, PTPN1, CASP8, XIAP, NCSTN, ATP1A1, ITGB1, PRKCD, IGF1R, CASP3, LGALS8, BCL2L1, MME, PSEN1, IMPDH2, HTR2C, IMPDH1, GLI1, HLA-A, ITGAV, ITGB3, PTPRA	Neuroactive ligand-receptor interaction, Calcium signaling pathway, AGE-RAGE signaling pathway in diabetic complications, Rennin- angiotensin system, c-GMP PkGsignaling pathway, Pathways in cancer, PI3K-AkT TNF signaling pathway, Apoptosis, Autophagy, Complement and coagulation cascades, P53 signaling pathway, T-cell receptor signaling pathway, NF-Kbsignaling pathway, JAK-STAT pathway, Alcoholism, Chemokine signaling pathway, Tuberculosis, HIF-1 signaling pathway, MAPK signaling pathway
95779	Thymohydroquinone	HSP90AA1, HDAC1, AURKA, AKT1, PARP1, ERBB2, ABL1, HDAC6, CDK1, HDAC3, ADRB2, GAPDH, MTNR1A, MAPK14, ALB, PTPN1, MTNR1B, PRKCA, LDHA, NOS2, LDHB, BRAF	Neuroactive ligand-receptor interaction, Calcium signaling pathway, AGE-RAGE signaling pathway in diabetic complications, Relaxinsignaling pathway, MAPK signaling pathway, Regulation of lipolysis in adipocytes, Oxytocin , Alcoholism, mTOR signaling pathway, PI3K-AkT TNF signaling pathway, FOXO signaling pathway, Inflammatory mediator regulation of TRP channels, VEGF signaling pathway, HIF-1 signaling pathway, Pathways in cancer, Natural killer cell mediated cytotoxicity, Tuberculosis, Colorectal cancer, Influenza A, Apoptosis, Amphetamine addiction, TNF signaling pathway, Hepatocellular carcinoma
398941	Dithymoquinone	HSP90AA1, SRC, AR, PRKDC, STAT3, NR3C1, MTNR1A, GAPDH, ESR2, ATM, MAPK8, TGFBR1, PTPN11, MTNR1B, EZR, NOS2, NCSTN, BRAF, CAPN1, PSEN1, CDC25A, PGR, PTK2B, ALOX5, PSEN2, OPRM1	Neuroactive ligand-receptor interaction, Calcium signaling pathway, AGE-RAGE signaling pathway in diabetic complications, Relaxinsignaling pathway, GABAergic synapse, Fluid shear stress and atherosclerosis, Apoptosis, Antigen processing and presentation, Chemokine signaling pathway, Morphine addiction, FOXO signaling pathway, Inflammatory mediator regulation of TRP channels, Cocaine addiction, Th17 cell differentiation, IL-17 signaling pathway, Alcoholism, c-GMP PkGsignaling pathway, Tuberculosis, Amphetamine addiction, Natural killer cell mediated cytotoxicity, TNF signaling pathway

(*continued*)

Table 3. Continued.

PubChem ID	Compound Name	Hub Nodes	Pathways targeted
637563	trans-Anethole	APP, EGF, RXPO1, ESR1 LRRK2 AR AURKA, RELA, PARP1, PRKDC, TUBB3, STAT3 MAPK14 KAT2B, PRKCA, NOS2, CDK5 KIF11, MAPT, JAK1, JAK2, CDC25B, TYK2	Nitrogen metabolism, Calcium signaling pathway, Primary immunodeficiency, TNF signaling pathway, T-cell receptor signaling pathway, Proximal tubule bicarbonate reclamation, Morphine addiction, IL-17 signaling pathway, NF-Kbsignaling pathway, Tuberculosis, VEGF signaling pathway, PI3K-AkT TNF signaling pathway, JAK-STAT pathway, Cocaine addiction, HIF-1 signaling pathway, Th17 cell differentiation, Pathways in cancer, Viral carcinogenesis
11402337	Nigellicine	HDAC1, EP300, AR, PPP1CA, CSNK2A1, RPA1, MAPK1, HDAC6, HSPA1A, CSNK2A2, MAP2K1, MCL1, TP53, NR4A1, ICAM1, TERT, EPRS, SORT1, FEN1, PGR	Neuroactive ligand-receptor interaction, cAMP signaling pathway, Nitrogen metabolism, Apoptosis, Lysosome, TNF signaling pathway, NF-Kbsignaling pathway, Influenza A, Autophagy, Alcoholism, Rheumatoid arthritis, HIF-1 signaling pathway, IL-17 signaling pathway, VEGF signaling pathway, Renin- angiotensin system, FOXO signaling pathway, Transcriptional misregulation in cancer, c-GMP PkGsignaling pathway, Oxytocin signaling pathway, Viral carcinogenesis, Nitrogen metabolism
136828302	Nigellidine	ESR1, CDK2, VCP, AKT1, ERBB2, HTT, GSK3B, RAF1, PLK1, CDK1, CFTR, KIT, MTNR1A, ILK, PPARG, CCNA2, CCNB1, MTNR1B, PDGFRB, CCNE1, PRKACA, FGFR1, CDK5, PSEN1, OPRM1	Pathways in cancer, Neuroactive ligand-receptor interaction, Calcium signaling pathway, MAPK signaling pathway, Chemokine signaling pathway, c-GMP PkGsignaling pathway, Ras signaling pathway, HIF-1 signaling pathway, VEGF signaling pathway, Arachidonic acid metabolism, Autophagy, Apoptosis, FOXO signaling pathway, Tuberculosis, AGE-RAGE signaling pathway in diabetic complications, Morphine addiction, Cocaine addiction, T-cell receptor signaling pathway
1796220	Longifolene	ESR1, AR, ESR2, PTPN1, UBA2, NR1H3, SAE1, SHBG, PPARA, POLB, NR1I3, NR1H4, BCHE, AKR1B10, HSD11B1, SLC6A4, NCOA3, EP300, NCOA1, ACTB, ACHE, MED1, NCOR1	Steroid hormone mediated signaling pathway, response to oxygen- containing compound, lipid metabolic process, regulation of inflammatory response, regulation of cytokine mediated signaling pathway, regulation of innate immune process, blood circulation, regulation of insulin receptor signaling pathway, regulation of toll- like receptor signaling pathway

19 as a global pandemic and no drug or antiviral treatment has yet been formulated to combat the disease. Hence, repurposing drugs available for other diseases could be a potential treatment strategy against SARS-CoV-2 and can be processed further for COVID-19 trials (Rosa & Santos, 2020) . SARS-CoV-2 is known to affect lung alveolar epithelial cells via the angiotensin-converting enzyme II (ACE2) as an entry receptor, using receptor-mediated endocytosis. ACE2 (Angiotensin converting enzyme 2) is an important player in mediating the viral entry into the host cell. ACE2 is expressed in GI tract, endocrine tissues, kidneys, liver/gall bladder, testis and to smaller extents in lungs and is known to regulate cardiovascular functions, renal functions, and fertility. The newly conferred function for ACE2 is, it being a receptor for the S-protein *i.e.* spikes glycoprotein of all the human coronavirus such as SARS-CoV, HCoV-NL63 and SARS-CoV-2 (COVID-19 virus). The Receptor Binding Domain (RBD) of spike proteins and ACE2 receptors come in direct contact and initiate fusion with cell membrane (Kim, 2020; Robson, 2020). Since this interaction is essential for SARS-CoV-2 entry into the host cell and infection, this S-RBD–ACE2 interface can be the main target for vaccine developers (Shang et al., 2020). Also, most importantly the next proteins in the signaling pathway after attachment of S-RBD-ACE2 will decide the fate of the infection and severity in body. The information about disturbed signaling pathways in COVID-19 is of utmost importance as this can guide the route and treatment plans. It is well known that attachment of virus to the receptor protein releases signals which help the virus to replicate and spread in body. Therefore, a protein interaction network was sketched around ACE2 to decipher the pathways which will be impacted instantly when virus will attach to ACE2.

In the present study cytoscape was used to visualise and analyse the main interacting partners of ACE2. A PPI master network was created around ACE2 and they key first line and second line receptors were identified. In addition, BiNGO analysis of the hub nodes was performed to determine the Gene Ontology categories which are statistically overrepresented in a set of obtained proteins to decipher the major molecular processes affected when ACE2 binds to the human receptors and when all the genes obtained in our network map interact with each-other. The top ten obtained hub nodes were ACE2, INS, AKT1, VEGFA, IL6, ALB, ACE, AGT, Renin and KNG1. IL-6 is a pro-inflammatory cytokine and is suggested to be associated with SARS severity (Zhang et al., 2004). IL-6 is being used as a potential biomarker whose measurements seem as a reliable measure to diagnose COVID-19. IL6 is also involved in host defence against bacteria related inflammation diseases (Narazaki & Kishimoto, 2018). In diseases, the IL-6 inhibitory strategy begins with the development of the anti-IL-6 receptor antibody, tocilizumab (TCZ). Clinical trials for Intravenous Tocilizumab for severe COVID-19 pneumonia are underway. TCZ therapy is being given to prevent/treat the cytokine storms emerged as an outcome of severe COVID-9 infections due to sharp rise in IL-6 levels. In moderately and critically ill patients, repeated TCZ therapy seems beneficial (Luo et al., 2020). Cytokine-mediated signaling pathways, which also have a key role in

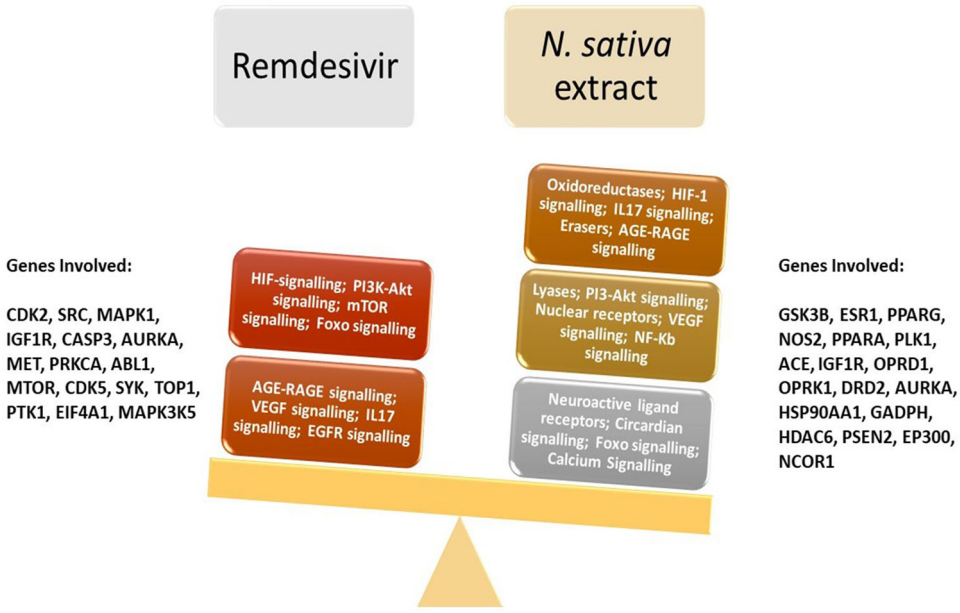

Figure 5. Comparison of Remdesivir and N. sativa extract with their genes and pathways.

Table 4. Docking analysis of *N sativa* constituents with ACE2 receptor.

Title	glide gscore	glide evdw	glide ecoul	glide energy	glide einternal	glide lipo	glide hbond
73296 (α-hederin)	−6.265	−25.96	−19.94	−50.00	0.783	−0.478	−1.487
95779 (Thymohydroquinone)	−5.466	−21.445	−7.586	−29.031	0.791	−0.417	−0.59
10281 (Thymoquinone)	−5.048	−24.979	−3.678	−28.657	0.028	−0.518	−0.32
10364 (Carvacrol)	−4.704	−20.361	−3.907	−24.268	0.064	−0.403	−0.446
11230 (Terpinen-4-ol)	−4.458	−15.737	−7.84	−23.577	0.884	−0.086	−0.16
637563 (trans-Anethole)	−4.287	−20.901	−0.824	−21.725	0.067	−1.433	0
7463 (P-Cymene)	−4.114	−19.554	−0.782	−20.336	0.044	−0.769	0
6654 (Alpha-Pinene)	−3.798	−17.04	−0.792	−17.832	0	−0.577	0

SARS-CoV-1 infection, are the most dysregulated pathways in early SARS-CoV-2 infection (Catanzaro et al., 2020). The change in cytokines' expression (i.e. IL6, IL8, IL17, CCL2) are indicative of pro-inflammation in early stages of SARS-CoV-2 infection. A moderate exposure to hypoxia combined with viral infection increases the lung VEGF levels. This increase is mediated by an elevation in the levels of Endothelin (ET) which further induces HIF1A mRNA expression. High VEGF expression allows the pulmonary vascular albumin extravasation which leads to the vascular permeability (Carpenter et al., 2005;Hicklin et al., 2005). HIF1A along with AKT1 are indicated to be upregulated by viral entry mediated by endocytosis, import/export and translation of viral mRNA which is fundamental for their multiplication (Cava et al., 2020). HIF1A activators may be used for relieving the symptoms of SARS-CoV-2 as it is responsible for the upregulation of glycolytic genes like phosphoglycerate kinase (*PGK*) and lactate dehydrogenase A (*LDHA*) under hypoxic conditions which help the tissues to adapt to oxygen deprived conditions and resort to anaerobic ATP formation (Hu et al., 2003; Lee et al., 2019). Oxidative stress induces AkT inactivation which eventually reduces HIF1A and VEGFA levels in the body causing lung impairment (Lee et al., 2019). Hence, compounds with Akt activating properties can be used to alleviate oxygen tension and improve the symptoms of SARS-CoV-2. BiNGO analysis of cytoscape generated protein networks helped to understand the role of major hub nodes in understanding the pathology of SARS-CoV-2 and hence could be used as druggable targets for the treatment of SARS-CoV-2.

To date, a lot of therapeutic benefits of *N. sativa* are known and it has been shown to be effective against a wide range of illnesses like neurologic disorders, Diabetes Mellitus, hypertension, dyslipidemia, inflammatory disorders, cancer, etc. (chronic non-infectious diseases) and bacterial, fungal, viral, and parasitic infections (infectious disease) (Yimer et al., 2019; Adamska et al., 2019). Thymoquinone (2-methyl-5-propan-2-ylcyclohexa-2, 5-diene-1, 4-dione) is a major bioactive constituent of *N. sativa*. It has anti-inflammatory effects besides providing protection against gastrointestinal problems, bronchial headache, asthma, and dysentery (Khader & Eckl, 2014). It possesses anti-inflammatory properties as it inhibits thromboxane B2 and leukotriene, the oxidative products of arachidonic acid by blocking the activity of cyclooxygenase and lipoxygenase enzymes (Majdalawieh & Fayyad, 2015; Chung et al., 2020). Therefore, controlling the overexpression of cytokines may help in managing the SARS-CoV-2 infection. In a study by Salem and Hossain (2000), *N. sativa* seed oil was found to suppress viral load caused by cytomegalovirus in mice to untraceable levels in the liver and spleen within a span of 10 days of intraperitoneal administration. This may be attributed to the increase in expression of CD-T cells and interferon-(INF-) gamma. Mu et al., 2015, reported that Thymoquinone acts as a potent chemosensitizer and apoptotic agent via downregulation of the PI3K/Akt/mTOR activation. A gradual decline in the downstream effector S6 ribosomal protein which is linked to the

chemoresistance of human malignancies to standard anticancer drugs was also reported by them (Fruman et al., 2017). As in SARS-CoV-2 infection an over expression of PI3K/Akt/mTOR signaling is reported therefore intervention of *N.sativa* may prove useful against SARS-CoV-2.

To study the effectiveness of *N.sativa* against SARS-CoV-2, protein interactions studies were carried out for receptors predicted via swiss target prediction for this plant's bioactive constituents, to understand their beneficial effect on SARS-CoV-2 in humans. Through literature search, the chemical constituents of *N. sativa* were retrieved and were allowed to undergo an ADME analysis. Through ADME studies false-positive compounds can easily be predicted and hence, can be excluded. It helps in determining the properties like absorption distribution, metabolism, excretion and toxicity of drug molecules, thereby, reduces the screening cost and also increases the rate of success of drug designing. In the current study ADME analysis was carried out as a preliminary test to find out whether the proposed drug candidate will work satisfactorily in the clinical trials or not, based on a thorough analysis (Liu et al., 2007). To find the binding receptors of the retrieved *Nigella* constituents inside the human body, Swiss target prediction tool was used. To understand which *N.sativa* constituent has the finest binding affinity (ΔG; Gibbs free energy) with the receptors present in human system molecular docking analysis was performed and also to determine the predominant binding mode(s) of a ligand with a purposed protein (ACE2). The most suitable binding ligand to ACE2 was found to be α-hederin (-6.265 kcal/mol), Thymohydroquinone (-5.466 kcal/mol) and Thymoquinone (-5.048 kcal/mol). Since ACE2 is the entry site of virus in the human system, the *N. sativa* bioactive constituents were taken further for a docking study. The receptor chosen was ACE2 (PDB ID: 1R4L). LigPrep v3.1 was used to prepare all the ligands. All the possible stereoisomers of all compounds were prepared, and their energy was minimized before docking. ACE2 was found to bind to the components- α-hederin and Thymohydroquinone with good binding energies. Hence, these 2 components of *Nigella* can be exploited therapeutically.

The chemo-proteomic analysis that emerges from this study not only highlights clinically actionable human proteins in the interactome, but also provides a context for interpreting their mechanism of action. This is the first study that has interpreted the role played by several proteins like ACE2, INS, AKT1, VEGFA, IL6, ALB, ACE, AGT, Renin, KNG1 *etc*. in the progression of SARS-CoV-2 pathology through cytoscape and BiNGO plugin. Therapeutically targeting these proteins can reveal the wide scope for designing the treatment against the disease so that to stop its progression at an early stage. Isolation of bioactive components of *N.sativa* and its oil and confirmatory clinical studies of their pharmacological effects are further recommended as the results obtained through swiss target prediction and docking studies are in favour of its use in SARS-CoV-2 pathology.

Conclusions

India is a land of Ayurveda. Indians have been consuming herbs and medicinal plants as daily routine spices in their households. As depicted in this study, *N. sativa* has the potential to be taken up as a treatment for COVID-19. This is the reason that lot of interest has been diverted by the Government of India to explore the potential of herbal drugs for the management and cure of COVID-19 in India. Currently, AYUSH (The Ministry of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy) as well as some of the pharmaceutical companies are coming forward to formulate and sell these herbal drugs and supplements. The current study is the first report and we have deciphered the mechanism of action of *N. sativa* bioactive constituents by protein interaction and docking studies as well as proven their binding efficiency with ACE2 receptor and now this can be studied further in wet lab and be formulated as the medicine to combat the deadly disease COVID-19.

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Disclosure statement

There is no conflict of interests to declare.

Author contributions

D.P.K and R.J.M designed the concept of study. R.J.M performed computational analysis and D.P.K, R.J.M and N.S analysed the data. R.J.M, N.S, N.D and S.S wrote the manuscript. D.P.K and R.J.M reviewed and finalized the manuscript.

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