Conceptual Analysis of Microbial Processes and Drug Delivery System by the Active Ingredients of *Nigella sativa*

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Abstract: Many natural plants have potential components having antiviral and antibacterial properties. *Nigella sativa* (*N. sativa*) components have been selected to observe the antiviral and antibacterial characteristics. Seed of *Nigella* contains thymoquinone (TQ), dithymo-quinone (DTQ), thymo-hydroquinone (THQ), p-cymene, and thymol (THY). The neutralization process of viral proteins by TQ and THQ has been demonstrated in this study. The interaction of *N. sativa* nanoparticles on bacteria cells was discussed. *N. sativa* electrospun nanofibrous membranes have diverse merits. These include easily adjustable structure, high surface area, and adjustable diameter, good pore connectivity, and high porosity, which give broad application prospects in drug delivery systems. Nanofibrous membrane produced from an electrospinning machine from nigella extract with polyvinyl alcohol (PVA) solution for potential application as the interaction of *N. sativa* nanoparticles on drug delivery systems and bacteria cells were discussed. The disk diffusion approach was applied to analyze the antimicrobial activity of the PVA-nigella membrane against the bacteria, namely *Staphylococcus aureus* (*S. aureus*), and the inhibition zone with an outcome of 15 mm is found.

Keywords: *Nigella sativa*; nanofibrous membrane; nanoparticles; targeted drug delivery; pharmaceutical applications.

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1. Introduction

A process in which microorganisms and their parts are used to make or modify products or new microorganisms are developed for special use is called a microbial process. These products made or modified by microorganisms are called microbe-based products [1]. Drug delivery systems are used to transport a pharmaceutical compound with the help of engineered devices to release its therapeutic cargo throughout the body in a controlled manner. The active compound's potential physical-chemical or enzymatic disruptions are diminished by encapsulating the molecules within a protective shell-like structure. Undesirable side effects
resulting from unspecific systemic distribution are reduced, and bioavailability of the active compound is increased [2-4].

Bioproducts from nature have been considered as a valuable source of adroit molecules. Their extracts and compounds of plant origin possess antiviral activity [5]. The World Health Organization (WHO) reckoned that more than 80% of the world's population is dependent on traditional medicinal plants to meet their health essentials [6]. The application of medicinally active plants predates modern history. Currently, plants in nature exploited for medicine have become a part of the modern world healthcare systems due to their distinctive ability to synthesize versatile compounds with diverse health-related benefits [7, 8].

Medicinal plants are exuberant in versatile of bioactive secondary metabolites such as alkaloids, terpenoids, phenolic, saponins, tannins, and flavonoids compounds which can generate an ascertained physiological action in the human body [9, 10] and schedule based taking the vegetables, fruits, legumes, nut increase immunity [11]. Among these various medicinal plants, (N. sativa has been contemplated as one of the most esteemed nutrients-opulent herbs worldwide, and manifold studies are in progress to ratify the traditionally claimed uses of a small seed of this species [12, 13]. N. sativa seeds contain fixed oils, alkaloids, saponin, proteins, and essential oil.

Most essential oil elements are characterized; however, the major components are TQ, trans-anethole, carvacrol, p-cymene, 4-terpineol, and longifolene. TQ can easily dimerize to form dithymoquinone [15]. Currently, clinical and experimental studies have been conducted to investigate the therapeutic effects of TQ, including anti-inflammatory, immunomodulatory, antimicrobial, and antitumor [16-18]. Various bioactive components from the seed of N. sativa have been studied in the literature; among those, the most important components are TQ. N. sativa has gained the potential therapeutic interest to cure immunosuppressive viral diseases with numerous biological targets and virtually no side effects. The essential oil composition studies investigated about forty various components; among the copious components reported are p- trans-anethole, carvone, cymene, α-thujene, limonene, THQ, TQ, DTQ, β-pinene, and carvacrol with varying composition concentrations [18-20]. Literature [12-20] has reported the antibacterial, antiviral, antioxidant, antihypertensive, anti-diabetic, anti-inflammatory, neuroprotective, anti analgesic, antifungal, antimicrobial, antiparasitic, anticancer properties of N. sativa. From our investigation, we found that there is no study to explain the mechanism to deactivate the virus and its consequences. Viruses are RNA or DNA based according to their genetic material. The nucleic acid could be single or double-stranded. The exhaustive infectious virus particle is stated as a virion composed of an outer shell of protein and nucleic acid [21]. A theoretical mechanism has been proposed to deactivate the viral proteins in the present study. This concept can be used in the future to make a drug against viruses for the survival of humans across the globe. The alternative environment-friendly natural herbs such as nigella seed have momentous effects due to their inherent biomedicinal properties. Thus, the fabrication of nanofibrous membrane by electrospinning technique extracted from natural polymers is reputed as an impeccable technique for applications in biomedical systems such as drug delivery and tissue engineering, leading to the coetaneous research field. Synergistic properties in a holistic approach of Nigella on nanofibrous membranes have not yet been studied comprehensively. The novelty of the present study is to fabricate a nanofibrous membrane with nigella extract to investigate its antibacterial and antiviral properties and useability in drug delivery systems. Similar works have been done in related literature [22-29].
The uniqueness of this work is that it conceptually shows the prospects of *N. sativa* in conventional and targeted drug delivery systems against bacteria cells, viruses. It would be helpful for others to focus on research in related fields and find suitable medicine by *N. sativa*.

2. Materials and Methods

*N. sativa* has been considered for this conceptual paper as it has several biomedical properties against viruses, bacteria, and diseases. Nanoparticles from *N. sativa* can be synthesized either by top-down or bottom-up methods and can be applied as a drug in a drug delivery system or targeted drug delivery system or by any other means. A scanning electron microscopy test of the *N. sativa* nanoparticle has been performed, which confirms the physical adsorption, nanoparticles assembly, and multilayer assembly properties.

Nanofibrous can be fabricated with a wide range of polymers. With regard to drug delivery systems, a polymer solution (*N. sativa*+ polymer) is prepared. After that, a prescribed proportion of the drug is homogenized, creating a suspension. This solution is electrospun to fabricate a nanofibrous membrane composed of a solid complex of polymer-drug.

3. Results and Discussion

3.1. *Nigella sativa* used in drug delivery systems.

Rapid, controlled release or dissolution have become significant for designing and developing novel techniques for drug delivery applications due to their advantages, such as controlling the rate, site of delivery, increasing the bioavailability, and drug solubility [30]. Most of the known delivery systems are conducted by enteral routes in the form of capsules, tablets, granules, and so on. In contrast, some are conducted by parenteral routes such as intrarterial, intravenous, intramuscular, and subcutaneous. These forms and routes of conduction have disadvantages, such as discomfort or pain and first-pass metabolism. These issues can be resolved by conducting the drugs in the buccal cavity. Incorporating the active pharmaceutical substances into the nanofibrous membrane is expedient for this motive. A nanofibrous membrane prepared by electrospinning can be wetted by salvia, disintegrating the patient's mouth and releasing drugs that absorb the buccal mucosa.

*N. sativa* or black cumin/black seed has been identified as a "miracle cure" because of its ability to cure various diseases. Researchers reported that black seeds have many therapeutic properties such as antimicrobial, antipyretic, antitumor, anti-diabetic, antihistaminic, antihypertensive, hepatoprotective, gastroprotective, and anti-inflammatory [31, 32]. Moreover, TQ is one of the important active substances of black cumin because it has many pharmacological activities such as antioxidant, anti-inflammatory, anticancer, and antibacterial properties [33]. Black cumin is hydrophobic, and therefore, it exhibits limited therapeutic effects on oral conduction.

The electrospun technique has shown in Figure 1 (a). Black cumin is used in matrix delivery systems where the drug can be either molecular dispersed or dissolved inside the black cumin membrane. The drugs in drug-loaded membranes are released through surface dissolution or diffusion, and pores are produced because of fibers' degradation, as shown in Figures 1 (b) and 1(c). The rate of drug release in a dissolution drug delivery system is mainly determined by the slow dissolution of the matrix of the dissolution medium. The matrix slowly dissolves in the dissolution medium when the matrix system is immersed in and thereby
releases the drug. Noyes and Whitney described the basic equation for matrix dissolution shown in equation 1.

\[
\frac{dM}{dt} = K (Cs - Cb)
\]  

(1)

Nernst and Brunner modified this basic equation afterward-

\[
\frac{dM}{dt} = \frac{DA}{h} (Cs - Cb)
\]

where, \(\frac{dM}{dt}\) is the dissolution rate, D is the coefficient of diffusion, A is the surface area, H is the stagnant layer thickness, Cs is saturation solubility, and C is the concentration in the dissolution medium.

Conversely, in a diffusion-based matrix system, the drug release rate is determined by the drug diffusion property of the drug in the matrix system. In this process, the dimensions of the matrix remain unchanged during the drug release. The diffusion process of molecules in a matrix can be described by Fick’s law of diffusion, represented in equation 2.

\[
J = D \left(\frac{dC}{dx}\right)
\]  

(2)

where J is the flux rate (kgm\(^{-2}\)s\(^{-1}\)), D is the diffusion constant (m\(^{2}\)s\(^{-1}\)) and \(\frac{dC}{dx}\) is concentration difference (kgm\(^{-4}\)).

![Figure 1. Depiction of the release of drugs from the electrospun fibers. (a) Schematic of electrospun technique; (b) Various physical adsorptions of drugs into electrospun nanofibers; (c) drug release from electrospun nanofiber.](https://biointerfaceresearch.com/)
3.2. Nigella sativa in targeted drug delivery systems.

In a targeted drug delivery system, *N. sativa* nanoparticles can be applied labeling with receptors or biomolecules to target cells or tissues, specifically in Figure 2. Antibodies are the most common targeted molecules in anticipation of epithelial growth factor receptors and anti-epidermal growth factor receptors (EGFR). When drugs are delivered to epithelial cells, they are specially targeted as EGFR is common in all epithelial cells. Different cancer cells are overexpressed by human epithelial receptors, and they are commonly targeted by nano-enabled targeted drug delivery [34-36]. In order to increase the therapeutic efficiency of anticancer drugs, enabling targeted drug delivery and reducing side effects, nanoparticle-based cancer therapies play an important role. Much preclinical evidence shows the potential therapeutic role of TQ against various cancers [37-40].

![Figure 2. Targeted drug delivery.](https://biointerfaceresearch.com/)

3.3. Interaction of *N. sativa* on bacteria cell.

Microbes reduce the effect of antibiotics by means of biofilm formation, and they can hide within the polymeric matrix [41]. Literature [42, 43] reported that TQ is a bioactive ingredient of *N. sativa* responsible for restraining biofilm formation among the bacteria. This is immensely beneficial in livestock production as the biofilm is generally the protective clothing used by pathogenic microbes to defend themselves from the effect of chemicals that could demolish them or antibiotics. TQ allows microbes to endure in extreme environmental situations [44]. In addition, TQ executed its antimicrobial function by blebbing and dents, preventing biofilm formation, cell lysis [45]. TQ has the capacity to generate ROS, which can damage the cellular electron transport, leading to extended oxidative stress that causes immutable damage to bacterial proteins, DNA, and membrane, effectuating cell death because of quick aging [46].

The components of *N. sativa* NPs with their manifold shapes, degree of selectivity sizes, chemical and physical stability, and high surface to volume ratios can unfold the cell membrane channels; therefore, opening the path of *N. sativa* NPs can reach their target sites. These *N.*
sativa nano encapsulations or nanoemulsions, including EOS can be adhered through hydrogen bonding, electrostatic and covalent interactions to generate antimicrobial systems.

When *N. sativa* NPs enter cell walls, they directly affect toxicity due to higher concentrations of *N. sativa* NPs redemp more ions and are distributed through the bacterial cell wall. The higher intentness of produced ions disrupts the cell membrane and ROS generation and also helps to penetrate the cell membranes [47]. DNA damages and Protein inhibition causes oxidative stress represented in Figure 3.

**Figure 3.** Proposed antibacterial mechanisms of *N. sativa*. It attacks the bacteria cell through multiple mechanisms; direct contact with the cell membrane by producing metal ions, cell membrane disruption, protein dysfunction, DNA damage, the electron transport chain inhibition, and the synchronization of bacterial metabolic processes.

**Figure 4.** Contact active antibacterial surface structure consisting of different parts.
The formation of biofilms is due to microbial contamination on artificial surfaces associated with various resilient infections and multi-resistant bacterial resistance [48]. In addition, biofilms also cause material damage because secretion can deteriorate artificial or manufactured materials [49]. These issues have intimidated human health and caused functional application of medical devices or implants [50, 51]. To conquer these issues, different contact-active antibacterial materials developed from antibacterial polymers [52, 53]. Most current contact active antibacterial surfaces can easily be blocked by residues of dead cells or biomolecules, locking intersections with pathogens and stimulating unwanted ambivalent effects [54]. Contact active antibacterial surface structure consisting of different parts is presented in Figure 4. Ecofriendly antibacterial materials that integrate anti-fouling properties and contact active biocidal are therefore propitious to prevent microbial contamination [55]. The inhibition zone of 15 mm was found in PVA- N. sativa nanofibrous membrane against S. aureus bacteria, whereas there is no inhibition zone in PVA membrane shown in Figure 5. This is due to the action of TQ and THQ.

![Figure 5](https://biointerfaceresearch.com/)  
**Figure 5.** Formation of inhibition zone or susceptibility profile (a) PVA; (b) PVA-N Sativa membrane.

### 3.4. Mechanism against viruses.

Nigella seed contains main active components such as TQ, DTQ, THQ, THY, p-cymene, and other components. TQ is an active ingredient isolated from N. sativa. It could be a favorable agent to deactivate the viral proteins since the peptides and proteins are functional groups in the side chains of the constituent amino acids (e.g., amino, imine, heterocyclic amine, thiol, and hydroxyl) and the terminal amino acid residues. It would be considered that proteins could react with TQ, although their difficulty is greater than free amino acids or related compounds. TQ can modify or damage the viral proteins. A proposed mechanism for the reaction involved is shown in Figure 4, and the generated idea was confirmed from the literature [56, 57].

THQ is willingly transformed into highly reactive TQ. TQs can modify a protein of a virus in three ways. Firstly, redox-cycling between TQs and the reduced THQs can assist in the generation of reactive oxygen species (ROS), which can be untreatable to biological events. The outcome of TQ redox-cycling would be the agent of the generation of ROS like superoxide anion radicals that can be the cause of oxidative damage of proteins. Oxidative
damage includes the formation of \( \text{H}_2\text{O}_2 \) from \( \text{O}_2^{-} \) which in turn produces either active hydroxyl radical or atomic oxygen capable of inactivating viruses.

Secondly, TQ can alkylate a protein through a nucleophilic attack. The reaction can be initiated by O, N, and S-containing nucleophilic amino acids in a protein. A proposed formation mechanism for the TQ or viral protein adduct follows the Michael addition \[58\]. Thirdly, TQs can induce protein cross-linking. In this pathway, TQs react with a nucleophilic amino acid, which is the residue of a protein deriving in amino acid oxidation, leading to the fabrication of aldehyde-containing amino acid, which can then condense with an amino acid residue from another protein molecule and generate intramolecular cross-linking. A reiterative cross-linking may cause the generation of oligomers and, ultimately, polymeric aggregates.

Generally, it is considered that the main mechanism for TQ-induced protein modifications occurs through abducting formation, as suggested by literature \[59, 60, 61, 62\],...
Proteins can be converted into ammonia and carbon dioxide in a series of biochemical reactions in the human body.

Some recent researches have been conducted on *N. sativa* for stating the opportunities of producing new medicine, antiviral face mask, anti-inflammatory, tissue growth stimulation, antioxidative approaches, anticancer effectiveness, covid-19 survival, toxicity reduction from nanoparticles, minimization of diabetic and cardiovascular diseases, and related health problems [64-76]. In the future, the mechanisms of different ingredients of *N. sativa* on different health problems need to be clearly understood for better treatment, and thus the research gaps from these recent studies in relation to the present study need to be investigated.

This research work shows the scopes of *N. sativa* in microbial process and drug delivery system, which will act as state of the art for the researchers to carry out more research in this field to invent medicine from *N. sativa* for the benefit of a human being. *N. sativa* is already used as an anticancer and anti-inflammatory agent in pharmaceutical applications. The cytotoxic impacts of various *N. sativa* seeds as an adjunct treatment to doxorubicin on human MCF-7 breast cancer cells were assessed [77]. Moreover, the carcinogenic influences of dimethyl-benz(a)anthracene carcinogen in mammary carcinoma decreased by the applications of these seeds [78]. Anti-inflammatory and analgesic properties are found in the aqueous extract of these seeds [79].

### 4. Conclusions

A mechanism for the deactivation of viruses by the interaction of TQ and THQ has been conceptually proposed and explained. A development of PVA-nigella nanofibrous membrane is also introduced that offers bacterial resistance against *S. aureus* bacteria, and thus it can be used as a wound dressing material. The potential of using *N. sativa* NPs in various fields increases the need to generate them on an industrial scale. Therefore, much effort has been made to utilize this natural resource and implement biological production processes with proven advantages such as being easy to scale up, environment friendly, and cost-effective. *N. sativa* nanofibrous membrane would be a proficient choice for targeted and controlled delivery of therapeutic agents with their potential applications in tissue engineering, pharmaceuticals, surgical implants, cancer therapeutics, and wound dressing. Although, it is considered that TQ has a comparatively poor capacity to douse free radicals due to its oxidized form. In the future, there is room for further scientific research on components of Nigella to boost human immunity along with proper drugs against corona and other viruses. Several studies revealed that *N. sativa* and its components have outstanding natural therapy for treating a significant range of illnesses such as neurologic disorders, dyslipidemia, hypertension, and cancer. Both human and animal studies found that *N. sativa* and TQ have antioxidant properties useful as dietary supplements with minimum side effects.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Behera, B.K.; Varma, A. Concept of Microbiological Processes. Microbial Biomass Process Technologies and Management 2017, 1–43, https://doi.org/10.1007/978-3-319-53913-3.
2. NIH. Drug Delivery Systems: Getting Drugs to Their Targets in a Controlled Manner. National Institute of Biomedical Imaging and Bioengineering. Science Education 2016.
3. Felice, B.; Prabhakaran, M.P.; Rodríguez, A.P.; Ramakrishna, S. Drug delivery vehicles on a nano-engineering perspective. Mater. Sci. Eng. C 2014, 41, 178–195, https://doi.org/10.1016/j.msec.2014.04.049.
4. Vega-Vásquez P.; Mosier N.S.; Joseph, I. Nanoscale Drug Delivery Systems: From Medicine to Agriculture. Frontiers in Bioengineering and Biotechnology 2020, 8, 79, https://doi.org/10.3389/fbioe.2020.00079.
5. Kondrová, Eliška, Stopka, P.; Souček, P. Cytochrome P450 destruction by benzene metabolites 1, 4-benzoquinone and 1, 4-hydroquinone and the formation of hydroxyl radicals in minipig liver microsomes. Toxicology in vitro 2007, 21, 566-575, https://doi.org/10.1016/j.tiv.2006.11.002.
6. Mullahy, Brooks. Plants That Save Lives: a report from an International Symposium on Medicinal Plants. Botanic Gardens Conservation News 1994, 3, 25-27, https://www.bgci.org/files/Worldwide/Publications/PDFs/medicinal.pdf.
7. Fabricant, Daniel, S.; Norman, R.; Farnsworth. The value of plants used in traditional medicine for drug discovery. Environmental health perspectives 2001, 69-75, https://doi.org/10.1289/ehp.01109a169.
8. Clardy, Jon, Walsh, C. Lessons from natural molecules. Nature 2004, 432, 829-837, https://www.nature.com/articles/nature03194.
9. Shakya, Kumar, A. Medicinal plants: future source of new drugs. International Journal of Herbal Medicine 2016, 4, 59-64, https://doi.org/10.13140/RG.2.1.1395.6085.
10. Atef, Nagwa, M.; Sanaa, M.; Shanab, Sahar, I.; Negm, Yasmeen, A.; Abbas. Evaluation of antimicrobial activity of some plant extracts against antibiotic susceptible and resistant bacterial strains causing wound infection. Bulletin of the National Research Centre 2019, 43, 144, https://doi.org/10.1186/s42269-019-0184-9.
11. Chowdhury, M.A.; Hossain, N.; Kashem, M.A.; Shahid, M.A.; Alam, A. Immune response in COVID-19: A review. Journal of Infection and Public Health 2020, 13, 1619-1629, https://doi.org/10.1016/j.jiph.2020.07.001.
12. Takruri, Hamed, R.H.; Majdoleen, A.F.; Dameh. Study of the nutritional value of black cumin seeds (Nigella sativata). Journal of the Science of Food and Agriculture 1998, 76, 404-410, https://doi.org/10.1002/SICID.1097-0010(199803)76:3&4<p>404:&gt;AID-JSFA964+3.0.CO;2-L</a>
13. Ramadan, Fawzy, M. Nutritional value, functional properties and nutraceutical applications of black cumin (Nigella sativa L.); an overview. International journal of food science & technology 2007, 42, 1208-1218, https://doi.org/10.1111/j.1365-2621.2006.01417.x.
14. Hannan, M.; Rahman, M.; Sohag, A. A. M.; Uddin, M.; Dash, R.; Sikder, M. H.; Timalsina B. Black Cumin (Nigella sativa L.); A Comprehensive Review on Phytochemistry, Health Benefits, Molecular Pharmacology, and Safety. Nutrients 2021, 6, 1784, https://doi.org/10.3390/nu13061784.
15. Ahmad, Aftab, Husain, A.; Mugeeb, M.; Khan, S.A.; Najmi, A.K.; Siddique, N.A.; Damanhour, Z.A.; Anwar, F. A review on therapeutic potential of Nigella sativa: A miracle herb. Asian Pacific journal of tropical biomedicine 2013, 3, 337-352, https://doi.org/10.1016/S2221-1691(13)60075-1.
16. Al-Mufarrej, S.I. Immune-responsiveness and performance of broiler chickens fed black cumin (Nigella sativa L.) powder. Journal of the Saudi Society of Agricultural Sciences 2014, 13, 75-80, https://doi.org/10.1016/j.jssas.2013.01.006.
17. Azeeem, T.; Zaib-Ur-Rehman, U.S.; Asif, M.; Arif, M.; Rahman. A. Effect of Nigella sativa on poultry health and production: a review. Science Letter 2014, 2, 76-82, https://doi.org/10.1155/2021/2070375.
18. Amine, T.Z.; Askoua, M.; Elkouali, M.; Talbi, S.; Lahsasni, I.; Warad, T.; Hadda, B. Chemical composition and antibacterial activity of essential oil of Nigella sativa seeds from Beni Mellal (Morocco): What is the most important part, Essential Oil or the rest of seeds. Journal of Materials and Environmental Science 2014, 5, 2017-2020, https://www.semanticscholar.org/paper/Chemical-composition-and-antibacterial-activity-of-Amine-Askoua/5e3a58c0d0c7b1ad52d4c71b659668afebeb7376c.
19. Benkaci-Ali, Farid, Akloul, R.; Boukenouche, A.; Pauw, E.D.; Chemical composition of the essential oil of Nigella sativa seeds extracted by microwave steam distillation. Journal of Essential Oil-Bearing Plants 2013, 16, 781-794, https://doi.org/10.1080/0972060X.2013.813275.
20. İŞİK, Selin, Kartal, M.; Erdem, S.A. Quantitative analysis of thymoquinone in Nigella sativa L. (Black Cumin) seeds and commercial seed oils and seed oil capsules from Turkey 2017, https://doi.org/10.1501/Eczfak_000000593.
21. Lodish, Harvey, Berk, A.; Zipursky, S.L.; Matsudaira, P.; Baltimore, D.; Darnell, J. Viruses: Structure, function, and uses. In Molecular Cell Biology 2000, https://www.ncbi.nlm.nih.gov/books/NBK21523/.
Nigella sativa (black cumin) seeds contain a variety of compounds that exhibit diverse biological and therapeutic effects. These seeds are known for their antioxidant, anti-inflammatory, and anti-microbial properties. Nigella sativa is also known for its potential in cancer therapy, as thymoquinone, a major monoterpenoid compound, shows significant antimicrobial activity against anaerobic bacteria.

Recent studies suggest that Nigella sativa seeds may have neuroprotective effects and could be beneficial in Alzheimer's disease treatment. Furthermore, the oil extracted from Nigella sativa seeds is being investigated as a sustainable technology for water cleansing.

In conclusion, the therapeutic potential of Nigella sativa seeds highlights their importance in contemporary research and development, and underscores the need for further investigations into their applications in medical and pharmaceutical fields.
42. Chaieb, Kamel, Koundhi, B.; Jrah, H.; Mahdouani, K.; Bakhrouf, A. Antibacterial activity of thymoquinone, an active principle of *Nigella sativa* and its potency to prevent bacterial biofilm formation. *BMC complementary and alternative medicine* **2011**, *19*, https://doi.org/10.1186/1472-6882-11-29.

43. Mah, Thien-Fah, C.; George, A; O'Toole. Mechanisms of biofilm resistance to antimicrobial agents. *Trends in microbiology* **2001**, *9*, 34-39, https://doi.org/10.1016/s0966-842x(00)01913-2.

44. Goel, Surbhi, Mishra, P. Thymoquinone inhibits biofilm formation and has selective antibacterial activity due to ROS generation. *Applied microbiology and biotechnology* **2018**, *102*, 1955-1967, https://doi.org/10.1007/s00253-018-8736-8.

45. Martinovich, G.G.; Martinovich, I.V.; Vcherashniaya, A.V.; Shadyro, O.I.; Cherenkevich, S. N. Thymoquinone, a biologically active component of *Nigella sativa*, induces mitochondrial production of reactive oxygen species and programmed death of tumor cells. *Biophysics* **2016**, *61*, 963-970, https://doi.org/10.1134/S0006350916060154.

46. Wu, Jieran, Shu, Q.; Niu, Y.; Jiao, Y.: Chen, Q. Preparation, characterization, and antibacterial effects of chitosan nanoparticles embedded with essential oils synthesized in an ionic liquid containing system. *Journal of agricultural and food chemistry* **2018**, *66*, 7006-7014. https://doi.org/10.1021/acs.jafc.8b01428.

47. Tuson, Hannah, H.; Douglas, B.; Weibel. Bacteria–surface interactions. *Soft matter* **2013**, *9*, 4368-4380, https://doi.org/10.1039/C3SM27705D.

48. Siedenbiedel, Felix, Joerg C. Tiller. Antimicrobial polymers in solution and on surfaces: overview and functional principles. *Polymers* **2012**, *4*, 46-71, https://doi.org/10.3390/polym4010046.

49. Asri, ATW, L.; Crismani, M.; Roest, S.; Chen, Y.; Ivashenko, O.; Rudolf, P.; Tiller, J.C.; Mei, H.C.; Loontjens, T.J.; Busscher, H.J. A shape-adaptive, antibacterial-coating of immobilized quaternary-ammonium compounds tethered on hyperbranched polyurea and its mechanism of action. *Advanced Functional Materials* **2014**, *24*, 346-355, https://doi.org/10.1002/adfm.201301686.

50. Kazemzadeh-Narbat, Mehdi, Lai, B.F.; Ding, C.; Kizhakkedathu, J.N.; Hancock, R.E.; Wang, R. Multilayered coating on titanium for controlled release of antimicrobial peptides for the prevention of implant-associated infections. *Biomaterials* **2013**, *34*, 5969-5977, https://doi.org/10.1016/j.biomaterials.2013.04.036.

51. Xiong, Meng-Hua, Li, Y.; Bao, Y.; Yang, X.; Hu, B.; Wang, J. Bacteria-responsive multifunctional nanogel for targeted antibiotic delivery. *Advanced Materials* **2012**, *24*, 6175-6180, https://doi.org/10.1002/adma.201202847.

52. Fuchs, Andreas, D.; Joerg, C.; Tiller. Contact-active antimicrobial coatings derived from aqueous suspensions. *Angewandte Chemie International Edition* **2006**, *45*, 6759-6762, https://doi.org/10.1002/anie.200602738.

53. Cheng, Gang, Xue, H.; Zhang, Z.; Chen, S.; Jiang, S. A switchable biocompatible polymer surface with self-sterilizing and nonfouling capabilities. *Angewandte Chemie* **2008**, *120*, 8963-8966, https://doi.org/10.1002/anie.200800800.

54. Sundaram, Subramanian, H.; Ella-Menye, J.; Brault, N. D.; Shao, Q.; Jiang, S. Reversibly switchable polymer with cationic/zwitterionic/anionic behavior through synergistic protonation and deprotonation. *Chemical Science* **2014**, *5*, 200-205, https://doi.org/10.1039/c3sc52233d.

55. Vaughn, Albert, R.; Caitlin, B.; Redman, Kang, S. M.; Kim, J. Biological implications of 2chlorocyclohexa-2, 5-diene-1, 4-dione toward ribonuclease A. *2013*, https://doi.org/10.4236/abb.2013.41004.

56. Kim, Joosook, Vaughn, A. R.; Cho, C.; Alba, T. V.; Carver, E. A.: Modifications of ribonuclease A induced by p-benzquinone. *Bioorganic chemistry* **2012**, *40*, 92-98, https://doi.org/10.1016/j.bioorg.2011.11.002.

57. Mihara, Satoru, Shibamoto, T. The role of flavor and fragrance chemicals in TRPA1 (transient receptor potential cation channel, member A1) activity associated with allergies. *Allergy, Asthma & Clinical Immunology* **2015**, *11*, 1-12, https://doi.org/10.1186/s13233-015-0074-0.

58. Hanzlik, Robert, P.; Shaw, P.; Harriman, Frauenhoff, M. M. Covalent binding of benzoquinone to reduced ribonuclease. Adduct structures and stoichiometry. *Chemical research in toxicology* **1994**, *7*, 177-184, https://doi.org/10.1021/tr00038a010.

59. McDonald, Thomas, A.; Waidyanatha, S.; Rappaport, S.M. Production of benzoquinone adducts with hemoglobin and bone-marrow proteins following administration of [13C6] benzene to rats. *Carcinogenesis* **1993**, *14*, 1921-1925, https://doi.org/10.1093/carcin/14.9.1921.

60. Fisher, Ashley, A.; Labenski, M. T.; Malladi, S.; Chapman, J. D.; Bratton, S. B.; Monks, T. J.; Lau, S. S. The frequency of 1, 4-benzoquinone-lysine adducts in cytochrome c correlate with defects in apoptosome activation. *Toxicological Sciences* **2011**, *122*, 64-72, https://doi.org/10.1093/toxsci/kfr085.

61. Person, Maria, D.; Mason, D.E.; Liebler, D.C.; Monks, T. J.; Lau, S. S. Alkylation of cytochrome c by (glutathion-S-y1)-1, 4-benzoquinone and iodoacetamide demonstrates compound-dependent site specificity. *Chemical research in toxicology* **2005**, *18*, 41-50; https://doi.org/10.1021/tr049873n.

62. Zaborska, Wieslawa, Krajewska, B.; Kot, M.; Karcz, W. Quinone-induced inhibition of urease: Elucidation of its mechanisms by probing thiol groups of the enzyme. *Bioorganic Chemistry* **2007**, *35*, 233-242, https://doi.org/10.1016/j.bioorg.2006.11.001.
63. Shahid, M.A.; Rahim, A.; Chowdhury, M.A.; Kashem, M.A. Development of antibacterial nanofibrous wound dressing and conceptual reaction mechanism to deactivate the viral protein by Nigella sativa extract. *Advances in Traditional Medicine* **2021**, *1*, 1–9, https://doi.org/10.1007/s13596-020-00538-3.

64. Chowdhury, M.A.; Shuvho, M.B.A.; Shahid, M. A.; Haque, A. M.; Kashem, M. K.; Lam, S. S.; Ong, H. C.; Uddin, M.A.; Mofijur, M. Prospect of biobased antiviral face mask to limit the coronavirus outbreak. *Environmental Research* **2021**, *192*, 110294, https://doi.org/10.1016/j.envres.2020.110294.

65. Sallehuddin, Nusaibah, Abid Nordin, Ruszymah Bt Hj Idrus, and Mh Busra Fauzi. *Nigella sativa* and its active compound, thymoquinone, accelerate wound healing in an in vivo animal model: a comprehensive review. *International journal of environmental research and public health* **2020**, *17*, 4160, https://doi.org/10.3390/ijerph17114160.

66. Mani, J.; Ruchi, Sehgal, N.; Dogra, N.; Saxena, S.; Katare, D.P.; 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. *Journal of Biomolecular Structure and Dynamics* **2020**, *1*-13, https://doi.org/10.1080/07391102.2020.1839560.

67. Arazmjoo, Sanaz, Es-haghi, A.; Mahmoodzadeh, H. Evaluation of anticancer and antioxidant properties of nanoemulsions synthesized by *Nigella sativa* L. tincture. *Nanomedicine Journal* **2021**, *8*, https://civilica.com/doc/1186847.

68. Hannan, M.; Rahman, M.; Sohag, A.A.M.; Uddin, M.; Dash, R.; Sikder, M. H.; Timalsina B. Black Cumin (*Nigella sativa* L.): A Comprehensive Review on Phytochemistry, Health Benefits, Molecular Pharmacology, and Safety. *Nutrients* **2021**, *13*, 1784, https://doi.org/10.3390/nu13061784.

69. Sharmin, Eram, Batubara, A.S.; Tamboosi, B.A.; Khozay, E.B.A.; Alamoudi, M.K.; Aidaros, O.Z.A.; Albenayan J.A. PVA nanocomposite hydrogel loaded with silver nanoparticles enriched *Nigella sativa* oil. *Inorganic and Nano-Metal Chemistry* **2021**, *1*-9, https://doi.org/10.1080/24701556.2021.1963277.

70. Ghamari, Ata, M.; Amirii, S.; Rezaazadeh-Bari, M.; Rezaazad-Bari, L. Physical, mechanical, and antimicrobial properties of active edible film based on milk proteins incorporated with *Nigella sativa* essential oil. *Polymer Bulletin* **2021**, *1*-21, https://doi.org/10.1007/s00289-021-03550-y.

71. Badary, Osama A.; Hamza, M.S.; Tikamdas, R.; Thymoquinone: A Promising Natural Compound with Potential Benefits for COVID-19 Prevention and Cure. *Drug Design, Development and Therapy* **2021**, *15*, 1819, https://doi.org/10.2147/DDDT.S308863.

72. Ahmad, Faruque, M.; Ahmad, F.A.; Ashraf, S.A.; Saad, H.H.; Wahab, S.; Khan, M.I.; Ali, M.; Mohan, S.; Hakeem, K.R.; Athar, M.T. An updated knowledge of Black seed (*Nigella sativa* Linn.): Review of phytochemical constituents and pharmacological properties. *Journal of herbal medicine* **2021**, *25*, 100404, https://doi.org/10.1016/j.jhermed.2020.100404.

73. Salehi, Bahare, Quispe, C.; Imran, M.; Ul-Haq, I.; Živković, I.I.; Abu-Reidah, I. A.; Sen S. *Nigella Plants–Traditional Uses, Bioactive Phytoconstituents, Preclinical and Clinical Studies*. *Frontiers in Pharmacology* **2021**, *12*, 417, https://doi.org/10.3389/fphar.2021.625386.

74. Hadi, Saeid, Daryabegyi-Khotbehsara, R.; Mirmiran, P.; McVicar, V.I.; Hadi, A.; Soleimani, D.; Askari, G.; Effect of *Nigella sativa* oil extract on cardiometabolic risk factors in type 2 diabetes: A randomized, double-blind, placebo-controlled clinical trial. *Phytotherapy Research* **2021**, https://doi.org/10.1002/ptr.6990.

75. Razmpoosh, Elham, Safi, S.; Nadjarzadeh, A.; Fallahzadeh, H.; Abdollahi, N.; Mazaheri, M.; Nazari, M.; Salehi-Abargouei, A. The effect of *Nigella sativa* supplementation on cardiovascular risk factors in obese and overweight women: a crossover, double-blind, placebo-controlled randomized clinical trial. *European Journal of Nutrition* **2021**, *60*, 1863–1874, https://doi.org/10.1007/s00394-020-02374-2.

76. Mahmoud, Sherif, S.; Torchilin, V.P. Hormetic/cytotoxic effects of *Nigella sativa* seed alcoholic and aqueous extracts on MCF-7 breast cancer cells alone or in combination with doxorubicin. *Cell biochemistry and biophysics* **2013**, *66*, 451-460, https://doi.org/10.1007/s12123-012-9493-4.

77. Zaky, Ahmed A.; El-Aty A.M.A review on extraction, characterization, and applications of bioactive peptides from pressed black cumin seed cake. *Frontiers in Nutrition* **2021**, *609*, https://doi.org/10.3389/fnut.2021.743909.

78. Shuid, Nazrun, A.; Mohamed, N.; Mohamed, I.N.; Othman, F.; Suhaimi, F.; Ramli, E.S.M.; Muhammad, N.; Soelaiman, I.N. *Nigella sativa*: A potential antiosteoporotic agent. *Evidence-Based Complementary and Alternative Medicine* **2012**, https://doi.org/10.1155/2012/696230.