Development of antibacterial nanofibrous wound dressing and conceptual reaction mechanism to deactivate the viral protein by *Nigella sativa* extract

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Received: 28 May 2020 / Accepted: 14 December 2020 / Published online: 13 January 2021
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Abstract

*Nigella sativa* (*N. sativa*) is extensively used as medicinal plant all over the world. It has the potential properties as the antiviral and antibacterial application. Its seed contain thymoquinone (TQ), thymohydroquinone (THQ), thymol (THY), p-cymene as major and other minor components. TQ and THQ exhibit broad spectrum of antimicrobial properties against the activity of bacteria, viruses, parasites, schistosoma and fungi. This work provides credence to the fabrication of antibacterial nanofibrous membrane by electrospinning machine from *N. sativa* extract with polyvinyl alcohol (PVA) solution for wound dressing. The morphology of the developed membrane is also characterized using scanning electron microscope. Fourier transform infrared spectroscopy (FTIR) data has been showed that the functional groups of *N. sativa* are present in the prepared PVA-*N. sativa* nanofibrous membrane and its antibacterial activity was investigated. The disk diffusion method has been used to evaluate the antibacterial activity of PVA-*N. sativa* nanofibrous membrane against *Staphylococcus aureus* (*S. aureus*) bacteria and the inhibition zone with a value of 10 mm is formed. Considering the inherent properties of *N. sativa*, a conceptual reaction mechanism has been proposed to deactivate the viral proteins by the action of TQ and THQ.

Keywords *Nigella sativa* · Electrospinning · Nanofibrous membrane · *Staphylococcus aureus* · Viral protein

Introduction

*Nigella sativa* is a famous and special plant that has been widely used in different cultures for many centuries (Saad et al. 2005; Mohammed and Babikir 2013). It is commonly known as black seed, posses several medicinal properties. In herbal medicine, this seed has been extensively reported to treat asthma, hypertension, gastric disorders, liver disorders, immune disorders, cancer, neurological disorders, and many other health conditions (Majeed et al. 2020; Mahmoud and Abdelraazek 2019). From the religious background, it is called the miracle curative herb for all ailments, except the death (Mahboubi 2018). Enhancement of immunity system in human body, *N. sativa* extract has inhibitory response on the human immune deficiency virus protease but the active principle happens for this activity was not determined. *N. sativa* seed and its oil are used as a wound healing in farm animals (Aljabre et al. 2015). The novel coronavirus 2019 (2019-nCoV), officially named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a newly emerged human infectious coronavirus that contains four proteins (Lai et al. 2020). Many different treatment options have been suggested and some older drugs are used to treat patients even though much more works are demanded. The supportive treatment, including oxygen therapy, conservation fluid management, and the use of broad-spectrum antibiotics to cover secondary bacterial infection remains to be the most important management (Huang et al. 2020). Considering the limitations of treatments of virus with
existing and new medicine, this study suggested the possibility of antiviral medicine from \textit{N. sativa} components. \textit{N. sativa} seeds contain 36–38% fixed oils, proteins, alkaloids, saponin and 0.4–2.5% essential oil. Many components of essential oil were characterized but the major ones were TQ (27.8–57.0%), \textit{p}-cymene (7.1–15.5%), carvacrol (5.8–11.6%), \textit{t}-anethole (0.25–2.3%), 4-terpineol (2.0–6.6%) and longifoline (1.0–8.0%). TQ can readily dimerizes to form DTQ (Ali and Blunden 2003). Recently, clinical and experimental studies have demonstrated many therapeutic effects of TQ including immunomodulatory, anti-inflammatory, antitumor and antimicrobial (Ahmad et al. 2013; Al-Mufarrej 2014; Azeem et al. 2014). Several bioactive compounds from the seed of \textit{N. sativa} have been reported in the literature; among those the most important bioactive ones are TQs. Due to a vast number of biological targets and virtually no side effects, \textit{N. sativa} has achieved the potential therapeutic interest to cure immunosuppressive viral diseases. The essential oil composition (0.4–0.45%) reported in various studies represented about forty different compounds, amongst the abundantly constituents identified are \textit{trans}-anethole, \textit{p}-cymene, limonene, carvone, \textit{\alpha}-thujene, TQ, THQ, DTQ, carvacrol, and \textit{\beta}-pinene with various concentrations (Ainane et al. 2014; Benkaci-Ali et al. 2013; Tohumiari 2017). Nanofibrous membrane have been produced by electrospinning technique from natural, modified natural and synthetic polymers has drawn a lot of attention for few years for their applications in biomedical fields like wound dressing, tissue engineering, filtration media and drug delivery. Natural polymers have drawn much attraction due to their excellent properties like biocompatibility, biodegradation, low toxicity, high porosity, light weight and most prominently large surface area (Guarino and Ambrosio 2018). Usually metal nanoparticles are incorporated to offer antibacterial properties in the nanofibrous materials. Due to the harmful effects of nanoparticles and synthetic antibiotics on the environment, human health and/or bacteria resistance issues, the use of natural antimicrobial compounds are still demanding because of keeping safe sustainable environment and less prone to create resistant bacteria (Abdullah et al. 2014; Agarwal et al. 2008; Venugopal et al. 2008). Hence, the development of nanofibrous membrane through electrospinning technique embedding the component of \textit{N. sativa} like natural biocide has a great importance in this field. This study investigates the antibacterial efficacy of the nanomembrane developed from the mixture of PVA and \textit{N. sativa} extract to nullify the use of conventional metal nanoparticles into such type of antibacterial nano-structures (Shahid et al. 2020; Ali and Shahid 2019; Ali et al. 2019). To the best of our knowledge, there is no details explanation of reaction mechanism to deactivate the virus and their consequences by the components of \textit{N. sativa} seed. In the present study, a theoretical mechanism has been proposed to deactivate the virus. This concept can be used in future to make a drug against virus for survival of human life of the world.

**Method**

**Material**

Seeds of \textit{N. sativa} were collected from the market of Gazipur city, Bangladesh. PVA (molecular weight 115,000, degree of polymerization 170-800, viscosity 26-32cps, 99% hydrolyzed granules was sourced from Loba chemical, India and absolute methanol of 99% purity was sourced from Merck, Germany. All of the chemicals were used without further purification or modification. Viral protein is considered to develop the conceptual reaction mechanism by the action of TQ and THQ.

**Fabrication of PVA-\textit{N. sativa} Nanofibrous Membrane**

The seeds of \textit{N. sativa} were washed thoroughly with mineral water and grinded to reduce its size and then immersed into methanol of sigma-aldrich at the ratio of 1: 2 (\textit{N. sativa}; methanol) for 8 h. This solution was filtered through a quadruple layer of nylon mesh fabric two times and it was magnetically stirred at 70 °C for 8 h in order to obtain the \textit{N. sativa} extracted solution. On the other hand, solution of 10% (w/v) PVA was prepared at 70 °C by dissolving the PVA grains in 50% aqueous acetic acid solution. Final solution to feed into the electrospinning machine was made by adding 17 g of \textit{N. sativa} extract to 30 mL of PVA solution and stirred until a homogeneous solution was obtained. Electrospinning machine of model: TL-01, Tong Li tech. China was used to fabricate the nanofibrous membrane by optimizing the process parameters of voltage, pressure, heater power, collector distance under ambient condition of 65% relative humidity and 27 °C temperature. The parameters were optimized by trial and error method at voltage of −12.3 kV, +23 kV; heater power of 0.45 kW; collector distance of 15 cm and flow rate of 1.5 mL to fabricate the PVA-\textit{N. sativa} nanofibrous membrane.

**Characterization of PVA-\textit{N. sativa} nanofibrous membrane**

Scanning electron microscopy (SEM) of model: SU 1510, Hitachi, Japan was used to observe the morphology of developed sample at 5 kV and 2kX magnification. FTIR (IR Prestige 21, Shimadzu Corporation, Japan) was used to analyze the spectra of the sample at 4000-698 cm\(^{-1}\) resolution. Antibacterial activity of the sample was studied using...
disc diffusion method (1.5X10^5 CFU/mL) against *S. aureus* bacteria as it is more responsible for wound infection and zone of inhibition (ZOI) was measured and compared with PVA nanofibrous membrane (Shahid et al. 2020).

**Results**

**SEM, FTIR and antibacterial test analysis**

The image of SEM is shown in Fig. 1. It reflects the formation of nanofibers having an average diameter of 215 nm and shows tiny pores in it. The presence of tiny pores in wound dressing materials is effective because diffusion and permeability of oxygen from air to skin is provided through tiny pores. The existence of such type pores does not only allow air ventilation but also restrict the penetration of bacteria in the wound area (Liu et al. 2010; Yang et al. 2009).

The FTIR spectra of PVA and PVA-*N. sativa* nanofibrous membrane are shown in Fig. 2. The characteristic peaks of PVA-*N. sativa* nanofibrous membrane in FTIR spectra have been found at 3009 cm⁻¹ (C–H stretching of vinyl group), 2927 cm⁻¹ (C–H stretching), 2858 cm⁻¹ (C–H stretching of aliphatic group), 1716 cm⁻¹ (C=O stretching for ester and ketone groups), 1463 cm⁻¹ (C–H absorption scissoring) and 1087 cm⁻¹ (C–O–C stretching) (Mansur et al. 2008). In FTIR analysis, various components and functional groups like C=O, C–O, CH₃, CH₂ chemical constituents are present in PVA-*N. sativa* nanofibrous membrane. FTIR data also showed the presence of O–H, C=O and C–O for acetate group in PVA nanofibrous membrane. These results indicate the presence of *N. sativa* components in PVA-*N. sativa* nanofibrous membrane.

Antibacterial activities of developed samples were investigated by using disc diffusion method against *S. aureus* bacteria and ZOI was measured. The ZOI of PVA and PVA-*N. sativa* nanofibrous membrane are shown in Fig. 3. ZOI of 10 mm is formed in PVA-*N. sativa* nanofibrous membrane whereas there is no inhibition zone in PVA nanofibrous membrane against *S. aureus* bacteria. The *S. aureus* Gram-positive bacteria is highly susceptible to TQ and THQ (Mohammed et al. 2019). This result is similar with literatures (Khan 2018; Park et al. 2015) that stated the mechanism of killing bacteria with components of *N. sativa*.

![Fig. 1 Morphology of PVA-*N. sativa* nanofibrous SEM image at 10 kV and 5kX magnification](image1)

![Fig. 2 FTIR spectra of PVA and PVA- *N. sativa* membrane scanned at 4000–698 cm⁻¹](image2)
Deactivation mechanism of viral proteins

*N. sativa* contains active ingredients like TQ, THQ, DTQ, THY, p-cymene and other minor components. Chemical structures of the main components are given in Fig. 4.

Some recent interesting research findings of *N. sativa* and its components are provided in Table 1.

TQ is an active ingredient which is widely used as medicinal compound isolated from *N. sativa*. This component of *N. sativa* will be a promising agent to deactivate the virus. Virus consists of different types of proteins (Lodish et al. 2000; Schoeman and Fielding 2019; Seebach et al. 2004; Shereen et al. 2020). If we can modify or damage the proteins by active components of *N. sativa* consequently virus will be deactivated. For clear understanding, we consider TQ and THQ components for the reaction with viral proteins. TQ may modify/damage the proteins. A proposed mechanism for the reaction involved is presented in Fig. 5 (Vaughn et al. 2013; Kim et al. 2012; Mohammed et al. 2019; Armutcu et al. 2018; Kundu et al. 2014).

THQ is converted into highly reactive TQ by oxidation and THQ formed by reduction of TQ. Formation of THQ...
### Table 1  Some recent interesting research findings using *N. sativa* and its components

| Form/chemicals | Dose/R.O./Test systems | Activity | References |
|----------------|-------------------------|----------|------------|
| TQ             | 1 μM/mL and 2 mg/200μL(s.c.) with olive oil in rats | Cure for polycystic ovary via NF-κB signaling pathway | Arif et al. (2016) |
| Oil            | 2.5 mL in infertile men(n = 34) for 2 months | Enhanced abnormal semen quality without producing any adverse effect | Kolahdooz et al. (2014) |
| Oil            | –                      | Lessen total cholesterol, LDL-C, and TG levels and increased HDL-C | Sahebkar et al. (2016) |
| Seeds ethanol extract | 250-mg/kg(p.o.) in female Wistar Albino rats | Remarkable nephroprotective activity on paracetamol-induced nephrotoxicity | Canayakin et al. (2016) |
| Essential oil  | 5-50 g/L for antioxidant assays 0.2-2.0 μg/mL for antimicrobial | Produced antioxidant activity and protected the Artemia spp. after experimental infection of Vibrio parahaemolyticus Dahv-2 | Manju et al. (2016) |
| Lipid (4%) and volatile (3%) fractions | In streptozotocin induce diabetes mellitus Sprague–Dawley rats for 56 days | Lessen toxicological and adverse consequences of diabetes mellitus | Sultan et al. (2014) |
| Oil            | p.o. Administration in 22-50yrs old patients | Lessen thyroid stimulating hormone(TSH) and anti-TPO antibodies in patients with Hashimoto’s thyroiditis | Tajmiri et al. (2016) |
| Essential oil Nanoemulsion (20-50 nm diameter) | 20-80μL/mL in MCF-7cells | Produced cell membrane blebbing, cytoplasmic vacuolation, marginalization of chromatin, and fragmentation of the nucleus | Periasamy et al. (2016) |
| Phenolic-Protein complexes | 100μL in vitro test | Antioxidant and ACE inhibitory properties | Alu’datt et al. (2016) |
| Diethyl ether extract(25-400 μg/disc) | Filter paper disc impregnated for antibacterial test | Fruitful result against Gram-positive(*Staphylococcus aureus*) Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*), *C. albicans*,(not effective on *Salmonella typhimurium*), also effective against *staphyloccocal* infection in mice | Hanafy and Hatem (1991) |
| Oil administered continuously for 3 months a dose (450 mg three times daily) | Patient with hepatitis C virus (HCV) infection who were not suitable for IFN-α therapy for antiviral test | The oil notably improved HCV viral load | Barakat et al. (2013) |
from TQ may occur by two steps. TQ can be converted to semiquinone radical by accepting one electron and one hydrogen ion. In the same way, THQ is formed from semi-quinone radical. TQs can react with viral proteins in two different ways. First, redox-cycling between TQs and the reduced form (THQs) can lead to the formation of reactive oxygen species (ROS) which can be fatal to biological events. The outcome of TQ redox-cycling would be the generation of ROS such as superoxide ion radical which can cause oxidative damage of proteins. Oxidative damage includes formation of H$_2$O$_2$ from O$_2^-$ which in turn produces hydroxyl radical in presence of metal ion that causes virus inactive and production of water. Second, the functional groups of peptides and proteins are those of the side chains of the constituent amino acids and the terminal amino acid residues, it would be expected that proteins could react with TQs, and such reactions do occur, although their complexity is greater than that observed for the free amino acid or related compound. TQs can alkylate a protein via a nucleophilic attack by the protein, undergoing adducts formation with the reacting protein. Characteristic reactions of TQ include the addition of nucleophilic groups to an unsaturated carbon of the TQ. Carbon carbon double bonds conjugated with electron sinks may serve as substrates for nucleophilic addition reactions. The reaction can be initiated by N, O, and S-containing nucleophilic amino acids in a protein. A proposed formation mechanism for the TQ/protein adduct follows the Michael addition (Mihara and Shibamoto 2015). The free carbon center of TQ is highly reactive center for nucleophiles of proteins' residues. As a result, the amount of adduct formation is higher than that of other reaction pathways. So the adduct formation with proteins follows the main mechanism of modifying proteins which were studied by researchers utilizing mass spectrometric approach, target enzyme activity assays, $^{14}$C labeling experiments and ESI–MS approach (Kondrová et al. 2007; Zaborska et al. 2007; Fisher et al. 2007, 2011; Person et al. 2003; Hanzlik et al. 1994). Total components obtained from *N. sativa* extract i.e., TQ, THQ, DTQ, THY, and p-cymene etc. may react with more proteins and deactivation of more viruses occur as well. In human body, proteins can be converted into ammonia and carbon dioxide in a set of biochemical reactions. Protein which is taken as diet is broken down to constituent amino acids, which are then converted to carbon dioxide, water, and ammonia in the liver. In human body, enzymes in the liver metabolize toxic ammonia and carbon dioxide to non-toxic urea. Urea is the product of a series of biochemical reactions, and urea produced in the liver is transported to the kidneys and finally excreted as a waste product in urine that is presented in Fig. 6.
Conclusion

This article introduces a new way of developing PVA-\textit{N. sativa} nanofibrous membrane by electrospinning technique that show the antibacterial resistance against \textit{S. aureus} bacteria. The morphological structure has been shown that having nanofibers with particular diameter and tiny pores. The existence of a variety of sharp, strong and weak peaks as well as crucial functional groups that correspond to C–H, –CH$_2$, –CH$_3$, C=O, C–O, C=C suggesting the presence of the components of \textit{N. sativa} have been assigned in the results of developed PVA-\textit{N. sativa} nanofibrous membrane. Supplementary components can be added to improve the functional properties of developed sample so that it can be used for personal protective equipment (PPE) e.g. mask, globes. The proposed mechanism shows that TQ and THQ components react with viral proteins and can modify/damage the proteins. As this process occurs with natural bio-elements, it reduces the risk factor of human health. In future, there is a room for further research on components of \textit{N. sativa} to improve the immunity of human body with proper drug delivery system against virus.

Acknowledgements We would like to thank Department of Textile Engineering and Institute of Energy Engineering, DUET, Gazipur 1707 Bangladesh for providing technical and financial support. Sincere thanks to Waffen research laboratory (WRL), Dhaka for providing the antibacterial testing facility. We also acknowledge the valuable guidelines of Professor (Supernumerary) Dr Md Nurul Islam, Department of Chemistry, DUET.

Author contributions Md Abdus Shahid: Conceptualization, Supervision, Investigation, Writing, Editing. Abdur Rahim: Conceptualization, Analysis, Writing, Editing. Mohammad Asaduzzaman Chowdhury: Conceptualization, Supervision, Analysis, Writing, Editing. Mohammad Abul Kashem: Supervision, Writing.

Funding Not applicable.

Compliance with ethical standards

Ethical statement This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of Interest Md Abdus Shahid has no conflict of interest. Abdur Rahim has no conflict of interest. Mohammad Asaduzzaman Chowdhury has no conflict of interest. Mohammad Abul Kashem has no conflict of interest.

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