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Chapter 16

Antiviral effects of black seeds: Effect on COVID-19

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Natural products as an important source of drug discovery

Natural products have been a rich source of drug discovery for humankind since the beginning of time. Although the exploration of natural products for new drugs has declined over the past few decades, in part, due to technical challenges in screening these products with high throughput assays, the recent technological advancement and the ever expanding list of functional assays and genetic screens have driven a resurgence in the use of natural products for drug discovery (Harvey et al., 2015). Due to their record safety and structural diversity relative to their synthetic counterparts, natural products remain a credible source of drug discovery against various conditions, including cancer, osteoporosis, neurodegenerative disorders, viral infections, among others. Due to the devastating health and economic impact of the coronavirus disease (COVID-19) pandemic and the dire need to identify efficient and effective antivirals for the treatment/management of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, we discuss the potential of *Nigella sativa* prophetic plant as a treatment strategy against COVID-19 and other pathogenic human/animal viruses.

Nigella sativa and its medicinal properties

N. sativa is an annual flowering plant belonging to the family Ranunculaceae. It is native to northern regions of Africa, Southwest Asia, and southern parts of Europe. *N. sativa* possesses a rich religious and medicinal backgrounds, and it is

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known by different names, including black seed, black cumin, kalonji, Hak Jung Chou, and many more (Majdalawieh et al., 2017). The plant has been widely used in traditional and folk medicine across the globe to treat various conditions. For example, in traditional Chinese and folk medicine, *N. sativa* is part of a plant formulation prepared to treat headache and make spleen tonic for kidney yin deficiency. The kidney yin, also called the primordial yin, is responsible for nourishing other organs, thereby maintaining their proper physiological functioning (Cai, 2019) (Wang, 2016).

Similarly, in the other parts of the world, the seeds of *N. sativa* are used to treat chronic migraine and headache, and as diuretics, diaphoretics, and tonics for liver and digestive functions. In Southeast and Middle East Asia, the plant has been traditionally used for centuries to treat inflammatory diseases like asthma, bronchitis, and rheumatism. In the Arabic folklore medicine, where the seeds of *N. sativa* are also referred to as the "seed of blessing," an extract prepared from the seeds of this plant is used to treat diarrhea, indigestion, appetite loss (stomachic), rheumatoid arthritis, edema, dysmenorrhea, and amenorrhea, and consumption of roasted seeds of *N. sativa is* believed to stop vomiting. Moreover, oil derived from *N. sativa* seeds is used as an antiseptic and for treating alopecia, eczema, freckles (Usmanghani et al., 1997), leprosy (Kapoor, 1990), and various skin diseases (Kinghorn, 2001). Thus, it appears that almost every part of *N. sativa* possesses numerous medicinal benefits, making the plant an excellent source of bioactives for therapeutic interventions against various conditions, including viral diseases.

Besides its medical properties, *N. sativa also* possesses numerous pharmacological and biological activities (Fig. 16.1) and has been used for nutraceutical purposes, a pharmaceutical alternative, similar to dietary supplements that claim to have physiological benefits that could protect against chronic diseases. In addition to its well-described antioxidant activity, *N. sativa* seed extracts display robust antiinflammatory activities by inhibiting the expression levels of the proinflammatory cytokines, interleukin 1 beta (IL-1β), interleukin 6 (IL-6)

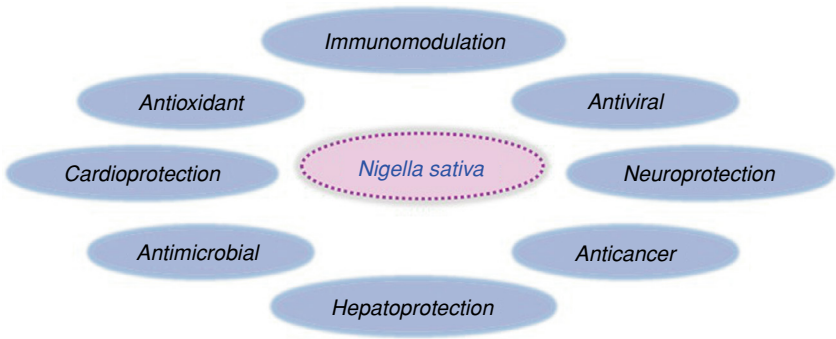


FIG. 16.1 Biological activities of *Nigella sativa.*

(Bordoni et al., 2019). Also, consumption of 500 μL of *N. sativa* daily for 30 days, ameliorated mucosal congestion, sneezing, and hypertrophy in patients with allergic arthritis (Nikakhlagh et al., 2011).

N. sativa and its constituents have also been documented to possess hepatoprotective and nephroprotective effects, mainly by suppressing oxidative stress and lipid peroxidation (Kanter et al., 2005; Ragheb et al., 2009; Yaman and Balikci, 2010). Besides, its primary compound, thymoquinone (TQ), exhibits antiproliferative and proapoptotic activities. *In vivo* studies found that TQ induces high expression of p53 and low expression of Bcl-2, resulting in apoptosis and cell cycle arrest (Ng et al., 2011). Consistent with the TQ-mediated induction of apoptosis and cell cycle arrest, *N. sativa* was reported to exert its anticancer activity by modulating the antioxidant, p53, and caspase pathways (Majdalawieh and Fayyad, 2016; Randhawa and Alghamdi, 2011).

N. sativa also exhibits cardioprotective effects and was reported to protect the cardiac myoblast cells, H9c2 cells against the toxicity induced by doxorubicin (Hosseini et al., 2014). The seed oil displayed a protective effect against lead-induced cardiotoxicity in Wistar albino rats by suppressing cardiac tissue damage, oxidative stress, and the induction of proinflammatory cytokines (Ahmed and Hassanein, 2013). Neuroprotective effects of *N. sativa* have also been reported. A study done on human volunteers showed that intake of 500 mg of the plant seeds twice a day resulted in a better attention span and enhanced memory and cognitive abilities (Bin Sayeed et al., 2013). Moreover, TQ was also reported to exhibit neuroprotective effect in 6-hydroxydopamine (6-OHDA)-induced Hemi-Parkinson rat models by suppressing oxidative stress and lipid peroxidation, and enhancing the midbrain superoxide dismutase (SOD) activity (Babazadeh et al., 2012; Sedaghat et al., 2014).

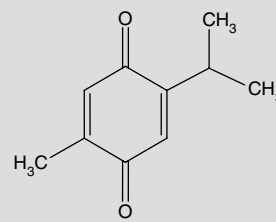
Immunomodulation is also one of the essential bioactivities displayed by this plant. A previous clinical study showed that the oil of *N. sativa* significantly ameliorated the swollen joints in the patients with rheumatoid arthritis, reduced the serum C-reactive proteins and $CD8^+$ cells, and increased the $CD4^+CD25^+$ T cell percentage (Kheirouri et al., 2016). Besides, *N. sativa* and its major compounds also modulate the cellular and humoral immune responses as indicated, among others, by their effects on allergies (antihistaminic impacts) and ability to induce seroreversion in HIV-infected patients (Gholamnezhad et al., 2015; Majdalawieh and Fayyad, 2015; Onifade, A.A. et al., 2013). Together, these findings pinpoint the *N. sativa* plant as a rich source of bioactives to treat various human ailments. Fig. 16.1.

Chemical constituents

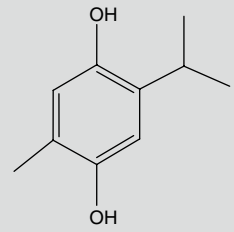
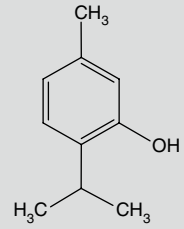
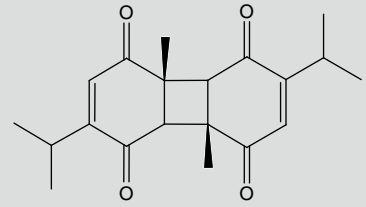
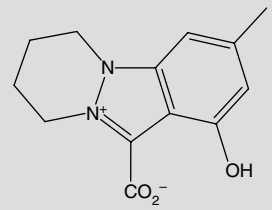
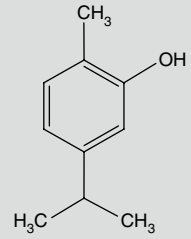
Chemical studies performed on *N. sativa* showed that the seeds approximately contain 39% fixed oils, which, in turn, contains volatile oils (0.7% approx.), with TQ constituting 19% to 24% of these volatile oils. TQ displays significant pharmaceutical potential against asthma, diabetes, cancer, and inflammation

(Goyal et al., 2017; Gupta et al., 2016), in addition to its antiviral and antimalarial effects (Fröhlich et al., 2018). Thymohydroquinone (THQ) is a TQ derivative and another major active compound of the plant, which exhibited antitumor activities *in vitro* and *in vivo* (Ivankovic et al., 2006). THQ also possesses antimicrobial and antioxidant activities (Fröhlich et al., 2018). Dithymoquinone (DTQ), also known as nigellone, is another derivative of TQ and is an inhibitor of 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF). CMPF is a uremic toxic that is commonly found in the human serum albumin (HSA) of renal failure patients, which damages the renal cells and is a marker for chronic renal failure. Given the technical challenges associated with removing CMPF using conventional hemodialysis, Faiza et al. hypothesized that an inhibitor that competes with CMPF to bind to HAS might facilitate its removal. Through molecular docking analysis, the authors demonstrated that DTQ had a higher binding affinity to HSA than CMPF, suggesting that it can occupy the docking pocket and inhibit the binding of CMPF (Faiza et al., 2017). Thymol improves digestion by relaxing the muscles, preventing menstrual cramps, acting as a vasorelaxant, and attenuating respiratory ailments (Nagoor Meeran et al., 2017). It is a component usually found in essential oils that is used for aromatic inhalation mixtures for the cold and nasal congestion relief. Thymol was also reported to be widely used for treating helminth infections. It is also used in antiseptic mouthwashes for the treatment of oral ulcers (Tisserand and Young, 2014a, b). Carvacrol is a structural isomer of thymol and a constituent of *N. sativa* that is abundant in a wide variety of herbs. It possesses antitumor, antiinflammatory, angiogenic, antiparasitic, analgesic, and antimicrobial activities (Baser, 2008), and it is also highly beneficial for animals and poultry health (Alagawany et al., 2015). Both thymol and carvacrol exert their antimicrobial activity by disintegrating the outer membrane, thereby damaging the cell membrane. Carvacol also increases the fluidity of the membrane, making it more permeable (Jafri et al., 2019). Besides these compounds, other compounds commonly isolated from *N. sativa* include nigellicine, nigellidine, nigellidine-4-O-sulfite, α-hederin, campestrol, carvone, taraxerol, and many more (Islam et al., 2017; Islam et al., 2019; Wajs et al., 2008). Table 16.1.

TABLE 16.1 Structures of some bioactive compounds of *Nigella sativa*.

Compound from *Nigella sativa*	Structure
Thymoquinone	

(continued)

Compound from *Nigella sativa*	Structure
Thymohyroquinone	
Thymol	
Dithymoquinone	
Nigellicine	
Cavacrol	

Viruses as important human pathogens

Viruses are obligate intracellular pathogens that impose a severe threat to global public health and socioeconomic development. Currently, several critical human viruses are without a prophylactic vaccine or effective antiviral treatment. The current ongoing unprecedented SARS-CoV-2 pandemic (aka COVID-19), which as of August 19, 2020, infected 22,308,044 people, killing 784,365 people globally, encapsulates the urgent need to continuously design/develop novel therapeutic strategies against these infectious agents. Plant-derived compounds have been a mainstay for drug discovery, with over 50% of all FDA-approved drugs in current use being derived from plants (Veeresham, 2012). *N. sativa* is an annual flowering plant that has been documented to possess numerous therapeutic effects against several conditions, including hypertension, diabetes, amenorrhea, and a host of other medical conditions (Forouzanfar et al., 2014; Yimer et al., 2019). A mounting body of evidence showed that the plant and its components also possess robust antimicrobial activities, including antibacterial and antiviral activities (Forouzanfar et al., 2014). Here, we summarize the antiviral activity of *N. sativa* and its active compounds against critical human viruses, focusing on the SARS-CoV-2.

Antiviral activity of *Nigella sativa* against the SARS-CoV-2 virus

Coronaviruses (CoVs) are important pathogens that typically cause respiratory diseases in humans and animals (Masters and Perlman, 2013). Based on their genomic structure and phylogenetics, this family of viruses can be divided into four genera, including Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus. The SARS-CoV, the Middle East respiratory syndrome coronavirus (MERS-CoV), and the novel SARS-CoV-2 coronavirus belong to the Betacoronaviruses (Su et al., 2016). CoVs are positive-sense single-stranded RNA viruses. The viral genome of 27–32 kb encodes 20 proteins, which include four structural proteins, spike (S), matrix (M), envelope (E), and nucleocapsid (N)) located at the 3' terminus, with two-third of the genome coding for the nonstructural proteins. Infection with SARS-CoV-2 mostly results in the development of mild-to-moderate symptoms, such as headache, fever, and dry cough, and patients recover without the need for hospitalization. However, a subset of patients, particularly the elderly and patients with preexisting medical conditions, can develop severe symptoms, including shortness of breath, chest pain or pressure, and loss of speech or movement. Patients experiencing such severe symptoms should seek urgent medical attention.

Since its emergence in December 2019, and due to its unprecedented spread and high mortality compared to the previous CoV pandemics, tremendous efforts have been made to develop effective vaccines and therapeutic strategies against the virus. However, to date, there is no FDA-approved prophylactic vaccine or treatment against the virus. Nonetheless, several promising repurposed drugs have been identified, including remdesivir and lopinavir/ritonavir,

and are undergoing different phases of clinical trials (Wang, Y. et al., 2020). Interestingly, none of the drugs currently undergoing clinical trials are natural products. Given the significance of nature-derived products in drug discovery, continuous research to identify natural products with potent anti-SARS-CoV-2 activity is urgently needed.

Given its documented medicinal effects, including antiinflammatory, antioxidant, immunomodulatory, and, most importantly, antiviral properties, several groups have explored *N. sativa* and its pure compounds as a potential treatment strategy against COVID-19. In an attempt to determine the anti-SARS-CoV-2 potential of *N. sativa's* main active constituents, Sajjad et al. generated a two-dimensional (2D) structure of these compounds and docked them to the chimeric SARS-CoV-2 receptor-binding domain on the S protein. The S protein is responsible for SARS-CoV-2 binding to the angiotensin-converting enzyme 2 (ACE2) receptor as well as the fusion of the viral membrane with the endosomal membrane for the release of the viral genome in the cytoplasm (Ahmad et al., 2020). They showed that among all the compounds tested, including chloroquine, which was included as a positive control, DTQ yielded the highest binding affinity (-8.6 kcal/mol) to the receptor binding domain (RBD) of SARS-CoV-2 S protein, thereby identifying DTQ as a potential inhibitor of SARS-CoV-2 interaction with the ACE2 receptor on the host cells and hence, its entry. Consistent with the above findings, *N. sativa*-derived compounds, including DTQ, THQ, thymol, and TQ were shown to dock with high affinity to the RBD of SARS-CoV-2 protein (Shaikh et al., 2020). The fact that many of *N. sativa*'s main compounds demonstrated a higher/comparable binding affinity to RBD relative to chloroquine, (Ahmad et al., 2020) which is widely known to block virus entry, including SARS-CoV-2 entry (Savarino et al., 2003; Wang, M. et al., 2020), is promising and shows the potential anti-SARS-CoV-2 activity of these compounds.

Using a different approach, Bouchentouf et al. (Bouchentouf and Missoum, 2020) docked the main chemical compounds of *N. sativa* to the crystal structure of SARS-CoV-2 main protease (SARS-CoV-2 M^{pro}) in complex with its inhibitor N3 (SARS-CoV-2 M^{pro}-N3) (Jin et al., 2020), which was developed using computer-aided drug design and shown to specifically inhibit M^{pro} from multiple coronaviruses, including MERS-CoV and SARS-CoV. The M^{pro} is the main SARS-CoV-2 protease responsible for the proteolytic processing of the SARS-CoV-2 replicase genes (Wu et al., 2020), playing a crucial role in both viral replication and transcription and hence, constituting an attractive target for antiviral development. They demonstrated that several *N. sativa*-derived compounds could dock to the SARS-CoV-2 M^{pro}-N3 complex, with nigellincine (-6.29734373 kcal/mol) and α-hederin (-5.25583553 kcal/mol) yielding the highest binding affinity. Consistent with these findings, nigellincine was shown to bind with high affinity to numerous essential SARS-CoV-2 proteins (Maiti et al., 2020). In support of the molecular docking studies, a recent randomized clinical trial (RCT) reported that *N. sativa* accelerates the recovery of

patients displaying mild COVID-19 symptoms (Koshak et al., 2020). Patients who received oral capsules of MARNYS Cuminmar (500 mg twice daily for 10 days), which is derived from *N. sativa*, recovered faster than patients on standard care alone. Although the finding is encouraging, it remains to be seen whether MARNYS Cuminmar capsules could similarly expedite recovery from COVID-19 severe disease. Together, these findings suggest that *N. sativa*-derived compounds can block several steps of the SARS-CoV-2 life cycle, including entry, replication, and transcription, making the plant a rich source of bioactive compounds against SARS-CoV-2. Nonetheless, further *in vitro* and *in vivo* studies are needed to holistically understand *N. sativa's* mechanism of action against SARS-CoV-2.

Antiviral activity of *Nigella sativa* against other medically important viruses

Being known for its medicinal properties, *N. sativa* has been widely explored as an antiviral agent against numerous medically important human viral pathogens, particularly HIV and hepatitis C virus (HCV).

Antiviral activity of Nigella sativa against human immunodeficiency virus

HIV is the etiologic agent of the acquired AIDS, a chronic, life-threatening condition that damages the host's immune system, leaving them susceptible to opportunistic infections. Since its emergence in 1981, the disease has spread to almost every region of the world, causing a global pandemic (Piot et al., 2001). Currently, over 36 million people are infected with HIV, the majority of whom live in the resource-constrained sub-Saharan Africa (WHO, 2006). The introduction of the antiretroviral therapy (ART), which is a combination of drugs that target different stages of the HIV life cycle, has monumentally helped in improving patient's quality of life and longevity, as well as, preventing opportunistic infections, thereby significantly decreasing the global AIDS-related deaths since the peak in 2004. Notwithstanding, to date, there is no effective prophylactic vaccine to prevent HIV infection. Therefore, identifying complementary or alternative options to treat HIV infection, such as using plant-derived compounds, could improve the management of HIV infection.

N. sativa was first explored as an anti-HIV agent in 2011 in a pilot study in Nigeria for patients seeking herbal remedies as a complementary or alternative treatment to ART at the α-zam therapist herbal centers. A total of 51 HIV patients were treated with α-zam, a herbal concoction that contains *N. sativa* and honey, for 5 months. Treatment with α-zam alone or in combination with the highly active antiretroviral therapy (HAART) for 4 months decreased the viral load to undetectable levels for 80% of the patients and significantly increased the cluster of differentiation 4 (CD4) counts for all patients (Onifade et al., 2011). Similar results were found in the author's subsequent study, wherein

α-zam treatment for 4 months decreased the patient's viral loads to undetectable levels and concomitantly increased their CD4 counts (Onifade, A. et al., 2013). Following these pioneering studies, the above authors reported a complete seroreversion in two patients, a 47-year-old man and a 27-year-old pregnant woman who were treated with α-zam for 6 and 12 months, respectively. After the cessation of α-zam treatment, serological tests showed undetectable viral load accompanied by normal CD4 counts in both patients (Onifade et al., 2015; Onifade, A.A. et al., 2013). These findings pinpoint *N. sativa* as an attractive natural product for the treatment of HIV. Although the extract and its active compound appear to be highly effective in treating HIV infection, the exact step(s) of the viral life cycle that it targets is mostly unknown. Further in-depth *in vitro* and *in vivo* studies could help clarify the mechanism of action of *N. sativa* against HIV.

Antiviral activity of Nigella sativa against hepatitis C virus

Hepatitis C virus (HCV) is an RNA virus that chronically infects over 179 million people globally, predisposing them to the risk of developing end-stage liver diseases such as liver cirrhosis and hepatocellular carcinoma (Alter and Seeff, 2000; Thrift et al., 2017). In contrast to the decade-old treatment strategy, which consists of pegylated interferon-alpha (IFN-α) in combination with the nucleoside inhibitor, ribavirin, the advent of the highly effective direct-acting antivirals (DAAs) have drastically changed the landscape of HCV treatment, achieving cure rates of over 90% in the most difficult to treat populations (Terrault, 2015). Despite this success, the DAAs are still costly, cannot induce sterilizing immunity, and could select resistant mutants. More importantly, there is still no effective vaccine that can prevent HCV infection. Thus, the continuous identification of alternative/complementary treatment strategies to complement the existing options is crucial.

Owing to the high prevalence of chronic HCV infection, the efficacy of *N. sativa* treatment in 30 patients who are not eligible for IFN-α treatment was evaluated in a prospective, single-armed, self-controlled pilot study in Egypt. After completing baseline evaluations, patients were given *N. sativa* seed oil capsules (450 mg) three times daily for 3 months. The administration of *N. sativa* significantly decreases the HCV viral loads relative to the baseline. Besides, the extract treatment also improved the total antioxidant capacity and decreased the blood glucose levels in these patients (Barakat et al., 2013). In a similar study in Egypt, the administration of *N. sativa* capsules resulted in a significant decrease in the patient's viral load and also decreased the markers of liver damage, including serum alpha-fetoprotein (AFP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactose dehydrogenase (LDH) (Abdel-Moneim et al., 2013). These findings suggest that *N. sativa* treatment can exert multiple benefits in HCV infected patients by decreasing viral replication, and at the same time, alleviating factors like oxidative stress and diabetes that contribute to HCV disease progression (Kawaguchi and Sata, 2010; Poli

and Parola, 1997), thereby making it an attractive candidate for development as an anti-HCV therapeutic agent. Following these clinical studies, in 2016, Oyero et al., using HCV genotype 1b full-length and subgenomic replicons, investigated the anti-HCV activity of α-zam, a *N. sativa* seed formulation *in vitro*. Consistent with the clinical studies, *N. sativa* treatment robustly impeded HCV replication, as demonstrated by the significant decrease in viral RNA levels and the luciferase signals in the full-length replicons and the subgenomic replicons, respectively (Oyero et al., 2016). However, whether *N. sativa* could inhibit the other steps of the HCV life cycle, including entry, assembly, and release have not been investigated in this study. Therefore, it would be interesting to know whether α-zam could modulate the other steps of the viral life cycle.

Antiviral activity of Nigella sativa against hepatitis B virus

Hepatitis B virus (HBV) approximately infects 2 billion people worldwide, 350 million of which suffer from chronic infection, which can predispose them to end-stage liver disease such as hepatocellular carcinoma (HCC). Chronic HBV infection is the 10th leading cause of death globally, with 500,000 to 1.2 million deaths per year attributed to its infection, making the virus a severe public health concern (Lavanchy, 2004). Similar to HCV and HIV, HBV is a bloodborne pathogen and can be transmitted vertically, from mother to child, which is the most common mode of transmission in Africa and Asia. The virus can also be transmitted through contact with contaminated products, such as during intravenous drug use and through sexual intercourse with an infected individual. Although a highly effective prophylactic vaccine has been available since 1982 to prevent HCV infection, which was instrumental in decreasing the global incidence of HBV infection (Abdel-Moneim et al., 2013), the current treatment strategies such as IFN-α, entecavir, and lamivudine although can help contain the virus and slow liver damage, they cannot completely eradicate the infection (Férir et al., 2008). Hence, there is a dire need to identify better treatment options against the liver pathogen.

Given the hepatoprotective effects of *N. sativa*, and the current lack of antivirals that can completely cure HBV infection, it is befuddling that the antiviral effect of *N. sativa* on HBV has not attracted much attention. Nonetheless, Haron and colleagues examined the anti-tumor effects of TQ on Hep3B liver cancer cells integrated with the HBV genome (Haron et al., 2018). TQ is a lipophilic compound and hence cannot be taken orally and has a low bioavailability. To overcome this challenge, the authors loaded TQ on nanocarriers (nanostructured lipid carrier [NLC]) before testing their antitumor activity. They demonstrated that TQ and its nanoformulated counterpart TQ-NLC time-dependently inhibited the growth of the HCV genome-integrated Hep3B cells. Moreover, TQ and TQ-NLC treatment-induced cell cycle arrest and apoptosis in these cells with TQ-NLC functioning as an antioxidant. These results show that TQ can impede liver cancer cells expressing the HBV genome and potentially also impede HBV replication. Given that the authors did not directly examine the

effect of TQ on HBV replication, it remains to be seen whether the compound, besides its antitumor activity against the Hep3B cells, could impede HBV replication. However, considering the hepatoprotective, immuno-potentiating, and antiinflammatory activity of *N. sativa*, it is tempting to theorize that the plant and its extract may exhibit antiviral effects against HBV.

In 2008, to determine whether or not *N. sativa* could protect against HBV infection, albino rats were divided into three groups (negative control, positive control, and test group) and injected intramuscularly with human serum containing HBV (positive control), prefed with *N. sativa* for 2 weeks before injection and fed for 8 weeks with *N. sativa* after injection (test group), or left uninjected (negative control), before collecting blood samples for serological testing. Although markers for active replication HBV such as HBV surface antigen (HBsAg) and HBV-e antigen (HBeAg) were positive for both positive control and the test group, the HBsAg, which is a marker for protection against HBV infection was positive for only the test group, but not for the control group. Moreover, *N. sativa* treatment significantly decreased the expression of the AST and ALT liver enzymes compared to the control group (Al Ghamdi, 2008). These results, therefore, suggest that *N. sativa* can protect against HBV infection. However, precisely how the extract protects against HBV infection in the albino rats was not clarified in the study. Whether the anti-HBV effects observed are due to a direct effect of the plant on the virus, for example, by blocking viral replication, or indirectly, by potentiating the immune response and blocking the HBV-mediated inflammatory response, remains to be elucidated. Nonetheless, these results provide compelling evidence for the anti-HBV activity of *N. sativa* and its constituents.

Antiviral activity of Nigella sativa against H9N2 avian influenza virus

The avian influenza virus (AIV) H9N2, which has been isolated from birds in different countries, including India, Pakistan, and Egypt, imposes a severe threat to poultry birds. H9N2 is a type A influenza virus belonging to the Orthomyxoviridae family, which contains a segmented RNA genome encoding 10 viral proteins and a host of accessory proteins (James et al., 2019). These viruses can be divided into highly pathogenic avian influenza (HPAI), which causes systemic severe lethal infections in birds, and low pathogenic avian influenza (LPAI) that typically cause mild infections in poultry. Although H9N2 belongs to the LPAI group, these viruses cause various symptoms in chickens and have been reported to pose a zoonotic threat to humans (Umar et al., 2016). Therefore, identifying antiviral strategies that could restrict H9N2 infection in birds and prevent their potential transmission to humans is of vital importance.

Given the importance of the immune response in preventing and controlling viral infections, including H9N2 infection (Rouse and Sehrawat, 2010), augmenting the host-mediated immune response, for example, using immune-potentiating agents could constitute an attractive antiviral strategy. Due to the

documented immuno-potentiating properties of *N. sativa*, in 2016, Sajid et al. evaluated the plant's effect on the pathogenesis of H9N2 in turkeys. Birds were left untreated and uninfected (negative control), infected with AIV without treatment (positive control), or infected and treated with increasing concentrations of *N. sativa* (2%, 4%, and 6%) (test groups), and observed daily for 12 days postinfection.

Examination of the clinical signs, including facial swelling, diarrhea, sneezing, and depression, was more severe in the control group than the *N. sativa* treated groups. Similarly, virus shedding and viral titer were higher in the positive control group than the test groups, with *N. sativa* treatment exhibiting a dose-dependent inhibition of viral shedding and viral titer, suggesting that the natural product could ameliorate the pathogenesis/virulence of H9N2 infection. However, there was no statistical difference in the mRNA expression of interferon gamma (IFN-γ), an immunomodulatory cytokine with well-established antiviral properties against AIV (Yuk et al., 2016), which indicate that the antiviral activity of *N. sativa* in the turkeys does not implicate the IFN-γ. Nonetheless, given the decrease in viral load and viral shedding, it is plausible to posit that the extract targets viral replication and/or release.

Antiviral activity of Nigella sativa against the cytomegalovirus

Human cytomegalovirus (HCMV) belongs to the Herpesviridae family of viruses, which also includes large DNA viruses such as varicella zoster virus (VSV), human simplex virus (HSV), and Epstein-Barr virus (EBV). Like the other members of the Herpesviridae family, HCMV is ubiquitous and belongs to the beta herpesvirus subfamily with a genome size of 235 kb, which encodes 165 genes (Davison et al., 2003). HCMV can be transmitted in multiple ways, such as through kissing, sexual intercourse, blood transfusion, and from an infected mother to her child, either through the placenta or during breastfeeding (Sia and Patel, 2000). Primary HCMV infection is generally asymptomatic in immunocompetent adults, but the virus can establish latent infection with periodic reactivations. However, the virus can cause severe clinical presentations in immunocompromised people, such as neonates, in whom it can induce impairments in neurological development and deafness (Fowler and Boppana, 2006; Gandhi and Khanna, 2004). Currently, there is no cure for CMV infection, and treatment is mainly supportive.

To determine whether *N. sativa* possess antiviral activities against CMV, Salem et al. employed the murine CMV, which causes fatal disease in immunocompromised animals, similar to the effect of HCMV infection in immunosuppressed human, making it an excellent surrogate model for studying the antiviral activities of *N. sativa* on CMV. Intraperitoneal administration of black seed oil (BSO) to BALB/c mice for 7 consecutive days before the MCMV challenge significantly decreased the viral titers in the spleen and liver 3 days postinfection with a concomitant increase in the IFN-γ serum levels, macrophages, and the CD4+ T cells. Moreover, in stark contrast to the control untreated mice, in

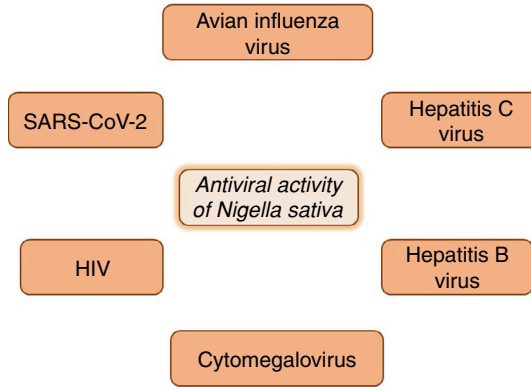


FIG 16.2 Effect of *Nigella sativa* on various viruses. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

which virus titer was detected at 10 days postinfection, no virus titer was seen in the BSO-treated mice (Salem and Hossain, 2000). These findings demonstrate BSO's robust antiviral activity against CMV, which is most likely mediated by its immune-potentiating effects (increase in the IFN-γ serum levels, macrophages, and CD4+ T cells), although a direct consequence of the extract on the virus itself cannot be ruled out. More importantly, these findings also support the use of BSO as a combination treatment in immunocompromised people, such as those with AIDS or cancer.

In addition to the above-mentioned viruses, *N. sativa* and its main active components have been recorded to exert potent antiviral activities against a diverse range of viruses (Shamim Molla et al., 2019), thereby identifying the plant as a broad-spectrum antiviral agent. Fig. 16.1. Fig. 16.2.

Concluding remarks

Since its emergence, the SARS-CoV-2 virus, the etiologic agent of the COVID-19 disease, has spread across the world, causing severe health threats, particularly to old patients and those with underlying health conditions, such as heart disease, liver diseases, and diabetes (Clark et al., 2020). Due to the severity of the pandemic and its socioeconomic impact, concerted efforts spearheaded by the World Health Organization (WHO) have been made to develop effective therapeutic strategies against the respiratory virus. However, to date, apart from the newly approved vaccine in Russia, the authenticity and efficacy of which is still obscure, there is no protective vaccine or therapeutic strategy against the virus. The current treatment of COVID-19 patients involves symptomatic treatment, supportive care, isolation, and experimental measures. Although several FDA-approved drugs are repurposed for use as anti-COVID-19 agents with

some already in the advanced stages of clinical trials (Wang, Y. et al., 2020), none of these drugs is approved yet, signaling an urgent need to identify complementary/alternative treatment options against the coronavirus. *N. sativa* is well known for its medicinal properties against a variety of conditions. Given its documented antiviral activity against several public heath important pathogens, numerous studies have explored the use of the plant and its active components as anti-SARS-CoV-2 agents. Indeed, the molecular docking analysis discussed above suggests that *N. sativa* could constitute an excellent anti-SARS-CoV-2 agent by targeting different stages of the viral life cycle, including viral entry and replication. However, further *in vitro* and *in vivo* studies are needed to verify the anti-SARS-CoV-2 effect of the prophetic plant. In addition to its robust antiviral effects, *N. sativa* is also well known to possess immune-potentiating, anti-inflammatory, and respiratory stimulation activities, making it a good candidate for the management of COVID-19 disease (Ali and Blunden, 2003; Swamy and Tan, 2000). Thus, *N. sativa* could have multiple benefits in treating COVID-19 disease by impeding viral replication, and at the same time, alleviating COVID-19 symptoms. Given its broad-spectrum antiviral activity, *N. sativa* could also be used in SARS-CoV-2 patients coinfected with other chronic viruses. Hence, the plant and its major components deserve further evaluation for use as an alternative/complementary therapy to treat the COVID-19 disease.

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References

Abdel-Moneim, A., Morsy, B.M., Mahmoud, A.M., Abo-Seif, M.A., Zanaty, M.I., 2013. Beneficial therapeutic effects of Nigella sativa and/or Zingiber officinale in HCV patients in Egypt. EXCLI J. 12, 943.

Ahmad, S., Abbasi, H.W., Shahid, S., Gul, S., Abbasi, S.W., 2020. Molecular docking, simulation and MM-PBSA studies of Nigella Sativa compounds: a computational quest to identify potential natural antiviral for COVID-19 treatment. J. Biomol. Struct. Dyn., 1–16.

Ahmed, M.A., Hassanein, K.M., 2013. Cardio protective effects of Nigella sativa oil on lead induced cardio toxicity: anti inflammatory and antioxidant mechanism. J. Physiol. Pathophysiol. 4 (5), 72–80.

Al Ghamdi, S., 2008. Nigella sativa seeds protect from viral hepatitis B virus infection. Curr. Top Pharmacol. 12, 101–104.

Alagawany, M., El-Hack, M., Farag, M.R., Tiwari, R., Dhama, K., 2015. Biological effects and modes of action of carvacrol in animal and poultry pro-duction and health-a review. Adv. Anim. Vet. Sci. 3 (2s), 73–84.

Ali, B., Blunden, G., 2003. Pharmacological and toxicological properties of Nigella sativa. Phytother. Res. 17 (4), 299–305.

Alter, H.J., Seeff, L.B., 2000. Recovery, Persistence, and Sequelae in Hepatitis C Virus Infection: A Perspective on Long-Term Outcome, Seminars in Liver Disease. Thieme Medical Publishers, pp. 0017–0036.

Babazadeh, B., Sadeghnia, H.R., Safarpour Kapurchal, E., Parsaee, H., Nasri, S., Tayarani-Najaran, Z., 2012. Protective effect of Nigella sativa and thymoquinone on serum/glucose deprivation-induced DNA damage in PC12 cells. Avicenna J. Phytomed. 2 (3), 125–132.

Barakat, E.M.F., El Wakeel, L.M., Hagag, R.S., 2013. Effects of Nigella sativa on outcome of hepatitis C in Egypt. World J. Gastroenterol. 19 (16), 2529.

Baser, K.H.C., 2008. Biological and pharmacological activities of carvacrol and carvacrol bearing essential oils. Curr. Pharm. Des. 14 (29), 3106–3119.

Bin Sayeed, M.S., Asaduzzaman, M., Morshed, H., Hossain, M.M., Kadir, M.F., Rahman, M.R., 2013. The effect of Nigella sativa Linn. seed on memory, attention and cognition in healthy human volunteers. J. Ethnopharmacol. 148 (3), 780–786.

Bordoni, L., Fedeli, D., Nasuti, C., Maggi, F., Papa, F., Wabitsch, M., De Caterina, R., Gabbianelli, R., 2019. Antioxidant and anti-inflammatory properties of Nigella sativa oil in human pre-adipocytes. Antioxidants (Basel) 8 (2), 51.

Bouchentouf, S., Missoum, N. https://doi.org/10.26434/chemrxiv.12055716.v1.

Cai, L., 2019. Chinese medicine for treating headache. Peop. Rep. China, p. 4.

Clark, A., Jit, M., Warren-Gash, C., Guthrie, B., Wang, H.H., Mercer, S.W., Sanderson, C., McKee, M., Troeger, C., Ong, K.L., 2020. Global, regional, and national estimates of the population at increased risk of severe COVID-19 due to underlying health conditions in 2020: a modelling study. Lancet Glob. Health.

Davison, A.J., Dolan, A., Akter, P., Addison, C., Dargan, D.J., Alcendor, D.J., McGeoch, D.J., Hayward, G.S., 2003. The human cytomegalovirus genome revisited: comparison with the chimpanzee cytomegalovirus genomeFN1. J. Gen. Virol. 84 (1), 17–28.

Faiza, M., Abdullah, T., Wang, P., 2017. Dithymoquinone as a Novel Inhibitor for 3-Carboxy-4-Methyl-5-Propyl-2-Furanpropanoic Acid (CMPF) to Prevent Renal Failure. arXiv preprint arXiv:1709.03813.

Férir, G., Kaptein, S., Neyts, J., De Clercq, E., 2008. Antiviral treatment of chronic hepatitis B virus infections: the past, the present and the future. Rev. Med. Virol. 18 (1), 19–34.

Forouzanfar, F., Bazzaz, B.S.F., Hosseinzadeh, H., 2014. Black cumin (Nigella sativa) and its constituent (thymoquinone): a review on antimicrobial effects. Iran. J. Basic Med. Sci. 17 (12), 929–938.

Fowler, K.B., Boppana, S.B., 2006. Congenital cytomegalovirus (CMV) infection and hearing deficit. J. Clin. Virology 35 (2), 226–231.

Fröhlich, T., Reiter, C., Saeed, M.E.M., Hutterer, C., Hahn, F., Leidenberger, M., Friedrich, O., Kappes, B., Marschall, M., Efferth, T., Tsogoeva, S.B., 2018. Synthesis of thymoquinone–artemisinin hybrids: new potent antileukemia, antiviral, and antimalarial agents. ACS Med. Chem. Lett. 9 (6), 534–539.

Gandhi, M.K., Khanna, R., 2004. Human cytomegalovirus: clinical aspects, immune regulation, and emerging treatments. Lancet Infect. Dis. 4 (12), 725–738.

Gholamnezhad, Z., Keyhanmanesh, R., Boskabady, M.H., 2015. Anti-inflammatory, antioxidant, and immunomodulatory aspects of Nigella sativa for its preventive and bronchodilatory effects on obstructive respiratory diseases: A review of basic and clinical evidence. J. Funct. Foods 17, 910–927.

Goyal, S.N., Prajapati, C.P., Gore, P.R., Patil, C.R., Mahajan, U.B., Sharma, C., Talla, S.P., Ojha, S.K., 2017. Therapeutic potential and pharmaceutical development of thymoquinone: a multi-targeted molecule of natural origin. Front. Pharmacol. 8 (656).

Gupta, B., Ghosh, K.K., Gupta, R.C., 2016. Chapter 39 - Thymoquinone. In: Gupta, R.C. (Ed.), Nutraceuticals. Academic Press, Boston, pp. 541–550.

Haron, A.S., Syed Alwi, S.S., Saiful Yazan, L., Abd Razak, R., Ong, Y.S., Zakarial Ansar, F.H., Roshini Alexander, H., 2018. Cytotoxic effect of thymoquinone-loaded nanostructured lipid

carrier (TQ-NLC) on liver cancer cell integrated with hepatitis B genome, Hep3B. Evid-Based Complement. Alternat. Med.

Harvey, A.L., Edrada-Ebel, R., Quinn, R.J., 2015. The re-emergence of natural products for drug discovery in the genomics era. Nat. Rev. Drug Discov. 14 (2), 111–129.

Hosseini, A., Shafiee-Nick, R., Mousavi, S.H., 2014. Combination of Nigella sativa with Glycyrrhiza glabra and Zingiber officinale augments their protective effects on doxorubicin-induced toxicity in h9c2 cells. Iran. J. Basic Med. Sci. 17 (12), 993–1000.

Islam, M., Guha, B., Hosen, S., Riaz, T., Shahadat, S., Da Rocha Sousa, L., De Oliveira Santos, J., Da Silva Júnior, J., De Lima, R., Braga, A., 2017. Nigellalogy: a review on Nigella sativa. MOJ Bioequiv. Availab. 3 (6), 00056.

Islam, M.T., Khan, M.R., Mishra, S.K., 2019. An updated literature-based review: phytochemistry, pharmacology and therapeutic promises of Nigella sativa L. Orient. Pharm. Exp. Med. 19 (2), 115–129.

Ivankovic, S., Stojkovic, R., Jukic, M., Milos, M., 2006. The antitumor activity of thymoquinone and thymohydroquinone in vitro and in vivo. Exp. Oncol.

Jafri, H., Ansari, F.A., Ahmad, I., 2019. Chapter 9 - Prospects of essential oils in controlling pathogenic biofilm. In: Ahmad Khan, M.S., Ahmad, I., Chattopadhyay, D. (Eds.), New Look to Phytomedicine. Academic Press, pp. 203–236.

James, J., Sealy, J.E., Iqbal, M., 2019. A global perspective on H9N2 avian influenza virus. Viruses 11 (7), 620.

Jin, Z., Du, X., Xu, Y., Deng, Y., Liu, M., Zhao, Y., Zhang, B., Li, X., Zhang, L., Peng, C., 2020. Structure of M pro from SARS-CoV-2 and discovery of its inhibitors. Nature, 1–5.

Kanter, M., Coskun, O., Budancamanak, M., 2005. Hepatoprotective effects of Nigella sativa L and Urtica dioica L on lipid peroxidation, antioxidant enzyme systems and liver enzymes in carbon tetrachloride-treated rats. World J. Gastroenterol. 11 (42), 6684–6688.

Kapoor, L., 1990. Handbook of Ayurvedic Medicinal Plants. CRC Press.

Kawaguchi, T., Sata, M., 2010. Importance of hepatitis C virus-associated insulin resistance: therapeutic strategies for insulin sensitization. World J. Gastroenterol. 16 (16), 1943.

Kheirouri, S., Hadi, V., Alizadeh, M., 2016. Immunomodulatory effect of Nigella sativa oil on T lymphocytes in patients with rheumatoid arthritis. Immunol. Invest. 45 (4), 271–283.

Kinghorn, A.D., 2001. Pharmacognosy in the 21st century*. J. Pharm. Pharmacol. 53 (2), 135–148.

Koshak, A.E., Koshak, E.A., Mobeireek, A.F., Badawi, M.A., Wali, S.O., Malibary, H.M., Atwah, A.F., Alhamdan, M.M., Almalki, R.A., Madani, T.A., 2020. Nigella Sativa supplementation accelerates recovery from mild COVID-19: first randomized controlled clinical trial. Trails 21 (1), 1–2.

Lavanchy, D., 2004. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J. Viral. Hepat. 11 (2), 97–107.

Maiti, S., Banerjee, A., Nazmeen, A., Kanwar, M., Das, S., 2020. Active-site molecular docking of nigellidine to nucleocapsid/Nsp2/Nsp3/MPro of COVID-19 and to human IL1R and TNFR1/2 may stop viral-growth/cytokine-flood, and the drug source Nigella sativa (black cumin) seeds show potent antioxidant role in experimental rats.

Majdalawieh, A.F., Fayyad, M.W., 2015. Immunomodulatory and anti-inflammatory action of Nigella sativa and thymoquinone: a comprehensive review. Int. Immunopharmacol. 28 (1), 295–304.

Majdalawieh, A.F., Fayyad, M.W., 2016. Recent advances on the anti-cancer properties of Nigella sativa, a widely used food additive. J. Ayurveda Integr. Med. 7 (3), 173–180.

Majdalawieh, A.F., Fayyad, M.W., Nasrallah, G.K., 2017. Anti-cancer properties and mechanisms of action of thymoquinone, the major active ingredient of Nigella sativa. Crit. Rev. Food Sci. Nutr. 57 (18), 3911–3928.

Masters, P., Perlman, S., 2013. In: Knipe, DM, Howley, PM (Eds.), Fields Virology, (vol. 2). Lippincott Williams & Wilkins, pp. 825–858.

Nagoor Meeran, M.F., Javed, H., Al Taee, H., Azimullah, S., Ojha, S.K., 2017. Pharmacological Properties and Molecular Mechanisms of Thymol: Prospects for Its Therapeutic Potential and Pharmaceutical Development. Front. Pharmacol 8 (380).

Ng, W.K., Yazan, L.S., Ismail, M., 2011. Thymoquinone from Nigella sativa was more potent than cisplatin in eliminating of SiHa cells via apoptosis with down-regulation of Bcl-2 protein. Toxicol. In Vitro 25 (7), 1392–1398.

Nikakhlagh, S., Rahim, F., Aryani, F.H.N., Syahpoush, A., Brougerdnya, M.G., Saki, N., 2011. Herbal treatment of allergic rhinitis: the use of Nigella sativa. Am. J. Otolaryngol. 32 (5), 402–407.

Onifade, A., Jewell, A., Ajadi, T., Rahamon, S., Ogunrin, O., 2013. Effectiveness of a herbal remedy in six HIV patients in Nigeria. J. Herb. Med. 3 (3), 99–103.

Onifade, A., Jewell, A., Onifade, A., 2011. Virologic and immunologic outcome of treatment of HIV infection with a herbal concoction, α-zam, among clients seeking herbal remedy in Nigeria. Afr. J. Tradit. Complement. Altern. Med. 8 (1).

Onifade, A.A., Jewell, A., Okesina, A., 2015. Seronegative conversion of an HIV positive subject treated with Nigella sativa and honey. Afr. J. Infect. Dis. 9 (2), 47–50.

Onifade, A.A., Jewell, A.P., Adedeji, W.A., 2013. Nigella sativa concoction induced sustained seroreversion in HIV patient. Afr. J. Tradit. Complement. Altern. Med. 10 (5), 332–335.

Oyero, O.G., Toyama, M., Mitsuhiro, N., Onifade, A.A., Hidaka, A., Okamoto, M., Baba, M., 2016. Selective inhibition of hepatitis C virus replication by Alpha-zam, a Nigella sativa seed formulation. Afr. J. Tradit. Complement. Altern. Med. 13 (6), 144–148.

Piot, P., Bartos, M., Ghys, P.D., Walker, N., Schwartländer, B., 2001. The global impact of HIV/AIDS. Nature 410 (6831), 968–973.

Poli, G., Parola, M., 1997. Oxidative damage and fibrogenesis. Free Radic. Biol. Med. 22 (1-2), 287–305.

Ragheb, A., Attia, A., Eldin, W., Elbarbry, F., Gazarin, S., Shoker, A., 2009. The protective effect of thymoquinone, an anti-oxidant and anti-inflammatory agent, against renal injury: A review. Saudi J. Kidney Dis. Transpl. 20 (5), 741–752.

Randhawa, M.A., Alghamdi, M.S., 2011. Anticancer activity of Nigella sativa (black seed)—a review. Am. J. Chin. Med. 39 (06), 1075–1091.

Rouse, B.T., Sehrawat, S., 2010. Immunity and immunopathology to viruses: what decides the outcome? Nat. Rev. Immunol. 10 (7), 514–526.

Salem, M.L., Hossain, M.S., 2000. Protective effect of black seed oil from Nigella sativa against murine cytomegalovirus infection. Int. J. Immunopharmacol. 22 (9), 729–740.

Savarino, A., Boelaert, J.R., Cassone, A., Majori, G., Cauda, R., 2003. Effects of chloroquine on viral infections: an old drug against today's diseases. Lancet Infect. Dis. 3 (11), 722–727.

Sedaghat, R., Roghani, M., Khalili, M., 2014. Neuroprotective effect of thymoquinone, the nigella sativa bioactive compound, in 6-hydroxydopamine-induced hemi-parkinsonian rat model. Iran. J. Pharm. Res. 13 (1), 227–234.

Shaikh, Y.I., Shaikh, V.S., Ahmed, K., Nazeruddin, G.M., Pathan, H.M., 2020. The revelation of various compounds found in Nigella sativa L. (black cumin) and their possibility to inhibit COVID-19 infection based on the molecular docking and physical properties. Eng. Sci.

Shamim Molla, M., Azad, A.K., Al Hasib, M.A.A., Hossain, M.M., Ahammed, M.S., Rana, S., Islam, M.T., 2019. A review on antiviral effects of Nigella Sativa L.

Sia, I.G., Patel, R., 2000. New strategies for prevention and therapy of cytomegalovirus infection and disease in solid-organ transplant recipients. Clin. Microbiol. Rev. 13 (1), 83–121.

Su, S., Wong, G., Shi, W., Liu, J., Lai, A.C., Zhou, J., Liu, W., Bi, Y., Gao, G.F., 2016. Epidemiology, genetic recombination, and pathogenesis of coronaviruses. Trends Microbiol. 24 (6), 490–502.

Swamy, S., Tan, B., 2000. Cytotoxic and immunopotentiating effects of ethanolic extract of Nigella sativa L. seeds. J. Ethnopharmacol. 70 (1), 1–7.

Terrault, N., 2015. Difficult-to-cure populations with chronic hepatitis C: vanishing in the direct-acting antiviral era? Hepatol. 62 (1), 4–7.

Thrift, A.P., El-Serag, H.B., Kanwal, F., 2017. Global epidemiology and burden of HCV infection and HCV-related disease. Nat. Rev. Gastroenterol. Hepatol. 14 (2), 122.

Tisserand, R., Young, R., 2014. 13 - Essential oil profiles. In: Tisserand, R., Young, R. (Eds.), Essential Oil SafetySecond Edition. Churchill Livingstone, St. Louis, pp. 187–482.

Tisserand, R., Young, R., 2014. 14 - Constituent profiles. In: Tisserand, R., Young, R. (Eds.), Essential Oil SafetySecond Edition. Churchill Livingstone, St. Louis, pp. 483–647.

Umar, S., Munir, M., Kaboudi, K., Rehman, A., Asif, S., Usman, M., Ali, A., Shahzad, M., Subhan, S., Shah, M., 2016. Effect of route of inoculation on replication of avian influenza virus (H9N2) and interferon gene expression in guinea fowl (Numida meleagridis). Br. Poult. Sci. 57 (4), 451–461.

Usmanghani, K., Saeed, A., Alam, M., 1997. Indusyunic medicine. Department of Pharmacognosy, Faculity of Pharmacy. University of Karachi, Pakistan, pp. 363–364.

Veeresham, C., 2012. Natural products derived from plants as a source of drugs. J. Adv.Pharm. Technol. Res. 3 (4), 200–201.

Wajs, A., Bonikowski, R., Kalemba, D., 2008. Composition of essential oil from seeds of Nigella sativa L. cultivated in Poland. Flavour Fragr. J. 23 (2), 126–132.

Wang, J., 2016. A traditional chinese medicine formulation for treating kidney yin deficiency and spleen deficiency. Peop. Rep. China.

Wang, M., Cao, R., Zhang, L., Yang, X., Liu, J., Xu, M., Shi, Z., Hu, Z., Zhong, W., Xiao, G., 2020. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res. 30 (3), 269–271.

Wang, Y., Zhang, D., Du, G., Du, R., Zhao, J., Jin, Y., Fu, S., Gao, L., Cheng, Z., Lu, Q., 2020. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. Lancet.

WHO, U., 2006. Joint United Nations Programmer on HIV/AIDS and World Health Organization. AIDS epidemic update.

Wu, F., Zhao, S., Yu, B., Chen, Y.-M., Wang, W., Song, Z.-G., Hu, Y., Tao, Z.-W., Tian, J.-H., Pei, Y.-Y., 2020. A new coronavirus associated with human respiratory disease in China. Nature 579 (7798), 265–269.

Yaman, İ., Balikci, E., 2010. Protective effects of nigella sativa against gentamicin-induced nephrotoxicity in rats. Exp. Toxicol. Pathol. 62 (2), 183–190.

Yimer, E.M., Tuem, K.B., Karim, A., Ur-Rehman, N., Anwar, F., 2019. *Nigella sativa* L. (black cumin): a promising natural remedy for wide range of illnesses. Evid.-Based Complement. Alternat. Med., 1528635.

Yuk, S.-S., Lee, D.-H., Park, J.-K., Tseren-Ochir, E.-O., Kwon, J.-H., Noh, J.-Y., Lee, J.-B., Park, S.-Y., Choi, I.-S., Song, C.-S., 2016. Pre-immune state induced by chicken interferon gamma inhibits the replication of H1N1 human and H9N2 avian influenza viruses in chicken embryo fibroblasts. Virol. J. 13 (1), 1–10.