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Novel Coronavirus-2019 (2019-nCoV): Perspectives of emergence, prophylaxis and predicted treatment approaches

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Abstract: Emergence of novel coronavirus-2019 has become an international health concern. The objective of this review is to focus on 2019-nCoV emergence, prophylaxis and to predict the treatment approaches. The first case of 2019-nCoV was noted in Wuhan, China. The WHO has announced this epidemic as pandemic. The 2019-nCoV has +ve ssRNA (29903bp), lipid-bilayer envelope spiked with glycoprotein and bears genome sequences similar to bat coronavirus RaTG13. Antiviral agents like Interferon, Darunavir, Ribavirin, Lopinavir, Remdesivir, Chloroquine and Camostat mesylate may be considered for clinical trials. Chinese herbals may be effective against 2019-nCoV. These include Saikosaponins (triterpene glycosides), Amentoflavone, Scutellarein, Myricetin, extracts of *Isatis indigotica*, and *Houttuynia cordata*. Another treatment approach is to administer plasma from COVID-19 recovered patients. RNA vaccines, recombinant vector based vaccine and ACE-2 receptor like molecules may be employed for immunization against COVID-19. Moreover, immunity can be boosted against 2019-nCoV by regular exercise. We have checked Thymoquinone as ligand for various targets of 2019-nCoV (receptor binding domain of spike, RNA polymerase, protease, Nsp9 RNA binding protein, nucleocapsid phosphoprotein, endoribonuclease) by protein-ligand docking server SwissDoc. Thymoquinone can bind effectively to the targets of 2019-nCoV. Hence, it may be an effective candidate for the treatment of COVID-19.

Keywords: Emergence of 2019-nCoV, SARS coronavirus-2, 2019-nCoV, COVID-19, anti-coronavirus agents, Thymoquinone

INTRODUCTION

Coronaviruses are famous for their crown-looked spikes. They are single stranded, plus-sense RNA viruses that exist in family Coronaviridae, sub-family Coronavirinae, and order Nidovirales. They are further classified into four major genera; alphacoronavirus, betacoronavirus, gammacoronavirus and deltacoronavirus (Rabi *et al*., 2020). However, betacoronaviruses are responsible for infections in mammals and produce respiratory and gut-associated complications (Zhou *et al*., 2018; Cui *et al*., 2019). Just before the end of 2019, there were only six known human coronaviruses. Only four of these viruses (HKU1, HCoV-NL63, HCoV-OC43 and HCoV-229E) were responsible for less severe cold-like manifestations in humans, while remaining two were the main sources of viral pandemics and epidemics in last 20 years. The earliest, severe acute respiratory syndrome coronavirus-1 (SARS-CoV-1) was the outbreak agent in Guandong Province of China (during 2002 and 2003) and was responsible for 10% fatalities (Zhong *et al*., 2003). A zoonotic viral strain, the Middle-East respiratory syndrome coronavirus (MERS-CoV) was the originator of disastrous pandemic with 37% fatality ratio in Middle East during 2012 (Zaki *et al*., 2012; Cui *et al*., 2019; Rabi *et al*., 2020).

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Origin and emergence of SARS Coronavirus-2 (SARS-Cov-2)

Evolving and re-evolving pathogens (viruses etc.) are the international threat for everyone health (Weiss and Leibowitz, 2011; Gao, 2018). One of them is novel SARS-coronavirus-2 (SARS-CoV-2) nowadays. SARS-CoV-2 has lipid-bilayer envelope spiked with glycoprotein, plus-sense, single stranded RNA genome (29903bp). It causes severe acute respiratory syndrome hence, known as or SARS-CoV-2 or 2019-nCoV. Entire generations of coronaviruses (known so far) associated with human ailments have originated from animals, mainly from rodents and bats (Fan *et al*., 2019). MERS-CoV and SARS-CoV-1 were contracted precisely by man from domestic camels and cats respectively. Spikes of SARS-coronaviruses have a variable or diversified receptor binding domain (RBD) for the attachment with angiotensin-converting enzyme-2 (ACE-2) receptor, located on the cells of heart, GI-tract, kidneys and lungs (Ksiazek *et al*., 2003). Genome sequences revealed that RBD of 2019-nCoV is closely related to the sequences of bat coronavirus RaTG13. Thus, the origin of 2019-nCoV is from bat. After getting mutations in bats, it became able to invade other animals. Such mutations have enhanced the affinity of virus RBD not only with men's ACE-2 receptor but also with that of Pangolins and Ferrets. Therefore, Pangolins are considered as intermediate host of 2019-nCoV. Interestingly, after mutations this virus

has lost its RBD affinity with ACE-2 receptor of civets and rodents (Cyranoski, 2020).

The first case of this viral strain was reported in Wuhan City of China, in the end of December, 2019, thence, often called Wuhan coronavirus. The genomic sequence of this *de novo* virus is 79% analogous to SARS-CoV-1 (Zhou *et al*., 2020). This time to time re-emergence and extended dissemination of coronaviruses is due to their broad genetic varieties, recurrent recombination of genetic material, error prone replication system, human-animal linked activities and periodic cross-species infections (Wong *et al*., 2015; Cui *et al*., 2019). In November and December, 2019, many local hospitals of China (Wuhan) observed the groups of patient having pneumonia of strange etiologic agent and were handled or treated according to influenza treatment criteria. These patients were connected with seafood market in Wuhan, Hubei Province, China (Zhu *et al*., 2020). According to Chinese Centre for Disease Control and Prevention (China CDC) investigation; etiological agent was the novel coronavirus-2, patients showing fever ranging 37-38 °C, cough with chest pain, and respiratory distress. However, none of the patients were positive for human specific coronaviruses (HCoV-NL63, HCoV-229E, HCoV-HKU1, and HCoV-OC43). Bronchoalveolar-lavage (BAL) viral RNA extract sequencing (Combo of Nanopore and Illumina) matched to the genome of betacoronavirus and displayed 85 percent similarity with SARS-related coronavirus of Bat (bat-SL-CoVZC45, MG772933.1). The novel coronavirus was inoculated on human epithelial cells, Huh-7 and Vero E6 cell lines and was entitled as 2019-nCoV. After 96 hours, cytopathic effect (inhibition of cilium beating) in electron microscope was noticed in human epithelial cells but not in Huh-7 and E6 cell lines even after 6 days. After the recognition of 2019-nCoV, the disease was nominated as coronavirus disease 2019 (COVID-19) (Chen *et al*., 2020; Huang *et al*., 2020; Lu *et al*., 2020; Tan *et al*., 2020; Zhu *et al*., 2020). Negatively stained electron micrograph of 2019-nCoV has revealed spherical, pleomorphic particles of 60-140 nm diameter, and absolutely unique spikes of 9-12 nm. The genome of 2019-nCoV consists of; a 3′ untranslated region (UTR), replicase (orf1ab), S, E M and N genes, 5′ untranslated region (UTR), and various anonymous open reading frames (non-structural). It belongs to the family coronaviridae, genus betacoronavirus, and subgenus sarbecovirus (Zhu *et al*., 2020). The genome sequence of 2019-nCoV (GenBank accession number MN985325) of USA differs in three nucleotides and one amino acid of open reading frame 8 from Wuhan 2019-nCoV (NC_045512.2) (Holshue *et al*., 2020). The epidemic of 2019-nCoV (COVID-19) has disseminated very quickly and unrestrained from Wuhan, not only to other cities of China but also to a number of countries or almost the entire world. In this scenario, World Health Organization (WHO) has proclaimed this epidemic as global public health emergency (on 30th January, 2020) and as pandemic (on 11th March, 2020) (Rabi *et al*., 2020; WHO, 2020). Therefore, there is a need to find out therapeutic and prophylactic agents and approaches to treat and control this threat.

Treatment and prophylactic options

The present favorite treatment approach for the patients of COVID-19 is totally supportive. The healthcare specialists suggest admitting the COVID-19 patients in isolated intensive care units and recommend pursuing the infection control protocols and practices to reduce the COIVD-19 spread (Wax and Christian, 2020).

Antiviral candidates for 2019-nCoV

There are various agents and approaches to be investigated for accurate treatment of COVID-19. The most effective way to treat COVID-19 is to examine the current active antiviral agents. Certain antiviral agents (Interferon, Darunavir, Ribavirin and Lopinavir) were analyzed and found effective in previous outbreaks of SARS and MERS coronaviruses (Chu, 2004; Falzarano *et al*., 2013). Similarly, in an in vitro study, analogue of adenosine (Remdesivir) was efficiently active against MERS-CoV, SARS-CoV-1, SARS-CoV-2 and other RNA viruses (Mulangu *et al*., 2019; Wang *et al*., 2020a). Fortunately, progress in recovery of COVID-19 patients was witnessed after the administration of Remdesivir. However, further trials regarding the effectiveness of Remdesivir are in progress (Holshue *et al*., 2020). Chloroquine (anti-malarial drug) contributes in hindering the interface between RBD of SARS-CoV and ACE-2 receptor by inhibiting their glycosylation. Thereby it prevents the virus attachment to host cell surface (Vincent *et al*., 2005). An in vitro investigation has witnessed the effectiveness of Chloroquine in inhibiting the 2019-nCoV entrance in host cell (Wang *et al*., 2020b). Camostat mesylate/(FOY 305) is prescribed to treat chronic pancreatitis. It binds with and inactivates type 2 trans-membrane protease enzyme (TMPRSS2) responsible for the processing (cleavage of ACE-2 and activation of 2019-nCoV spike) of ACE-2-RBD complex necessary for the viral entrance. Therefore, Camostat mesylate also impedes the 2019-nCoV entry (Zhou *et al*., 2015; Ramsey *et al*., 2019; Yamawaki *et al*., 2019). Modes of action of various antiviral agents are depicted in table 1.

It has been disclosed by the researchers that a Chinese herbal like Saikosaponins (triterpene glycosides) plants extract counter the HCoV-22E9 adsorption and entrance in host cell (Cheng *et al*., 2006). Moreover, the extracts of *Lindera aggregate*, *Artemisia annua*, *Lycoris radiata* and *Pyrrosia lingua* have also shown adverse effects on SARS-coronavirus (Li *et al*., 2005). Phenolic compounds of *Torreya nucifera* and *Isatis indigotica*, Scutellarein and Myricetin have been reported as essential inhibitors of SARS-coronaviral protease (3CL) and helicase (nsP13).

Table 1: Antiviral modes of action of natural and synthetic compounds or agents against SARS-CoV, HCoV-22E9

Name of agents	Mechanisms of action	References
Interferon, Darunavir, Ribavirin and Lopinavir	Block viral replication and protein synthesis.	Chu, 2004; Falzarano *et al*., 2013
Remdesivir	Analogue of Adenosine base and blocks transcription and replication.	Tchesnokov *et al*., 2019
Chloroquine (conventionally anti-malarial)	Inhibits the entrance and attachment of virus to host cell surface.	Vincent *et al*., 2005
Camostat mesylate/(FOY 305)	Blocks viral entrance by targeting enzyme type 2 trans-membrane protease (TMPRSS2)	Hoffmann *et al*., 2020
Phenol containing extracts of *Isatis indigotica*	Inhibition of protease (3CL)	Lin *et al*., 2005
Amentoflavone extracted component of *Torreya nucifera*	Inhibition of protease (3CL)	Ryu *et al*., 2010
Saikosaponins (A-D)	Blocks the adsorption and entrance of virus.	Cheng *et al*., 2006
Scutellarein	Inhibition of helicase enzyme	Yu *et al*., 2012
Myricetin	Inhibition of helicase enzyme	Yu *et al*., 2012
Aqua extracts of *Houttuynia cordata*	Inhibition of polymerase and protease (3CL) enzymes	Lau *et al*., 2008
Thymoquinone from oil of *Nigella sativa*	Antioxidation, inhibition of apoptosis, raised levels of interferon (gamma) and CD4+ cells	Forouzanfar *et al*., 2014

Amentoflavone (extracted component of *Torreya nucifera*) can hinder the protease (3CL) only (Lin *et al*., 2005; Ryu *et al*., 2010; Yu *et al*., 2012). Similarly, the extract of *Houttuynia cordata* exerts inhibitory effects on RNA polymerase and protease (3CL) of SARS-CoV (Lau *et al*., 2008; Lin *et al*., 2014). These compounds could be tested against 2019-nCoV. Antioxidant and antiviral activities are interconnected with each other because, virus induced apoptosis (programmed cell death) may cause deficiency of lymphoid cells while antioxidants can prevent viral related programmed cell death by blocking the virus reproduction (Peterhans, 1997). Component (Thymoquinone) in the oil of *Nigella sativa* is responsible for increased gamma interferon and CD4+ cells level and decreases the count and activity of macrophages in control mice (table 1) (Forouzanfar *et al*., 2014).

Passive Immunization and vaccines

Another competent treatment approach is to employ plasma from COVID-19 recovered patients. Such individuals have particular antibodies against the virus which can neutralize the 2019-nCoV upon administration in a COVID-19 suffering humans. Aforementioned approach has been employed against various viral infections e.g. Ebola virus infection etc. Still this way of treatment has limitation in COVID-19 as the number of infected individuals is rising rapidly than the number of recovered one as the requirement of plasma is higher than its availability (Kraft *et al*., 2015; Walker and Burton, 2018; Rabi *et al*., 2020). Initially, this procedure can be applied as the use of ACE-2 receptor specific antibodies which will inhibit the binding of virus. Target specific monoclonal antibodies could be considered for better cure (Wong *et al*., 2004; Elshabrawy *et al*., 2012; Arbabi-Ghahroudi, 2017). The antibody dependent therapy would be effective only before the beginning of the infection hence could be applicable within specified time duration and it accounts for the major disadvantage of this strategy (Kuba *et al*., 2005). Furthermore, after-effects of ACE-2 receptor inhibition should be taken into consideration as these are the functional counterparts of lungs cells.

The world is looking at the marvelous vaccine for mass immunization against COVID-19. Information regarding the spike proteins of 2019-nCoV may be helpful in rapid improvement in the special vaccine development (Wrapp *et al*., 2020). RNA vaccines technology is capable of inducing strong immunity against certain infections and malignancies (Sahin *et al*., 2014). The conventional vaccines provoke the development of antibodies through the introduction of purified proteins from infectious agents or by employing entire pathogen (attenuated/live vaccine). To be most useful, production of a novel vaccine may be of several years work. In contrast, RNA vaccines employs messenger RNA transcript that introduces in cells and is translated in to antigenic protein, which induces immune response. This strategy can effectively eradicate COVID-19 and cancers. Several experiments are in progress for various other cancers. Moreover, synthesis of RNA vaccine in lab or industry is very quick and less costly than conventional vaccines. This property batter fits for the current requirement of COVID-19 pandemic (Diken *et al*., 2017). Recombinant vector based vaccine may be constructed and utilized to cope with COVID-19. In 2005, similar concept was employed to control SARS-CoV-1 outbreak. Accordingly,

rabies virus based vectors were designed by using spike and nucleocapsid genes of SARS-CoV-1. These genes were genetically engineered between rabies virus glycoprotein and polymerase genes. In this approach, cDNA of both viruses (SARS-CoV-1 and rabies virus) genes were constructed by polymerase chain reaction (PCR) and were cloned in a plasmid vector (pSPBNGA) (McGettigan *et al*., 2001). Study indicated that rabies virus based recombinant vaccines were capable of producing the antigenic proteins of SARS-CoV that successfully stimulated the relevant neutralizing antibodies (Faber *et al*., 2005). Another efficient technique is to design molecules mimicking ACE-2 receptor that could attach itself to 2019-nCoV RBD. Previously, similar investigation that used ACE-2 like soluble proteins has been conducted and found to hinder adequately viral infectivity (Moore *et al*., 2004). This may provide extra advantage in reducing the chances of pulmonary edema of COVID-19 (Reilly *et al*., 2019). However, animal and human based research trials are needed (Maxmen, 2020).

Effects of physical exercise

The immunity is much sensible to physical exercise (Nieman and Wentz, 2019). Exercise spells of <60 min provide strong protection and curative benefits (Adams *et al*., 2011). In this phase, enhanced anti-infectious agent activity of phagocytes takes place in compliance with improved circulation of antibodies, natural killer cells, naive B-cells, cytokines, cytotoxic cells and neutrophils (Bigley *et al*., 2014; Simpson *et al*., 2017). Inflammation of respiratory tract is originated by >200 viruses (coronaviruses and rhinoviruses) (Fendrick *et al*., 2003). Researchers found that duration and symptoms of upper respiratory tract infections (URTIs) was more curtailed in the group 1 (doing aerobic exercise regularly) than the group 2 (sedentary/without exercise) (Nieman *et al*., 2011). Another controlled mice-based study indicated the increase in survival rate of mice after the exposure to H1N1 (Martin *et al*., 2009). This strategy of moderate daily exercise may be beneficial in preventing and reducing the cases of COVID-19.

Predicted ligand (Thymoquinone) for various targets of 2019-nCoV

In this review we have checked the affinity of Thymoquinone (listed in table 1) as ligand for spike, RNA-binding protein, protease, endoribonuclease and phosphoprotein of SARS-coronavirus-2, using SwissDoc (an online server for molecular docking). The three dimensional structure of selected ligand (Thymoquinone) is shown in fig. 1 and is available at (https://pubchem.ncbi.nlm.nih.gov/compound/10281#section=3D-Conformer). The molecular formula for Thymoquinone (compound CID: 10281) is $C_{10}H_{12}O_2$ and molecular weight is 164.2 g/mol.

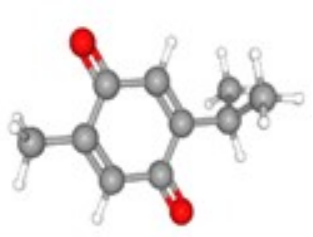
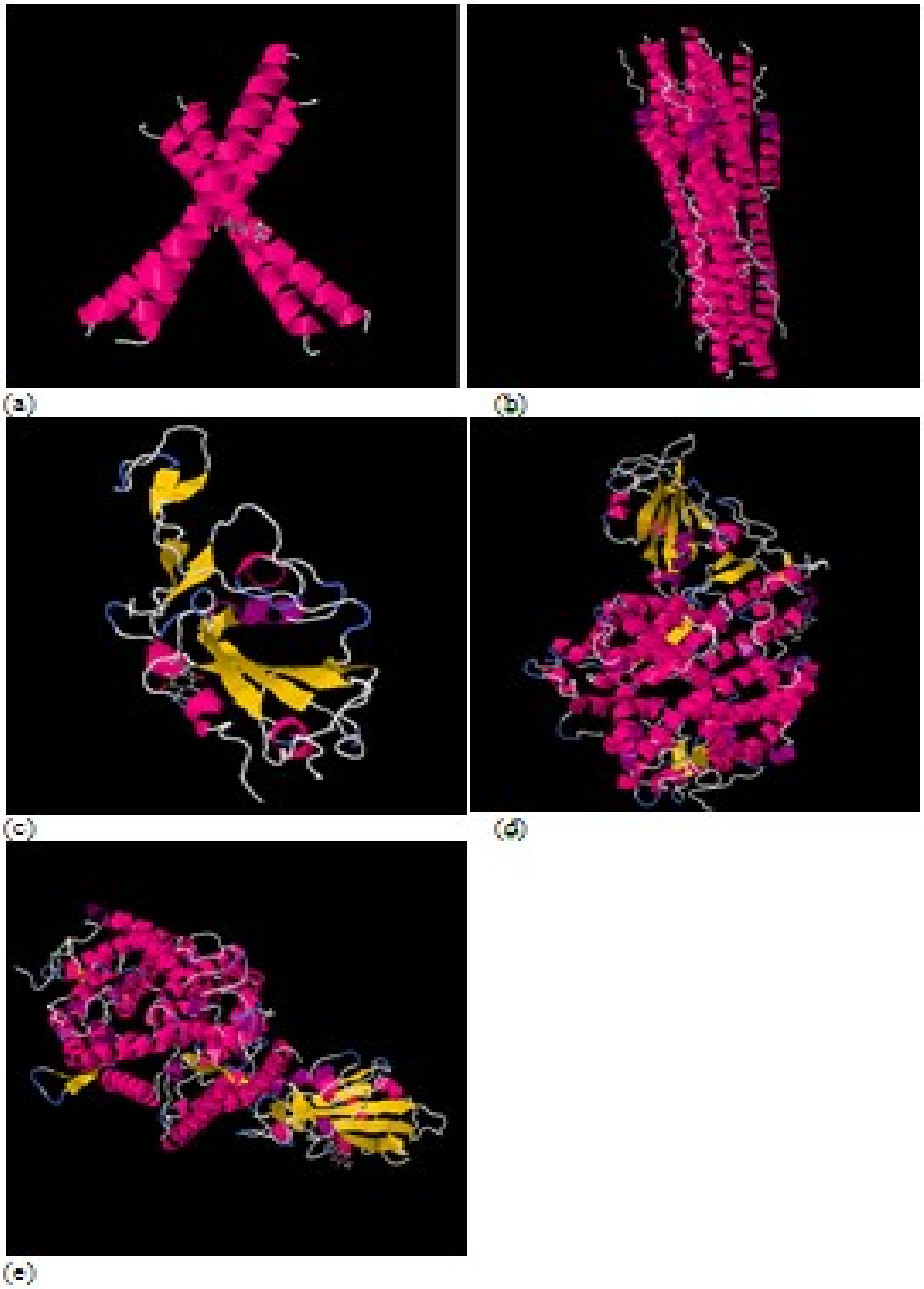


Fig. 1: Three dimensional (3D) structure of Thymoquinone

Protein sequence files were recruited from protein data bank and were run for protein-ligand binding models in Swiss Doc. Various clusters were generated by Swiss Doc but cluster 0 was the best for all the binding modes. Fixed parameters like passive flexibility distance = 0, clustering radius = 2.0, and max cluster size = 8 were selected for all binding models. Picture format is JS mol. We have noted the strong binding affinity of Thymoquinone (binding models) with the receptor binding domain of 2019-nCoV spike by using SwissDoc (fig. 2). The binding mode of Thymoquinone with the domain (HR2) of spike S2 subunit (PDB Id: 6VLN) showed -6.05 kcal/mol estimated free energy (ΔG) and -1420.59 kcal/mol for full fitness of the model (fig. 2a). The binding model of Thymoquinone with the S2 subunit core (PDB Id: 6LXT) (Xia *et al*., 2020) of spike after its fusion with the receptor was resulted with -6.41 kcal/mol estimated free energy (ΔG) and -5323.32 kcal/mol for full fitness of the model (fig. 2b). The binding mode of Thymoquinone with the unbound receptor-binding spike domain (PDB Id: 6LZG) (Wang *et al*., 2020b) displayed -6.10 kcal/mol estimated free energy (ΔG) and -785.36 kcal/mol for full fitness of the model (fig 2c). The binding model of Thymoquinone with the receptor ACE2 when receptor was engaged with the spike (PDB Id: 6LZG) (Wang *et al*., 2020b) offered -6.38 kcal/mol estimated free energy (ΔG) and -3431.17 for full fitness of the model (fig 2d). The binding design of Thymoquinone with receptor binding spike domain when spike was hooked with ACE-2 receptor (PDB Id: 6M0J) (Lan *et al*., 2020) exhibited -6.12 kcal/mol estimated free energy (ΔG) and -3347.19 for full fitness of the model (fig. 2e).

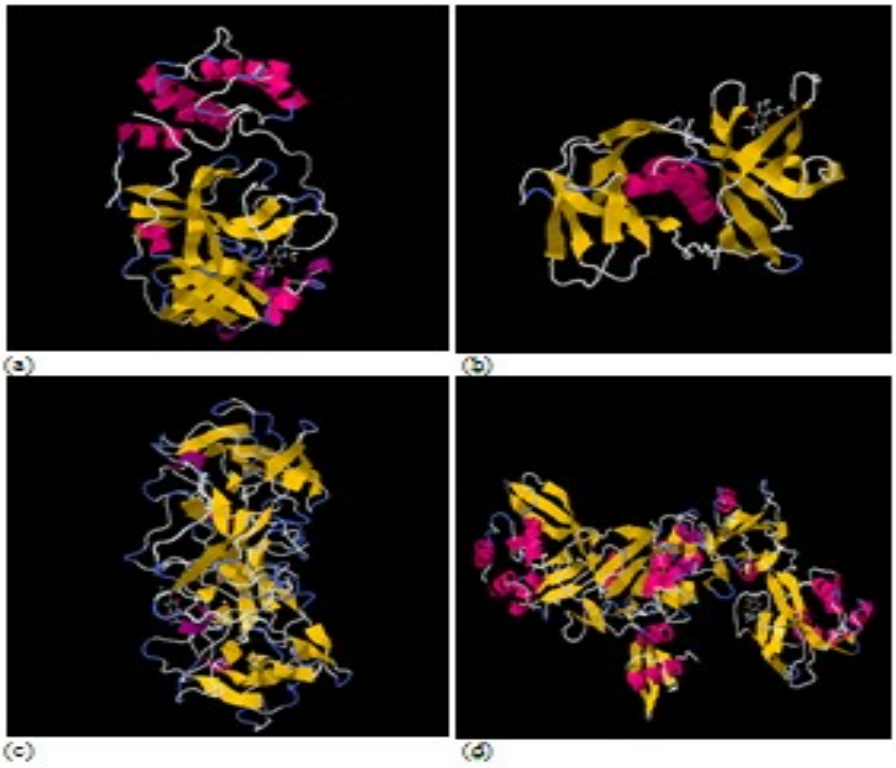
In addition to targeting ACE-2 receptor binding domain of 2019-nCoV spike, Thymoquinone was also capable to bind strongly or target main protease (PDB Id: 6Y2E) (Zhang *et al*., 2020), Nsp9 RNA binding protein (PDB Id: 6W4B), RNA binding domain nucleocapsid phosphoprotein (PDB Id: 6VYO) and endoribonuclease Nsp15 (PDB Id: 6VWW) of 2019-nCoV (Kim *et al*., 2020). In SwissDoc determined binding model of Thymoquinone (fig. 3a) with protease, results indicated -6.56 kcal/mol estimated free energy (ΔG) and -1169.78 kcal/mol for full fitness of the model. Binding mode of Thymoquinone with Nsp9 RNA binding domain (fig. 3b) indicated the -6.29 kcal/mol estimated free energy (ΔG)



Keys: (a) Thymoquinone attached with domain (HR2) of spike (S2 subunit), (b) linking of Thymoquinone after fusion of S2 subunit with cell receptor, (c) Thymoquinone bound with receptor-binding spike domain (unbound with receptor), (d) Attachment of Thymoquinone with the receptor ACE2 while receptor complexed with spike (e) Thymoquinone bonded with receptor binding spike domain while spike intricate with receptor ACE-2

Fig. 2: Binding modes of Thymoquinone (small ring structure in grey color) with the spike of 2019-nCoV

level and -1290.68 kcal/mol for full fitness of the model. Binding model of Thymoquinone with nucleocapsid phosphoprotein RNA binding domain revealed -6.53 kcal/mol estimated free energy (ΔG) level and -2696.80 kcal/mol for full fitness of the model (fig. 3c). The final binding model of Thymoquinone with endoribonuclease Nsp15 disclosed the -6.38 kcal/mol estimated free energy (ΔG) level and -3764.58 kcal/mol for full fitness of the model (fig. 3d). The molecular docking based research has indicated that the low free energy (ΔG) (in minus) could make spontaneous and stable binding of ligand with protein (Xing *et al*., 2016; Malau and Azzahra, 2020). So that, the above results suggest Thymoquinone as a candidate for blocking or inhibiting receptor binding domain of spike, protease, Nsp9 RNA binding protein, nucleocapsid phosphoprotein RNA binding domain, and endoribonuclease Nsp15 of 2019-nCoV.



Keys: (a) Thymoquinone bound with main protease of 2019-nCoV, (b) Thymoquinone attached with Nsp9 RNA binding protein, (c) Binding of Thymoquinone with nucleocapsid phosphoprotein RNA binding domain, (d) Attachment of Thymoquinone with Endoribonuclease Nsp15

Fig. 3: Binding models of Thymoquinone with protease, Nsp9 RNA binding protein, nucleocapsid phosphoprotein and endoribonuclease of 2019-nCoV

CONCLUSION

Review of the literate has indicated that human coronaviruses have emerged from animals and precisely, on the basis of genomic similarity 2019-nCoV has originated from bat. The earliest case of 2019-nCoV was emerged in Wuhan, China. Antiviral agents like Interferon, Darunavir, Ribavirin, Lopinavir, Remdesivir, Chloroquine and Camostat mesylate/(FOY 305) could be the treatment options. Extracted components of Chinese herbs like Amentoflavone, Saikosaponins (A-D), Scutellarein, Myricetin etc. may be effective against 2019-nCoV. Administration of plasma (passive immunization), RNA and recombinant vaccines, ACE-2 receptor analogous molecules and daily exercise may prevent and exclude COVID-19. Thymoquinone, an active component of *Nigella sativa* (black seeds) was found as a potent ligand with strong binding for various targets of 2019-nCoV. According to Swiss Doc, a ligand versus target protein docking server, Thymoquinone attaches itself effectively and spontaneously (without requiring energy/with minus energy) to various domains of 2019-nCoV spike, ACE-2 receptor convoluted with spike, protease, Nsp9, nucleocapsid phosphoprotein and endoribonuclease Nsp15 of 2019-nCoV. Such interaction of Thymoquinone with 2019-nCoV targets may block the viral attachment to ACE-2 receptor, release and replication or transcription machineries. Therefore, it may be a competent candidate for the cure of COVID-19.

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