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Electrospun nanofibrous membrane with antibacterial and antiviral properties decorated with Myoporum bontioides extract and silver-doped carbon nitride nanoparticles for medical masks application

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**A B S T R A C T**

Public health safety issues have been plaguing the world since the pandemic outbreak of coronavirus disease (COVID-19). However, most personal protective equipments (PPE) do not have antibacterial and anti-toxicity effects. In this work, we designed and prepared a reusable, antibacterial and anti-toxicity Polyacrylonitrile (PAN) based nanofibrous membrane cooperated with Ag/C\textsubscript{3}N\textsubscript{4} (Ag-CN), Myoporum.bontioides (M. bontioides) plant extracts and Ag nanoparticles (NPs) by an electrospinning-process. The SEM and TEM characterization revealed the formation of raised, creased or wrinkled areas on the fiber surface caused by the Ag nanoparticles, the rough surface prevented the aerosol particles on the fiber surface from sliding and stagnating, thus providing excellent filtration performance. The PAN/M. bontioides/Ag-CN/Ag nanofibrous membrane could be employed as a photocatalytic bactericidal material, which not only degraded 96.37\% of methylene blue within 150 min, but also exhibited the superior bactericidal effect of 98.65 $\pm$ 1.49\% and 97.8 $\pm$ 1.27\% against E. coli and S. aureus, respectively, under 3 h of light exposure. After 3 cycles of sterilization experiments, the PAN/M. bontioides/Ag-CN/Ag nanofibrous membrane maintained an efficient sterilization effect. Molecular docking revealed that the compounds in M. bontioides extracts interacted with neo-coronavirus targets mainly on Mpro and RdRp proteins, and these compounds had the strongest docking energy with Mpro protein, the shortest docking radius, and more binding sites for key amino acids around the viral protein targets, which influenced the replication and transcription process of neo-coronavirus. The PAN/M.bontioides/Ag-CN/Ag nanofibrous membrane also performed significant inhibition of influenza A virus H3N2. The novel nanofiber membrane is expected to be applied to medical masks, which will improve human isolation and protection against viruses.

### 1. Introduction

Public health safety has been plagued by the 2019 global coronavirus disease (COVID-19) pandemic caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). Most coronaviruses and influenza viruses are transmitted from infected to uninfected individuals through tiny droplets in the air exhaled during coughing and sneezing or in oral secretions [1,2]. Personal protective equipment (PPE) [3,4], which is currently widely promoted for use, provides effective protection for individuals [2,5-7]. Since the viability in vitro of SARS-CoV-2 has a prolonged duration, the pathogens intercepted and captured at the surface of PPE still have persistent infectious activity, which can be susceptible to cross-infection and virus transmission [8-12]. Therefore, it is further in need of a protective masks with efficient filtration properties to prevent the entry of viruses, while killing them effectively and rapidly.
The nanofibrous membranes prepared by electrospinning technology have been subjected to wide studies in air filtration [2,3,5,13-19]. The nanofibrous membranes with micro-and nano-diameters, high porosity, and large surface area to volume ratio can dramatically intercept micro- and nano-particles, which promotes high filtration efficiency and low-pressure drop [14,20-23]. Meanwhile, the nanofibrous membrane surface will carry electrostatic ions, which is able to attract airborne pathogens and particles during the filtration process, reducing the risk of diseases [24-29]. Polyacrylonitrile (PAN) is a typical polar polymer that is frequently employed as a filter membrane for respirators, offering good filtration performance and recyclability, and effective isolation of airborne bacteria and viruses [30-32], as well as it has low toxicity and good biocompatibility [33-35]. Consequently, it is one of the ideal materials for new medical masks in the future.

So far, there are few effective drugs that are available for SARS-CoV-2, where the safety of the new emerging drugs still remained to be verified. Doctors successfully worked with Chinese herbs to help patients with mild symptoms improve their constitution to overcome the virus, which was prevalent in China at the time. Subsequently various herbs (baicalin, Panax notoginseng, and licorice roots [6,27,36], etc.) were found to be effective against coronavirus. Among them, Myoporum bontioides (M. bontioides) was a traditional folk medicine whose main components were terpenoids and their derivatives, flavonoids and trace metal elements (Fig. S1) [37-40]. The terpenoids and flavonoids of M. bontioides were found to have antibacterial effects against a variety of bacteriais, as effective as inhibitory abilities against various viruses [37-40]. For instance, the essential oil of the fresh leaves of M. bontioides showed 80% antiviral activity against herpes simplex virus type 1 (HSV-1) [41]. In test tube experiments, the antiproliferative or cytotoxic activity of the compound epingaione at a concentration of 50 pg/mL was 79.24% and 50.83% against human SH-SY5Y neuroblastoma and TE-671 sarcoma cells [42]. It is probably due to the important role played by terpenoids and flavonoids contained in M. bontioides.

Silver is considered one of the most important elements because of its excellent antibacterial properties and relatively reasonable cytotoxic levels [43,44]. Alternatively, polymer hybrid materials embedded with silver nanoparticles have received considerable attention for their superior properties (photovoltaic, catalytic). When applied in reasonable dosages, Ag nanoparticles (NPs) are regarded as safe and effective topical antimicrobial agents for the skin wound dressing [34,45]. Some studies have found that surface modification of electrospin nanofibers with nanoparticles is a simple and effective way to build structures on the surface. The rough surface formed by projections, creases or wrinkled areas allows aerosol particles on the fiber surface to not slide and stagnate, thus enabling excellent filtration performance [46,47]. AgNPs are applied to commercial air filters to improve resistance to MS2 and H1N1 viruses [46,48]. Researches have proven that the utilization of AgNPs in air filtration against viruses is feasible [48,49]. However, more research on related nanofiber membranes is still needed.

Currently, one of the effective methods is the addition of photocatalysts into the protective mats, which can effectively kill surrounding pathogens and reduce their adhesion [50-52]. The mechanism of its inhibitory activity is demonstrated by inducing the release of reactive oxygen species (ROS), disrupting protein function and DNA, as well as damaging bacterial cell membranes [53,54]. The typical photocatalysts include TiO2 [22,55], ZnO [16], Bi3W6O24 [56] and g-C3N4. Among them, the graphitic carbon nitride (g-C3N4) is a non-toxic and chemically stable non-metallic polymer, a photocatalyst with a narrow bandgap of 2.7 eV that can degrade environmental pollutants when driven by visible light [57]. g-C3N4 is remarkable in photocatalytic degradation and photocatalytic hydrogen production. However, the catalytic efficiency of a single g-C3N4 is low, so it is usually combined with metals, such as the well-known Ag, Pt and Au [58,59]. Noble metal nanoparticles can act as active sites and play an important role in effective visible light absorption and subsequent photocatalytic reactions [60]. The possible reason is that noble metal nanoparticles can strongly absorb visible light because their localized surface plasmon resonance can be tuned by changing their size, shape and surroundings. Hence, by adding photocatalysts to the protective material to self-clean in the presence of light is an essential modification for the reusability of the protective material [53].

In this study, we present a functional approach for the fabrication of multifunctional membranes for protective mats modification by a direct electrospinning of membrane decorated with Ag/g-C3N4 (Ag-CN) and AgNPs together (Fig. 1). The manufacturing was accomplished in one step by electrospinning of PAN solution containing Ag-CN and Ag nanoparticles. The nanofibrous membrane structure was investigated, as well as the particle filtration performance. E. coli and S. aureus were employed to evaluate the antibacterial properties of the novel nanofibrous membrane. The antiviral properties of the nanofibrous membrane were evaluated with influenza A virus H3N2. We believe that this electrospin nanofibrous membranes might own antiviral, antibacterial and efficient filtration properties, which is important for capturing air pollutants and preventing infection and transmission of respiratory infectious diseases. It will be promising for application in medical protective materials.

2. Experimental section

2.1. Materials

Polyacrylonitrile (PAN, Mw = 150,000 g/mol) was purchased from Pingjiang Chemical Reagent Co., Ltd. (Guangdong, China), N, N-Dimethylformamide (DMF, AR) was brought from Tianjin Yongda Chemical Reagent Co., Ltd. (Tianjin,China), AgNO3 (AR) was obtained from Tianjin Qilun Chemical Technology Co., Ltd. (Tianjin, China), Melamine (AR) was purchased from Aladdin, Anhydrous ethanol (AR) was gotten from Tianjin Damao Chemical Reagent Factory.

2.2. Synthesis of Ag-CN nanoparticles

The metal-free g-C3N4 powder was synthesized by heating melamine in a muffle furnace. 1 g g-C3N4 was added to 100 mL of deionized water, the suspension was mixed for 30 min, and sonicated for 30 min. Subsequently, 3 wt% AgNO3 was rapidly added to the above suspension, whereupon the suspension was irradiated under a xenon lamp and magnetically stirred for 2 h. The suspension was filtered, washed with deionized water, and dried at 80 °C for 12 h.

2.3. Extraction of M. bontioides

The fresh leaves of M. bontioides were picked fresh from the planting fields at the agricultural training base of South China Agricultural University and dried in a cool place for 7d, then dried at 45 °C for 8 h and crushed to obtain the powder. Then, the powder was cold soaked twice (14d, 7d) before with 60% ethanol solution, filtered, combined filtrate, distilled under reduced pressure (Rotary Evaporator RE-2000A, Shanghai Yarong Biochemical Instrument Factory) to obtain the infusion, which was kept at low temperature.

2.4. Fabrication of the PAN/M. bontioides/Ag-CN/Ag nanofibrous membrane

To prepare the PAN/M. bontioides/Ag-CN/Ag nanofibrous membrane, Ag-CN (1 wt%) was first dispersed in 10 mL of N, N-dimethylformamide (DMF) and after this, M. bontioides (3, 5, 7 wt%) mixture was added in a continuous sonication for 30 min to disperse the carbon nitride nanoparticles. After obtaining a homogeneous mixture, PAN (Mw = 150,000 g/mol, 8 wt%) was mixed with the solution using a magnetic stirrer (1000 rpm). The solution was kept under stirring for 1 h until the polymer was completely dissolved in DMF. Finally, different concentrations of silver nitrate (3, 5, 7 wt%) were added to the DMF.
solution. After that, the solution was kept overnight with stirring until its color turned to dark brown.

Fabrication of the PAN/\textit{M. bontioides}/Ag-CN/Ag nanofibrous membrane was carried out by the electrospinning machine (TL-01, Shenzhen Tongli Micro-Nano Technology Co., Ltd.). The homogeneous solution was transferred to a 20 mL syringe that had an 18G metal needle. The distance from the needle to the collector was 13 cm, the applied voltage was 15 kV, the flow rate was 1 mL/h, and the electrospinning time was 1 h. The room temperature is maintained at 25 °C.

2.5. Characterization

The surface morphology of nanofibrous membrane was examined under a scanning electron microscope (SEM, ZEISS EVO MA 15). The diameter distributions of the nanofibers were determined by image J analysis using manometry on > 90 slices from SEM images. A transmission electron microscope (TEM, FEI/Talos L120C) was used to examine the surface morphology of the nanofibrous membrane. Wide-angle X-ray diffraction (XRD) analysis was performed using a Bruker D8 Advance (Cu Kα irradiated radiation) diffractometer to assess the physical state of the nanofibrous membrane. Fourier transform infrared spectroscopy (FT-IR) was employed to determine the structure and stability of the drug in the nanofibrous membrane by a Nicolet IS10 FTIR spectrometer (Thermo Fisher). Measured the viscosity of the nanofibrous membrane by a rotary viscometer (NDJ-5S, Shanghai Changji Geological Instruments Co., Ltd.). The conductivity of the nanofibrous membrane was monitored by a conductivity meter (DDS-11A, Shanghai INESA Scientific Instrument Co., Ltd.). Three different positions were conducted for each membrane and the average value was calculated.

2.6. Photocatalytic degradation and self-cleaning studies

The photocatalytic properties of the prepared the PAN/\textit{M. bontioides}/Ag-CN/Ag nanofibrous membranes was investigated using methylene blue (MB) as a probe molecule. The 2 cm × 2 cm size nanofibrous membranes were placed in 100 mL of 10 mg/L MB solution and stirred for 150 min under a xenon lamp (PLS-SXE300D, Beijing Bofeilai Technology Co., Ltd.) with a light intensity of 75,000 lx. The absorbance of the purified solution at OD_{663} was recorded at 10 min intervals. The reduction rate was calculated by the following equation [53,61,62]:

\[
\text{Reduced rate}(\%) = \frac{\ln C_t}{\ln C_0} \times 100\%
\]

\[
\ln \frac{C_t}{C_0} = kt
\]

where \(C_t\) represents OD_{663} after different illumination times, \(C_0\) represents OD_{663} before illumination, and K is the first-order rate constant given by the slope of ln (\(C_t/C_0\)) versus t [53,61,62].

\[
\ln 2 = k t_{1/2}
\]

where \(t_{1/2}\) is the time needed to reduce the initial concentration of the analyte to half of its initial concentration value.

2.7. Particulate filtration efficiency (PFE) and pressure difference

The particulate filtration efficiency of the material was measured by filtering NaCl aerosols that simulate viral and bacterial filtration. An automated filter tester (Model 8130, TSI, Shoreview, Minnesota, MN. USA) was used to measure submicron particle filtration efficiency of the PAN/\textit{M. bontioides}/Ag-CN/Ag nanofibrous membrane. NaCl particle loading experiments were also performed during the period, with a loading time of 1 min and a loading mass of 50 mg.

The submicron particulate filtration efficiency is defined as follows [2,15,16,21,63]:

\[
\text{PFE: The ratio of aerosol concentration captured by the mask to the original upstream aerogel concentration.}
\]
Permeation efficiency (%) = (aerosol concentration through the mask)/(upstream aerosol concentration in the air) × 100%

Protection efficiency (%) = 100 – penetration efficiency (%) (5)

Inhalation resistance: the ventilation resistance generated by a certain airflow in the inhalation direction of the mask.

The quality factor (QF) could be calculated by the following Eq.6 [2,46,64].

\[
QF = -\frac{\ln(1 - \eta)}{\Delta P}
\]

where \(\eta\) and \(\Delta P\) represented the filtration efficiency and pressure drop across the filter, respectively.

2.8. Antimicrobial activity assays

The antibacterial experiment of nanofibrous membranes was conducted by colony counting method. Gram-negative Escherichia coli (E. coli, OD\(_{600}\) = 0.33, 1OD = 1.0 × 10\(^9\) cfu/mL) and Gram-positive Staphylococcus aureus (S. aureus, OD\(_{600}\) = 0.14, 1OD = 1.0 × 10\(^9\) cfu/mL) were selected as representative microorganisms and cultured in a medium in an incubator. To examine the inhibitory effect of nanofibrous membranes, M. bontioides (2, 46, 64) were cut into 1 cm\(^2\) squares, then incubated in 10 mL bacterial solution diluted 5 times for 24 h (dark, 37 °C). 50 μL of the solution was finally taken in solid medium and the number of colonies was observed after 12 h.

The PAN/M. bontioides/Ag-CN/Ag nanofibrous membranes were similarly cut into 1 cm\(^2\) pieces and placed in a 12-well culture plate. A drop (20 μL, 1.47 × 10\(^9\) cfu/mL) of the bacterial liquid solution was applied directly onto the nanofibrous membrane, respectively. Then, they were incubated in a dark environment for 3 h and irradiated with a xenon lamp (PLS-SXE300D, Beijing Bofillay Technology Co., Ltd.) at a light intensity of 75,000 lx for 3 h at a room temperature of 20 °C, respectively. After that, 2 mL of normal saline was added to rinse the bacteria. One drop (50 μL) of the eluate was applied uniformly to the plate of solid medium and then placed in a 37 °C incubator overnight (12 h, dark).

For the cyclic sterilization test, 20 μL of bacterial solution was applied directly to the nanofiber membrane and irradiated for 3 h. After illumination, another 20 μL of bacterial solution was deposited on the above membrane and irradiated for another 3 h for cycle 2. For cycle 3, another 20 μL of bacterial solution was coated on the above-mentioned membrane for 3 h of light irradiation. After that, 2 mL of normal saline was injected to flush the bacteria. One drop (50 μL) of the eluate was spread evenly onto the solid culture plate and then kept in an incubator at 37 °C overnight (12 hr, dark).

The bacterial inhibition rate was calculated according to Eq. (7) [2,47].

\[
\text{Bacteria inhibition rate} = \left(1 - \frac{\text{Number of bacteria in the experimental group}}{\text{Number of bacteria in the control group}}\right) \times 100%
\]

2.9. Antiviral activity assays

The sterilized PAN/M. bontioides/Ag-CN/Ag nanofibrous membrane nanopowders and M. bontioides plant extracts were incorporated. The surface morphological features of the samples were observed by scanning electron microscopy as described in Fig. 2. Nanofibers without any

2.10. Molecular docking

Molecular docking of viral protein targets and potential target compounds was performed by the drug design software Discovery Studio 2017 to analyze the interactions between compounds and proteins. The relevant crystals of Mpro, RdRp and ACE2 were screened from the Research Collaboration for Structural Bioinformatics Protein Data Bank (RCSB PDB) in Table 1, imported the structures of the compounds into Discovery Studio 2017 and used the minimize module for drug structure optimization. The target protein was dehydrated and hydrogenated to convert the two-dimensional structure of the compound into a three-dimensional structure. The optimized small molecule structures were imported into the receptor- ligand interaction module for CDOCKER algorithm molecular docking. The parameters related to molecular docking were set as follows: Pose Cluster Radius was set to 0.5. Random Conformations was set to 10. Orientations to refine was set to 10. The rest of the settings were default. The binding ability of the compounds to the target protein was expressed by the CDOCKER Energy value.

3. Results and discussion

3.1. Surface morphology of the nanofibrous membranes

In this study, the PAN/M. bontioides/Ag-CN/Ag nanofibrous membrane were obtained by electrospinning, in which AgNPs, Ag-CN nanofibers were cut into 1 cm\(^2\) squares, then incubated in 10 mL bacterial solution diluted 5 times for 24 h (dark, 37 °C). The antibacterial experiment of nanofibrous membranes was conducted by colony counting method. Gram-negative Escherichia coli (E. coli, OD\(_{600}\) = 0.33, 1OD = 1.0 × 10\(^9\) cfu/mL) and Gram-positive Staphylococcus aureus (S. aureus, OD\(_{600}\) = 0.14, 1OD = 1.0 × 10\(^9\) cfu/mL) were selected as representative microorganisms and cultured in a medium in an incubator. To examine the inhibitory effect of nanofibrous membranes, M. bontioides (2, 46, 64) were cut into 1 cm\(^2\) squares, then incubated in 10 mL bacterial solution diluted 5 times for 24 h (dark, 37 °C). 50 μL of the solution was finally taken in solid medium and the number of colonies was observed after 12 h.

The PAN/M. bontioides/Ag-CN/Ag nanofibrous membranes were similarly cut into 1 cm\(^2\) pieces and placed in a 12-well culture plate. A drop (20 μL, 1.47 × 10\(^9\) cfu/mL) of the bacterial liquid solution was applied directly onto the nanofibrous membrane, respectively. Then, they were incubated in a dark environment for 3 h and irradiated with a xenon lamp (PLS-SXE300D, Beijing Bofillay Technology Co., Ltd.) at a light intensity of 75,000 lx for 3 h at a room temperature of 20 °C, respectively. After that, 2 mL of normal saline was added to rinse the bacteria. One drop (50 μL) of the eluate was applied uniformly to the plate of solid medium and then placed in a 37 °C incubator overnight (12 h, dark).

For the cyclic sterilization test, 20 μL of bacterial solution was applied directly to the nanofiber membrane and irradiated for 3 h. After illumination, another 20 μL of bacterial solution was painted on the nanofibrous membrane, respectively. Then, they were incubated in a dark environment for 3 h and irradiated with a xenon lamp (PLS-SXE300D, Beijing Bofillay Technology Co., Ltd.) at a light intensity of 75,000 lx for 3 h at a room temperature of 20 °C, respectively. After that, 2 mL of normal saline was added to rinse the bacteria. One drop (50 μL) of the eluate was applied uniformly to the plate of solid medium and then placed in a 37 °C incubator overnight (12 h, dark).

The bacterial inhibition rate was calculated according to Eq. (7) [2,47].
Fig. 2. SEM images and diameter analysis of PAN nanofibrous membrane (a, c), PAN/M.bontioides nanofibrous membrane (b, d), PAN/M.bontioides/Ag nanofibrous membrane (e, g), PAN/M.bontioides/Ag-CN/Ag nanofibrous membrane (f, h).
6

beads were noticed in all samples. The mean and average diameters and standard deviations of PAN, PAN/M. bontioides, PAN/M. bontioides/Ag and PAN/M. bontioides/Ag-CN/Ag Nanofibers were 447.48 ± 119.71 nm, 213.18 ± 60.52 nm, 465.74 ± 118.19 nm, and 726.93 ± 140.50 nm, respectively. As a special southern natural plant, the extract of M. bontioides contains a certain amount of solvent, mainly water or anhydrous ethanol [37,39]. When M. bontioides was included in the precursor solution, it reduced the viscosity of the solution, resulting in a smaller diameter of PAN/M. bontioides Nanofibers containing M. bontioides. The Ag ions were reduced to AgNPs in the presence of DMF, which will be dispersed around in the solution. Under the influence of the high conductivity of metallic silver, the ions of the precursor solution became exceptionally active when loaded with high-voltage current, and the attraction between the ions was enhanced, so that the diameter of the obtained nanofibers became larger than that of the pure PAN nanofibers [43,44,65,66]. In contrast to PAN nanofibers, PAN/M. bontioides/Ag-CN/Ag nanofibers had a rougher surface and a more heterogeneous structure, and aggregates of Ag-CN and AgNPs were observed on the surface. This increase in roughness indicated that the Ag-CN NPs were not only inside the PAN Nanofibers but also on the surface of the fibers. A difference in the diameters of the NPs was observed. Especially, the NPs containing Ag-CN were explained as a result of the enhanced viscosity and conductivity of the polymer solution and favorably reduced the ionic mobility and whipping instability during electrospinning (Table 2) [43]. It also caused an increase in the surface roughness of the nanofibers and the formation of various irregular structural features, which improved the interception of tiny particles.

3.2. SEM-mapping and TEM analysis of the nanofibrous membranes

The distribution of C, N, O, Ag and Mg elements in the PAN/M. bontioides/Ag-CN/Ag nanofibrous membrane was observed by SEM-Mapping to investigate the distribution of the additive inside and outside the nanofibrous membrane. As Fig. 3, all elements were uniformly distributed in the nanofibrous membrane. The carbon element was mainly derived from PAN and g-C₃N₄. While the nitrogen was originated dominantly in g-C₃N₄, there were several distinct bright spots (Fig. 3c), showing agglomeration of larger clusters. It was primarily owed to the mutual attraction of electrons. Fig. 3d-e) illustrated that M. bontioides contains a large number of trace metals (e.g. Mg) and active oxides. Mg was mainly derived from chlorophyll in plants, while Ag was added by the addition of AgNO₃. This indicated that the process of electrospinning was successfully applied to make the substances (e.g. M. bontioides extract, AgNPs and Ag-CN) uniformly dispersed on the electrospun nanofibrous membranes, thus effectively enhancing the ability and effectiveness of inhibiting and killing pathogens, and contributing to the enhancement of the protective material for the human body.

The Ag-CN powders and nanofibers were observed by transmission scanning electron microscopy (TEM, FEI/Talos L120C). Fig. 4 was the TEM image of Ag-CN powders and PAN/M. bontioides/Ag-CN/Ag nanofibers at different positions. As Fig. 4a, a significant amount of AgNPs were found to be bound to the g-C₃N₄ surface, which was mainly derived by photodeposition. Simultaneously, it was observed in Fig. 4b that both the surface and the interior of the nanofibers were encapsulated by many particles of different sizes. The large particles may be Ag-CN particles or clusters formed by Ag-CN and AgNPs, while the small particles may be AgNPs. The rough surface formed by such raised, folded or wrinkled areas caused by nanoparticles prevents aerosol particles on the fiber surface from sliding and stagnating, so as to obtain excellent filtration performance [46,67]. It also enhanced the contact area with pathogens to multiply the killing effectiveness. Furthermore, it was susceptible to receive more energy from sunlight and stimulate electron production of ROS [53,63].

3.3. Structural properties of the nanofibrous membranes

The fabricated different electrospun fiberous membrane composites were analyzed by XRD and FT-IR as presented in Fig. 5. To reveal the crystalline and phase structures of the prepared nanocomposites, XRD patterns were measured as demonstrated in Fig. 5a. No specific peaks were observed for the pure PAN nanofibrous membrane. The characteristic metallic silver peaks at 2θ = 38.1°, 46.2°, 64.5° and 77.3° corresponding to (111), (200), (220) and (311) crystal planes, respectively, were observed in all samples containing cubic Ag crystal structure (JDPDF 41-0607). The characteristic g-C₃N₄ peak at 27.9°, corresponding to the (002) plane, was in existence for all the photocatalysts. The peak was attributed to the strong structural interlayer stacking of the conjugated aromatic system along the c-axis within the lattice plane. The small angular peak at 17.3° corresponding to (100) was presumably ascribed to the occurrence of a small tilt angle with the interlayer stacking structure. The mean crystal size (D) of Ag NPs and Ag-CN was estimated by Debye-Scherrer Eq. (8).

$$D = \frac{0.9\lambda}{\beta \cos \theta}$$

where $\lambda$, $\theta$, and $\beta$ were the wavelength, peak Bragg angle and the half-height width of the peak, respectively. The mean crystal size of Ag-CN was calculated to be 20.1 nm, while the average crystal size of Ag NPs was 19.1 nm. No other characteristic peaks were found in the XRD patterns apart from g-C₃N₄ and Ag, which affirmed the high purity of the PAN/M. bontioides/Ag-CN/Ag nanofibrous membrane.

In the FT-IR spectrum (Fig. 5b), a broad absorption peak of 3433 cm⁻¹ was observed, which was the stretching vibration of the N-H and O-H groups of the physically adsorbed water molecules. The peak at 1200–1650 cm⁻¹ corresponded to the typical stretching pattern of the C-N heterocycle. The signal at 822 cm⁻¹ was the characteristic absorption peak of the triazine unit. The bands around 1000 and 1150 cm⁻¹ were the specific absorption vibration peaks of PAN. With the addition of Ag-CN and M. bontioides, the intensity of the individual absorption peaks had changed significantly. This illustrated that the nanofiber materials of complex systems prepared by electrospinning method can still maintain the stability of the structure and components of various additives without reducing the reduction of their effective functions.

3.4. Photocatalysis performance and mechanism of the nanofibrous membranes

The photocatalytic activity of the pure PAN, PAN/M. bontioides, PAN/M. bontioides/Ag, and PAN/M. bontioides/Ag-CN/Ag nanofibrous membranes was evaluated by the photodegradation of the MB solutions, as described in Fig. 6. The degradation efficiency of MB was determined by investigating the difference in the absorption value of MB at 663 nm. In Fig. 6a, the decrease in absorbance of the MB solution was exhibited for the period 0–150 min. The degradation efficiency of pure PAN for MB

| Samples | PAN | M. bontioides | Ag | Ag-CN | Viscosity/(mPa s) | Conductivity/(ms cm⁻¹) |
|---------|-----|---------------|----|-------|-----------------|-----------------------|
| PAN     | 8 wt% | /             | /  | /     | 1650 ± 0.82     | 0.0891 ± 0.0037       |
| PAN/M. bontioides | 8 wt% | 5 wt%         | /  | /     | 266 ± 1.63      | 0.1023 ± 0.0005       |
| PAN/M. bontioides/Ag | 8 wt% | 5 wt%         | 5 wt% | 1 wt% | 340.33 ± 2.87   | 1.7537 ± 0.0021       |
| PAN/M. bontioides/Ag-CN/Ag | 8 wt% | 5 wt%         | 5 wt% | 1 wt% | 405.67 ± 2.87   | 2.4267 ± 0.0170       |

Table 2
Viscosity and Conductivity of the electrospinning precursor fluid.
was only 3.86% (Fig. 6c), with no significant photocatalytic activity. This was attributed to the fact that pure PAN nanofiber membranes prepared by the electrospinning process carried electrostatic charges and attracted free MB molecules in the MB solution [61,68]. When \textit{M. bontioides} and Ag were included, 81.05% and 89.29% (Fig. 6c) of the MB degradation efficiency was achieved, respectively. PAN nanofibrous membrane with \textit{M. bontioides} and Ag-CN had revealed the highest MB photodegradation efficiency of 96.37% (Fig. 6c).

The slope of \(\ln(C_0/C_t)\) plotted as a linear function of time for the fabricated mats (Fig. 6b) corresponded to the pseudo-first-order rate constant \(k\) \((\text{min}^{-1})\) (Fig. 6d), and the half-life was calculated by Eq. (3) to (5). The value of slope \(k\) for pure PAN was close to 0 and negligible. The calculated half-life and rate constants of the PAN/\textit{M. bontioides}, PAN/\textit{M. bontioides}/Ag, PAN/\textit{M. bontioides}/Ag-CN/Ag nanofibrous membrane were 64.18 min and 0.0108 min\(^{-1}\), 47.15 min and 0.0147 min\(^{-1}\), 32.24 min and 0.0215 min\(^{-1}\), respectively. When xenon light was shone on the nanofiber membrane and the MB solution, the MB molecules became more active and easily bound to the trace metals (Ag, Fe) and Ag from \textit{M. bontioides} extract in the nanofiber membrane, while the nanofiber membrane also carried the electrostatic charge, causing the MB molecules to cling to the nanofiber membrane and a decrease in the absorbance of the solution. The MB degradation rate was accelerated up to 1.46–1.99 times after the addition of the photocatalyst g-C\(_3\)N\(_4\). This meant that the PAN/\textit{M. bontioides}/Ag-CN/Ag nanofibrous membrane had an effective photocatalytic degradation capability. In addition, the transient photocurrent response captured the change of photocurrent before and after binding Ag.

The photocurrent of g-C\(_3\)N\(_4\) after binding Ag increased by several times compared to that of pure g-C\(_3\)N\(_4\) (Fig. 6e), and the electrochemical impedance of g-C\(_3\)N\(_4\) after binding Ag was lower than that of pure g-C\(_3\)N\(_4\) (Fig. 6f), which indicated that Ag-CN had a low energy barrier and could more easily convert the solar energy, into other energy, such as...
chemical energy. It was also confirmed that Ag-CN can be used as a photocatalytic antibacterial agent. In conclusion, the PAN/M.bontioides/Ag-CN/Ag nanofibrous membranes were proven the ability of photocatalytic degradation and could rapidly clean the surface of the fiber by removing the contaminants.

Various reactive oxygen species were observed to have an inhibitory inactivating effect on pathogens such as bacteria and viruses, and the addition of photocatalyst Ag-CN to the nanofiber membrane facilitates the production of reactive oxygen species ROS. When the PAN/M.bontioides/Ag-CN/Ag nanofibrous membrane was irradiated by sunlight, the photon energy was higher than the bandgap of g-C3N4, the conduction band (CB) while leaving the same number of photogenerated electrons (e\textsuperscript{−}) from the valence band (VB) to the conduction band (CB) while leaving the same number of photogenerated holes (h\textsuperscript{+}) in the VB. The optically excited electrons can be transferred from g-C3N4 to the CB of Ag, which was narrower than the CB of g-C3N4. Electrons accumulated at the CB of Ag or g-C3N4 can be transferred to oxygen molecules adsorbed on the surface to form radicals such as \cdot O\textsuperscript{2−}, \cdot OH and DMPO-\cdot OH radicals (Fig. 7). The various reactions were explained with Eq. (9) to (14) [54,58-60]. A large amount of the reactive oxidation species being produced will react with the surrounding dyes and other contacts, or enter the interior of the pathogen, destroying protein function and DNA, as well as disrupting bacterial cell membranes, which will cause the death of the pathogen and result in antibacterial and antiviral effects [61,68].

\[
\begin{align*}
g - C_3N_4 + hv &\rightarrow g - C_3N_4(h + e) \quad (9) \\
g - C_3N_4(e) + Ag &\rightarrow g - C_3N_4 + Ag(e) \quad (10) \\
Ag(e) + O_2 &\rightarrow O_2^+ + Ag \quad (11) \\
O_2^+ + e^- + 2H^+ &\rightarrow H_2O_2 \quad (12) \\
H_2O_2 + e^- &\rightarrow OH + OH^- \quad (13) \\
OH^- + h^+ &\rightarrow OH \quad (14)
\end{align*}
\]

The electron paramagnetic resonance (EPR) spectra of xenon lamp irradiation for 10 min were recorded to investigate the production of ROS. The intense signals of DMPO-\cdot OH and DMPO-\cdot O\textsuperscript{2−} were successfully captured in the solution after 10 min of xenon lamp light as depicted in Fig. S2. However, no signal was detected in the EPR spectrum under dark conditions. The EPR results confirmed that the radicals produced by PAN/M.bontioides/Ag-CN/Ag nanofibrous membrane were mainly \cdot OH and \cdot O\textsuperscript{2−}, which also supported the conclusion of the photocatalytic mechanism.

3.5. Particulate filtration efficiency

The filterability and breathability were measured by filtering NaCl aerosols that simulate viral and bacterial filtration. To effectively evaluate the filterability of the nanofibrous membranes, we used sodium chloride aerosol with diameters of about 70–80 nm, even smaller than SARS-CoV-2 virus with diameters of about 100 nm [18,63]. The efficiency of the filter was tested with an automatic filter tester (Model 8130, TSI, Shoreview, Minnesota, MN, USA). It was observed as Table 3 that the gas flow rate was 85 L/min, the concentration of NaCl aerosol was 20 mg/m\textsuperscript{3}, while the experimental temperature was kept at 21.3 °C and the relative humidity was 36%. The filtration efficiency of the nanofibrous membranes reached 99.82 ± 0.016% in the loading experiment, while that of the unloaded experiment reached 99.97 ± 0.013%. That indicated that the nanofibrous membranes satisfied the basic requirement of high filtration efficiency for masks. The pressure drop was the differential pressure value generated by the filtration process. The smaller the value, the better the comfort level [17,46]. With the addition of M.bontioides and Ag-CN, the filtration efficiency of the nanofibrous membranes was improved and the pressure drop was reduced from 133 Pa to 64 Pa shown in Table 4. The overall filtration performance of an air filter considering efficiency and pressure drop was evaluated by the quality factor (QF). A higher QF value normally meant better overall filtration performance. The calculated QF values of the membranes based on Eq.14 were listed in Table 4. The QF value of PAN/M.bontioides/Ag-CN/Ag (0.097 Pa\textsuperscript{−1}) was significantly more than that of PAN (0.029 Pa\textsuperscript{−1}). It meant that the PAN/M.bontioides/Ag-CN/Ag nanofibrous membranes had promising overall filtration performance and was expected to be an alternative for mask filtration.

As shown in Fig. 8a, the nanofibrous membranes were laid on an automatic filtration tester to determine the upstream aerosol above the membranes and the downstream aerosol after the mat was filtered to calculate the filtration efficiency. Concurrently, the airflow and pressure through the mat were detected by sensors to represent the breathability of the mat. The filtering mechanism of the nanofibrous membranes was described in Fig. 8b, where the large particles were physically captured by inertial impact and interception of the fiber network [21], while small particles were hindered by diffusion. The microstructure of the obtained electrospun nanofibrous membranes was composed of numerous nanofibers with greater microporous porosity and larger

Fig. 5. XRD and FT-IR of the nanofibrous membranes. (a) XRD patterns of pure PAN, PAN/M.bontioides, PAN/M.bontioides/Ag and PAN/M.bontioides/Ag-CN/Ag, (b) FT-IR spectra of pristine PAN, PAN/M.bontioides, PAN/M.bontioides/Ag and PAN/M.bontioides/Ag-CN/Ag.
Fig. 6. Photocatalytic activity of the nanofibrous membranes. (a) UV–Vis absorbance of MB aqueous solutions transferred on the PAN/M.bontioides/Ag-CN/Ag nanofibrous membrane at time intervals from 0 to 150 min. (b) Plot of photocatalytic activity of the nanofibrous membrane as a function of time. (c) Photocatalytic degradation (%) of MB on the pure PAN, PAN/M.bontioides, PNA/M.bontioides/Ag and PAN/M.bontioides/Ag-CN/Ag nanofibrous membrane. (d) Half-life time of the photocatalytic degradation rate. The inset presents the pseudo-first-order reaction rate constant. (e) Photocurrent responses of Ag-CN and g-C₃N₄. (f) Electrochemical impedance spectra of Ag-CN and g-C₃N₄.
particles. The filtration performances of the nanofibrous membranes. Table 4

| Samples Filtration performances of the nanofibrous membranes. |
|---------------------------------------------------------------|
| Table 4                                                       |
| Test Condition                                               | Test Results | Average and Standard Deviation |
| S. aureus filtration performance (% of Ag-CN)                 | 1# 99.801%   | 99.82 ± 0.019% |
| S. aureus filtration pressure drop (Pa)                       | 1# 58 Pa     | 59 ± 5.57 Pa |
| S. aureus filtration performance (% of Ag-CN)                 | 2# 99.839%   | 99.90% ± 0.013% |
| S. aureus filtration pressure drop (Pa)                       | 2# 56 Pa     | 65 Pa |
| S. aureus filtration performance (% of Ag-CN)                 | 3# 99.822%   | 99.90% ± 0.013% |
| S. aureus filtration pressure drop (Pa)                       | 3# 54 Pa     | 65 Pa |
| S. aureus filtration performance (% of Ag-CN)                 | 4# 99.989%   | 99.90% ± 0.013% |
| S. aureus filtration pressure drop (Pa)                       | 4# 74 Pa     | 74 Pa |
| S. aureus filtration performance (% of Ag-CN)                 | 5# 99.958%   | 99.90% ± 0.013% |
| S. aureus filtration pressure drop (Pa)                       | 5# 66 Pa     | 66 Pa |
| S. aureus filtration performance (% of Ag-CN)                 | 6# 99.968%   | 99.90% ± 0.013% |
| S. aureus filtration pressure drop (Pa)                       | 6# 72 Pa     | 72 Pa |

Samples 4#, 5#, and 6# were not subjected to loading test.

M. bontioides contained a variety of terpenoids and flavonoids that were proved to have inhibitory effects on various bacterias [37-39]. It was evaluated by E. coli (Gram-negative bacteria) and S. aureus (Gram-positive bacteria) to determine the antibacterial and antimicrobial effects of M. bontioides. Pure PAN and M. bontioides with 3 wt%, 5 wt%, and 7 wt% PAN/M. bontioides nanofibrous membranes were studied (Fig. 9).

With the accumulation of the amount of M. bontioides, the antibacterial effect became increasingly apparent. When the amount of M. bontioides was 3 wt%, the inhibitory effect on E. coli was 64.68%, while the inhibitory effect on S. aureus was 69.23%. At the content of 5 wt%, the inhibitory effects on E. coli and S. aureus were 97.37% and 95.99%, respectively. Once the content reaches 7 wt%, it showed a strong inhibitory effect on both E. coli and S. aureus, up to 99.50% and 98.08%, respectively. The inhibitory activity of M. bontioides was principally connected with the number of hydroxyl groups on the aromatic ring of flavonoids and the position of hydroxyl groups [69-71]. The specific mechanism may be (Fig. 10): (1) by controlling the tricarboxylic acid cycle pathway in the sugar metabolism pathway of the test strain; (2) by impeding the uptake of energy substances and inhibiting their growth and recovery; (3) by suppressing the activity of reductase to inhibit the synthesis of the cell membrane, as well as by generating hydrogen peroxide to damage the cytoplasmic membrane to achieve the inhibition; (4) by causing protein coagulation or denaturation.

To evaluate the antimicrobial properties of the prepared nanofibrous membrane, PAN/M. bontioides/Ag-CN/Ag was evaluated against E. coli and S. aureus. The filter layer of the commercial mask was selected as the control group. 20 μL of bacterial solutions were dropped on nanofiber membranes and incubated in the dark and under a xenon lamp with a light intensity of 7500 lx for 3 h, respectively. In the dark environment, the PAN/M. bontioides/Ag-CN/Ag nanofibrous membrane had an inhibitory effect on E. coli, as in Fig. 11a, as well as on S. aureus, in Fig. 11b. It was the AgNPs and M. bontioides in the nanofiber membrane played an important role to inhibit the growth amount of bacteria. The inhibition results of E. coli were better than that of S. aureus, the reason was that the growth conditions of E. coli required low and fast proliferation, and the maximum bacterial capacity was easily reached after 12 h of incubation, and when AgNPs and M. bontioides were released, a large number of bacteria could be killed quickly. While S. aureus proliferates slowly, 12 h of incubation did not reach the maximum bacterial capacity, and when AgNPs and M. bontioides were delivered, there were still some bacteria kept dividing and proliferating. No live E. coli (Fig. 11a) and S. aureus (Fig. 11b) were detected in all samples after 3 h of irradiation using a xenon lamp, which indicated that the prepared nanofibrous membranes were more effective in killing bacteria under light conditions. The experimental results under light conditions were better compared to those under dark conditions, because the photocatalyst Ag-CN added played a crucial role in the photocatalytic reaction, producing a lot of active ROS that caused the death of massive bacteria.

### Table 3

**NaCl particle filtration test results of the nanofibrous membranes.**

| Test items                        | Test Conditions | Test results | Average ± Standard deviation |
|-----------------------------------|----------------|--------------|------------------------------|
| NaCl particle matter filtration efficiency (%) | Gas flow rate: 85 L/min | 1# 99.801% | 99.82 ± 0.019% |
|                                   | Aerosol particles: NaCl Aerosol | 2# 99.839% | 99.90% ± 0.013% |
|                                   | Concentration: 20 mg/m³ | 3# 99.822% | 99.90% ± 0.013% |
|                                   | Temperature: 21.3°C | 4# 99.989% | 99.90% ± 0.013% |
|                                   | Relative humidity: 36% | 5# 99.958% | 99.90% ± 0.013% |
|                                   |                    | 6# 99.968% | 99.90% ± 0.013% |
| Pressure drop (ΔP)/[Pa]            | Gas flow rate: 85 L/min | 1# 58 Pa | 59 ± 5.57 Pa |
|                                   |                       | 2# 65 Pa | 65 Pa |
|                                   |                       | 3# 54 Pa | 54 Pa |
|                                   |                       | 4# 74 Pa | 70.67 ± 4.16 Pa |
|                                   |                       | 5# 66 Pa | 66 Pa |
|                                   |                       | 6# 72 Pa | 72 Pa |

Samples 4#, 5#, and 6# were not subjected to loading test.
The antibacterial effect of the samples was verified by monitoring the viability of *E. coli* (Fig. 11c) and *S. aureus* (Fig. 11d) at different light times. The reason why we chose 3 h of illumination was to ensure that the illumination time was long enough to kill all bacteria before the next cycle test. It can be evident that the number of *E. coli* growing on the samples gradually decreases with passing time, such as Fig. 11c. The average inhibition rates for 1 h, 2 h and 3 h were calculated as 76.97 ± 0.71%, 88.58 ± 2.80% and 98.65 ± 1.49% after three cycles, correspondingly. In addition, the PAN/*M.bontioides*/Ag-CN/Ag nanofibrous membrane had demonstrated excellent antibacterial activity against *S. aureus* (Fig. 11d). After 3 h of illumination, *S. aureus* was substantially eliminated. The inhibition rates for 1 h, 2 h and 3 h against *S. aureus* were 31.84 ± 2.33%, 79.26 ± 3.82%, and 97.8 ± 1.27%, respectively. Encouragingly, after three cycles, *E. coli* and *S. aureus* were virtually eliminated, which indicated that the PAN/*M.bontioides*/Ag-CN/Ag nanofibrous membranes had a remarkable cyclic bactericidal effect.

To closely match the application in practice, the nanofiber membrane was placed in an environment with 85% humidity for 30 min, and

![Fig. 8. Airflow, and particle filtration of the nanofibrous membranes. (a) Schematic diagram of a mask measuring filtration efficiency, (b) The mechanism of filtering particulate matter by a mask.](image1)

![Fig. 9. The antibacterial test of the PAN/*M.bontioides* nanofibrous membranes with different *M.bontioides* concentrations.](image2)
**Fig. 10.** Specific mechanism of the inhibitory activity of the PAN/M. bontioides nanofibrous membranes.

**Fig. 11.** Photocatalytic antibacterial test. Antibacterial results of the PAN/M.bontioides/Ag-CN/Ag nanofiber membranes in 3 cycles of experiments incubated under dark conditions for 3 h (a) and under xenon light irradiation for 3 h (b). Inhibition results of the PAN/M.bontioides/Ag-CN/Ag nanofiber membranes irradiated with xenon lamp for different times against *E. coli* (c) and *S. aureus* (d) in 3-cycle experiments. The control group are the filter layers of commercial masks.
then the antibacterial activity of the nanofiber membrane was tested under dark and light conditions. It was noticed that the nanofiber membranes still presented good antibacterial activity under dark and light conditions (as in Fig. S3). The main reason was that the environment with 85% humidity not only increased the chance of contact between substances such as AgNPs and bacteria, but also promoted the production of ROS.

The antibacterial mechanism was probably: (1) the sample was illuminated by xenon light, the Ag-CN took an electron leap, resulting in a large amount of ROS [53,63]. ROS could damage bacterial cell membranes together with AgNPs, and the destruction of cell walls or cell membranes could interfere with protein synthesis and processing, prevent DNA replication, and cause leakage of intracellular components. (2) the additives (M. bontioides, AgNPs and ROS) enter the interior of the pathogen, which disrupted protein function and DNA, and affected the uptake and conversion cycle of sugars, leading to the death of the pathogen.

3.7. Molecular docking

The novel coronavirus (SARS-CoV-2) is a beta coronavirus with 11 functional genes encoding structural, nonstructural, and accessory proteins [72,73]. Structural proteins include spike proteins (S), cytosolic proteins, membrane proteins, and nucleocapsid proteins [74,75]. The S proteins are the major antigens for coronavirus-induced neutralizing antibodies and are better proteins for vaccine studies [76-78]. The nonstructural proteins contain the important two proteins from SARS-CoV-2: Mpro and SARS-CoV-2 RNA-directed RNA polymerase (RdRp) [78,79]. These two proteins (Mpro, RdRp) are involved in the replication and transcription of the virus. After the virus infiltrates the cell, it enters the cell by interacting with the host cell proteins. The most clearly identified protein in the organism that interacts with neocoronavirus is angiotensin-converting enzyme 2 (ACE2). As a result, screening for small molecule active drugs had focused on Mpro, RdRp, and ACE2.

-CDOCKER Internation Energy is an indicator for the evaluation of the results of molecular docking under the CDOCKER algorithm [80]. The molecular docking is measured by the energy value, and it was commonly accepted that the smaller the docking radius and the higher the energy value, the better the result [81]. The compounds prunasin, acteoside, 5,7-dihydroxy flavanone, anthemisol, and 7-methoxyaromadenrin in M. bontioides (Fig. 12) were selected for molecular docking simulations with protein targets, where the target point of Mpro was 6LU7, the target point of RdRp was 6XQB, and the ACE2 target point was 2AJF. The docking between Mpro, RdRp and ACE2 with the compounds was shown in Table 5. It was concluded that the targets of compound action were mainly focused on two proteins, Mpro and RdRp, where the docking energy of the compound with Mpro was the highest and the docking radius was the shortest. The docking radius from longest to shortest was: 2AJF > 6XQB > 6LU7. The docking energies for targeting with 6LU7 were from highest to lowest: acteoside > anthemisol > 7-methoxyaromadenrin > prunasin > 5,7-dihydroxy flavanone. For the 6XQB target, the docking energy was in descending order: acteoside > anthemisol > 7-methoxyaromadenrin > 5,7-dihydroxy flavanone > prunasin. In descending order of docking energy with 2AJF target: 5,7-

![Fig. 12. Structure of selected compounds in M. bontioides.](image-url)
dihydroxy flavanone > anthemisol > 7-hydroxyaromadenrin > prunasin > acteoside. The compound acteoside had the highest docking energy with the 6LU7 target and the 6XQB target, implying that acteoside was most promising to affect the process of neo-coronavirus replication and transcription.

The 3D and 2D molecular binding models were presented in Fig. 13. 3D diagrams illustrated the 3D spatial binding model of a small molecule compound and a protein receptor 6LU7 targets, while 2D diagrams showed the structure of the compound with its surrounding key amino acid binding sites. Table 6 provided the number of amino acid binding sites for M.bontioides compounds at the 6LU7 target site (from largest to smallest): acteoside > prunasin = 7-methoxyaromadenrin > anthemisol > 5,7-dihydroxy flavanone. The compound acteoside had the maximum bound sites to amino acids around the 6LU7 target site. The 3D and 2D plots in Fig. 13 identified that the M.bontioides compound combined to multiple amino acids around the 6LU7 target site, which may prevent it from participating in the replication and transcription process of the virus and reduce the quantity of the virus. The results illustrated that the M.bontioides extract had an inhibitory effect on SARS-CoV-2 virus, which also indicated that the incorporation of the M.bontioides extract into nanofiber materials to counteract the virus was feasible. M.bontioides is an herbal plant that can be cultivated on a large scale and widely used,
with green features and great potential for future applications.

### 3.8. Antiviral activity

Influenza A H3N2 is a subtype of influenza A virus and is one of the major human seasonal influenza viruses. It is characterized by high frequency of influenza A mutation and rapid antigenic drift, which poses a serious threat to human health and brings great pressure to public health prevention and control. Therefore, influenza A virus H3N2 was selected as the virus source and cut into 25 mm diameter discs for a series of tests, and the results were listed in Table 7. The amount of H3N2 virus was determined by 50% of the tissue culture infectious dose (TCID50) in MDCK cells, and the antiviral activity of the nanofibrous membrane was assessed by the logarithmic decrease value of the control titer at the end of the indicated incubation period. Compared to the control, after 2 h exposure of the PAN/M.bontioides/Ag-CN/Ag nanofibrous membrane to H3N2 virus, a decrease in the log TCID50 of the mean virus titer was found after 2 h of test sample inoculation, with the log TCID50 of the mean virus titer per mL decreasing from 5.52 to 5.39; The log TCID50 of the mean virus titer per cm² decreased from 5.32 to 2.73. The reduction of H3N2 virus implied that the PAN/M.bontioides/Ag-CN/Ag nanofibrous membrane has antiviral activity against H3N2 virus.

The mechanism of this inhibition could be that the ions of M.bontioides extracts and AgNPs disrupt the structural integrity of the lipid bilayer membrane or the surface antigens of the viral particles, affecting

### Table 6

Number of amino acid binding sites of the M.bontioides compounds at the 6LU7 target site.

| Compounds | prunasin | acteoside | 5,7-dihydroxy flavanone | anthemisol | 7-methoxysaradirenin |
|-----------|----------|-----------|--------------------------|------------|----------------------|
| Number of amino acid binding sites to the 6LU7 target site | 8 | 13 | 4 | 5 | 8 |

### Table 7

Antiviral activity of the PAN/M.bontioides/Ag-CN/Ag nanofibrous membranes against H3N2 influenza virus.

| Experimental virus | No. | Virus titer (Vb) of control samples 0 h after inoculation | Virus titer (Vc) of control samples 2 h after inoculation | Virus titer (Vc) 2 h after test sample inoculation |
|--------------------|-----|----------------------------------------------------------|----------------------------------------------------------|--------------------------------------------------|
| Influenza A virus H3N2 (ATCC VR-1679. MDCK cells as host) | 1 | 6.2 | 5.57 | 5.46 |
| | 2 | 6.12 | 5.50 | 5.31 |
| | 3 | 6.12 | 5.5 | 5.4 |
| Log TCID50 of the mean virus titer / mL | 6.15 | 5.52 | 5.39 |
| Log TCID50 of the mean virus titer / cm² | 5.95 | 5.32 | 2.73 |
the ability of the viral particles to attach and subsequently enter the host cell with the required cell surface receptor interactions; or allow partial mutation of the amino acid sites of the viral surface glycoproteins, which may affect the replication of the viral genome and transcription. Meanwhile, molecular docking studies revealed that *M. bontioides* extracted compounds could associate with SARS-CoV-2 viral proteins and affect the replication and transcription of the virus. Therefore, PAN/*M. bontioides*/Ag-CN/Ag nanofiber membranes showed antiviral activity against H3N2 influenza virus and SARS-CoV-2 coronavirus.

### 3.9. Application of the PAN/*M. bontioides*/Ag-CN/Ag nanofibrous membrane.

For the purpose of examining its application in practice, we prepared electrospun fiber mats into masks. As in Fig. 15a, we cut a commercial mask and replaced the filter layer of the commercial mask with an electrospun fiber mat to obtain a photocatalytic mask (Fig. 15b). Our photocatalytic masks had the same facial coverage as the commercial mask, as well as our masks emit *M. bontioides* plant fresh fragrance to reduce adverse reactions and boost comfort (Fig. 15c-d).

![Prototype of photocatalytic mask and filtration efficiency after wearing. (a) A prototype mask is manufactured by substituting the filter of a commercial mask with PAN material. (b) Photographs of a prototype photocatalytic mask and a commercial mask. (c) Side view and (d) front view of the commercial mask (1) and the photocatalytic prototype mask (2) worn by the author.](image)

Table 8

| Evaluation          | Excellent | Good | General | Poor | Very poor |
|---------------------|-----------|------|---------|------|-----------|
| Appearance/ per     | 2         | 7    | 9       | 7    | 5         |
| Filtration effect / per | 4        | 11   | 10      | 2    | 3         |
| Comfort/ per        | 8         | 10   | 8       | 3    | 1         |

The cytocompatibility of the nanofibrous membranes was determined with bone marrow MSCs, and the value-added growth of the cells at 1, 3, and 5 days was observed. Fig. S6 exhibited the growth and proliferation of bone marrow MSCs on the surface of the nanofiber membrane. The ability of bone marrow MSCs to grow and proliferate normally on the surface of the nanofiber membrane as observed by live-dead cell staining indicates the low toxicity of the nanofiber membrane. Meanwhile, the absorbance of the cells was detected by Thermo Science Multiskan GO microplate instrument, and it was found that the number of bone marrow MSCs increased with the increase of culture time. The results indicated that the prepared samples had favorable cytocompatibility and were friendly to human skin.

We randomly asked 30 passersby to evaluate the appearance, filtration effect and comfort of the produced photocatalytic prototype masks. The evaluation was divided into 5 levels and the results were listed in Table 8. The appearance of our handmade masks was still quite different from commercial masks, so most passersby were not very satisfied with them. However, most passersby were pleased and approved of the filtration effect and comfort of the made masks. It demonstrated that PAN/*M. bontioides*/Ag-CN/Ag nanofiber films were expected to be used in medical masks.

### 4. Conclusion

In conclusion, this study has demonstrated a multifunctional composite material prepared from plant extracts and Ag-CN photocatalytic material with potential applications in personal protective materials. The electrospinning technology enhances both the filtration and breathability of the mask, in which the nanofibrous membrane produced
has a uniform diameter of 726.93 ± 140.50 nm and strong electrostatic attraction, endowing the mask with superior pathogen filtration capability. The micro-pores formed between the nanofibrous membrane enable excellent breathability and air permeability with an average particle filtration efficiency of 99.82% ± 0.019%. The addition of photocatalyst Ag-CN enables PAN/M.bontioids/Ag-CN/Ag nanofiber membranes to be used as photocatalytic bacterial materials. It not only enables the photocatalytic degradation of pollutants with a 96.37% degradation rate of methylene blue; but also the ROS generated could eliminate pathogens immediately. The bactericidal effects on E.coli and S.aureus are 98.65 ± 1.49% and 97.8 ± 1.27%, respectively, at 3 h of light exposure. It could reduce secondary infections and environmental pollution caused by discarded masks. The obtained nanofibrous membrane can achieve 3 cycles of sterilization to prolong its life span. Molar docking revealed that the compounds in M.bontioids extracts interacted with neo-coronavirus targets mainly on two proteins, Mpro and RdRp, in which the acteoside exhibited the highest docking energy and the smallest docking radius with the Mpro protein. Moreover, the high number of key amino acid binding sites around the target site of Mpro protein results in a high impact on the replication and transcription process of Hunan Provincial Key Laboratory of Environmental Photocatalysis and unsatisfied wearing resistance, we strongly believe that the prepared nanofibrous membranes would act as promising protective materials in the future.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.seppur.2022.121565.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.seppur.2022.121565.

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