

Article

Antibacterial Properties of Dandelion Extract-Based PVA/CTS/DAN/CuNP Composite Gel

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Abstract: Dandelion extract is a reducing agent, and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ was used as a carrier to create copper nanoparticles (CuNPs). A novel polyvinyl alcohol–chitosan–dandelion–CuNP (PVA/CTS/DAN/CuNP) gel was acquired by cross-linking Polyvinyl alcohol (PVA) and chitosan (CTS) solution. Its structure was analyzed using Fourier transform infrared spectroscopy, Scanning electron microscopy, and X-ray diffraction. The PVA/CTS/DAN/CuNP gels manifested good stability, recycling ability, swelling properties, and biocompatibility. Using the agar diffusion method, the diameters of the inhibition zone of the composite gel against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* could be over 21 mm. In conclusion, the PVA/CTS/DAN/CuNP composite gel had good antibacterial performance, which has a high potential for application in microbial contamination treatment and environmental protection.

Keywords: antibacterial ability; dandelion extract; copper nanoparticles; composite gel; antibacterial test



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1. Introduction

Industrial wastewater usually carries hazardous pollutants that cause several environmental issues such as microbial contamination [1–3]. Microbial pollution in water bodies refers to the presence of pathogenic microorganisms (e.g., bacteria, parasites, etc.) in water bodies that exceed environmental quality indicators, thereby causing harm to human health and aquatic ecosystems [4,5]. Accordingly, it is crucial to develop a material that has the function of inhibiting microbial growth in water bodies. The use of gel materials to inhibit harmful microorganisms in water bodies is beneficial to the protection of water quality compared to other methods, as gel materials are usually derived from natural or renewable resources, which have a low impact on the environment and the treatment process does not involve the use of hazardous chemicals, which do not introduce new pollutants into the water [6–10].

In recent years, metal nanomaterials have been increasingly used in different fields and have been applied to gas sensors, batteries, fuel cells, high-temperature superconductors, and microelectronic circuits, etc., and they can also be used as adsorbents, antimicrobial agents, and catalysts [11–13]. Distinct from other metallic nanomaterials, copper-based nanomaterials have gained special attention due to their unique physical, chemical, and biological properties [14,15]. Copper nanoparticles (CuNPs) are considered to be relatively biocompatible and can be degraded through biological pathways. In addition, CuNPs have been manifested to possess anti-inflammatory and antimicrobial properties, which can be utilized in medical devices, wound aids, and anti-infection materials [16–18]. In practical applications, CuNPs have a large surface energy and are prone to accumulation [19]. The dispersion of CuNPs is associated with the synthesis method of the nanoparticles, surface charge, and size, among which the synthesis of CuNPs using green synthesis is the most common method to increase dispersion [20]. Plant extracts contain a large number of

chemicals, which can be used as reducing and stabilizing agents for transforming metal ions into metal nanoparticles [21]. Dandelion is a widely distributed perennial herb belonging to the family Asteraceae, which contains a variety of medicinal metabolites including carotenoids, flavonoids, phenolic acids, polysaccharides, and sterols, etc. [22]. CuNPs can be acquired using dandelion flower extract as a reducing agent [23]. In addition, the dispersion of CuNPs can also be enhanced by the combination of CuNPs with gels [24]. The gels render a more stable chemical environment for CuNPs and reduce their oxidation in air, and the matrix in the gel can help to disperse the nanoparticles and decline the particle agglomeration phenomenon, thus promoting the overall performance [25].

Gels are physically or chemically crosslinked three-dimensional hydrophilic polymer networks that can quickly absorb a huge volume of water and retain large amounts of water. Due to their unique physical and chemical properties, hydrogels have a wide range of applications in the fields of biopharmaceuticals, nursing, agriculture, and environmental protection, which have attracted increasing attention [26–29]. Polyvinyl alcohol (PVA) is one of the very few water-soluble vinyl polymers, which is an attractive material due to its excellent film-forming and adhesive properties, chemical stability, and biocompatibility [30]. Chitosan (CTS) is a polymeric polysaccharide, which is one kind of biomaterial for wound care dressings, tissue engineering scaffolds, and drug carriers because of its excellent biocompatibility, biodegradability, nontoxicity, and antimicrobial properties [31–33]. As a natural chelating agent, CTS is rich in active amino groups, whose protons carry a positive charge that can be attracted by the hydroxyl groups in the PVA molecule and thus crosslinked, and the composite hydrogel of PVA and CTS manifests excellent antimicrobial activity against a wide range of bacteria, including Gram-positive and Gram-negative bacteria [34–36]. Soy protein isolate (SPI), a high-purity protein extracted from soybeans, is extensively used in the field of biomedical materials due to its good biocompatibility, degradability, and low cost [37]. Glutaraldehyde (GA) is a commonly used cross-linking agent that may react with the hydroxyl group of PVA to generate hemiacetals [38,39].

This research aimed to design novel composites with good antimicrobial activity. A green synthesis method was used to prepare CuNPs using dandelion extract as a reducing agent, and a novel composite hydrogel was synthesized using PVA, CTS, and SPI as carriers and GA as a cross-linking agent. The structure and morphology of the composite gel were characterized by Fourier infrared spectroscopy (FTIR), X-ray diffraction (XRD), and scanning electron microscopy (SEM). The bacteriostatic effect on Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Pseudomonas aeruginosa*, *Escherichia coli*) bacteria was testified through the analysis with agar diffusion method. In addition, the reusability and stability of the gels were investigated. Finally, the prepared composite gel held great promising potential as an antibacterial agent.

2. Materials and Methods

2.1. Chemicals and Materials

Copper sulfate pentahydrate (>99.0%), polyvinyl alcohol (PVA $\geq$ 98.5%), chitosan (CTS) (deacetylation degree $\geq$ 95%; CAS 9012-76-4, molecular weight 1526.45), Soy protein isolate (SPI, CAS 9010-10-0), polyvinylpyrrolidone (PVP, molecular weight 10,000–70,000), glutaraldehyde (GA $\geq$ 99%), and other chemicals were from Sinopharm Chem. Reagent Co., Ltd. (Shanghai, China). *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, and *Staphylococcus aureus* ATCC 6538 were from Shanghai Institute of Microbiology, China. Dandelion flowers were obtained from a farm in Changzhou (Jiangsu Province, China).

2.2. Synthesis of CuNPs

Dandelion flowers were thoroughly cleaned to remove surface dust particles and dried under shade at room temperature. After drying, the dried dandelion flowers were pulverized with a pulverizer and further passed through a 60-mesh sieve to obtain 60-mesh of dandelion flower powders. A total of 2.0 g of the prepared dandelion flower powder and 50 mL of 80% ethanol were mixed, and ultrasonication-assisted extraction was implemented

at 60 °C for 1 h. After the mixture was cooled to room temperature, the extract was filtered through filter paper to acquire the extract of dandelion flowers (DAN), which was stored at 4 °C for further experiments. A total of 1.0 g of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ was dissolved in 100 mL of deionized water, and 10 mL of DAN was added. Afterwards, this mixture was heated at 80 °C by stirring (500 rpm) for 60 min. The color change was observed, and the black particles formed in the solution were the generation of CuNPs.

2.3. Preparation of PVA/CTS/DAN/CuNP Gels

To acquire 2.0 wt% acetic acid solution, 1.0 g of CTS powder was dispersed in 49 g of acetic acid solution and stirred (800 rpm) for 30 min at room temperature. A total of 1.0 g of PVA was added to 49 mL of deionized water to dissolve for 15 min, and the dissolved PVA was stirred (800 rpm) at 90 °C in a water bath until the PVA was entirely dissolved, to form a PVA (2.0 wt%) solution. A total of 1.0 g of SPI was supplemented to 2.0 wt% PVA solution and mixed well. The 2.0 wt% CTS solution was then supplemented with the above solution and magnetically stirred (800 rpm) for 20 min. A total of 10 mL of CuNP solution was then added and continued to be magnetically stirred (800 rpm) for 10 min to mix well, and 0.05 g of PVP was supplemented to the mixed solution. Eventually, 1 mL of glutaraldehyde was supplemented with magnetic stirring until a PVA/CTS/DAN/CuNP gel was formed. By varying the incorporation of PVA, DAN, and CuNPs, PVA/CTS gels, CTS/DAN/CuNP gels, and PVA/CTS/DAN gels were obtained, respectively.

The preparation procedure of PVA/CTS/DAN/CuNP gels was given as Figure 1. Different concentrations of CTS solutions were prepared to make PVA/CTS/DAN/CuNP/X gels with different CTS contents, where X denotes the content of CTS (12.5, 15, 17.5, 20, and 25 g/L). Different weights of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ were added to deionized water to formulate $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solutions with different concentrations, and CuNP solutions with different concentrations were prepared by reducing copper sulfate solutions with dandelion extract. Different concentrations of CuNP solutions were prepared to acquire PVA/CTS/DAN/CuNP/Y gels, where Y was the CuNP content (0.01, 0.05, 0.1, 0.5, 1, 2, 4, and 6 g/L).

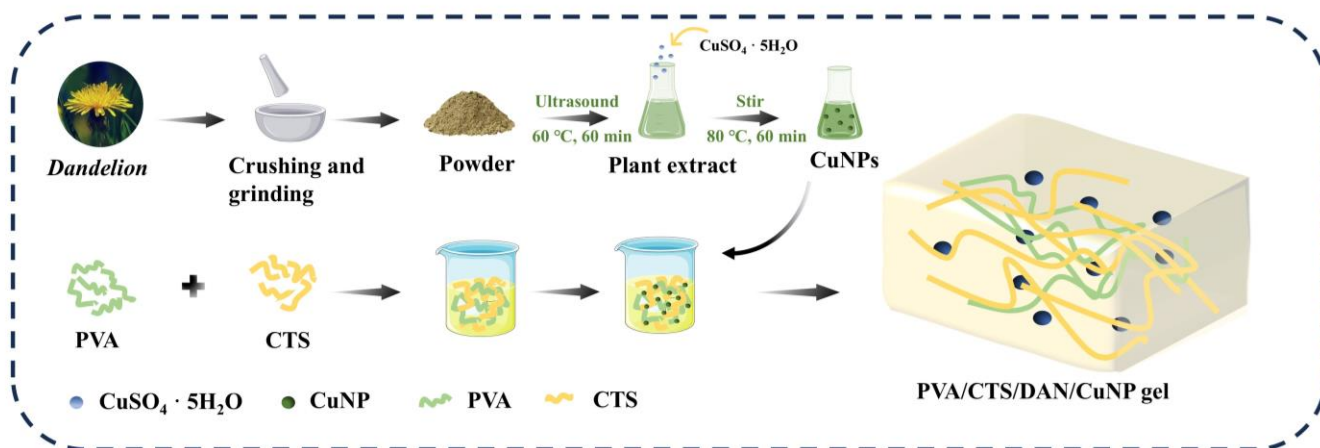


Figure 1. Procedure for the preparation of PVA/CTS/DAN/CuNP gels.

2.4. Characterization of PVA/CTS/DAN/CuNP Gels

Fourier Transform Infrared Spectrometer (FT-IR) was utilized to scan the PVA/CTS, CTS/DAN/CuNP, PVA/CTS/DAN, and PVA/CTS/DAN/CuNP gels in the range of $500\text{--}4000\text{ cm}^{-1}$ to study the changes occurring in the structure of different components of the gels. Through the surface morphology analysis with Scanning Electron Microscope (SEM), PVA/CTS, CTS/DAN/CuNP, PVA/CTS/DAN, and PVA/CTS/DAN/CuNP gels were scanned to observe the gel surface structure. X-ray diffraction (XRD) was imple-

mented over 5°–80° to characterize the binding state of the PVA/CTS, CTS/DAN/CuNP, PVA/CTS/DAN, and PVA/CTS/DAN/CuNP gels, respectively.

2.5. Antibacterial Testing

2.5.1. Optimization of Gel Preparation

The bacteriostatic activity of four composite gels with different components (PVA/CTS, CTS/DAN/CuNP, PVA/CTS/DAN, and PVA/CTS/DAN/CuNP gels) was investigated using *E. coli*, *S. aureus*, and *P. aeruginosa*. A total of 100 µL of bacterial suspension (about 10⁶ CFU/mL) was uniformly applied to a sterile Luria–Bertani agar plate and a hole (diameter 9 mm) was drilled in Luria–Bertani solid medium, then 0.30 g of the composite gel was supplemented to the hole, and the diameter of the antimicrobial zone was determined after incubation at 37 °C for 18 h [40].

2.5.2. Optimization of the Amount of CTS as well as CuNPs in PVA/CTS/DAN/CuNP Gels

Different CTS contents (12.5, 15, 17.5, 20, and 25 g/L) and different CuSO₄·5H₂O contents (0.01, 0.05, 0.1, 0.5, 1, 2, 4, and 6 g/L) were assessed using the same methodology as described above.

2.5.3. Stability and Reusability of Antibacterial Gels

E. coli, *P. aeruginosa* and *S. aureus* were used to assess the reusability and stability of the gel in the antibacterial experiments. Based on the plate counting method, 0.30 g of PVA/CTS/DAN/CuNP gel was supplemented into 50 mL of bacterial suspension (10⁸ CFU/mL), and the bacterial suspension without composite gel was used as a blank, and then incubated at 37 °C for 1 h. A total of 100 µL of bacterial suspension was withdrawn and further diluted to a certain concentration, and then uniformly coated on Luria–Bertani agar plates. The cultivation was conducted at 37 °C for 18 h. To evaluate the reusability and stability of the antibacterial gel to *E. coli*, *S. aureus*, and *P. aeruginosa*, plate counting was implemented to detect the reusability and stability of the antibacterial gel. After incubation at 37 °C for 18 h, plate counting was implemented to detect the bacterial content in the suspension. Afterwards, the recovered gel was carefully washed with water and the surface was dried for the next antibacterial test. The reused experiment was implemented eight times to test the effect of repeated use on the antibacterial ability of the gel and to determine the reusability of the gel [41].

The stability of the PVA/CTS/DAN/CuNP gel in the aqueous environment of different pH was analyzed using the plate counting method. The prepared PVA/CTS/DAN/CuNP gels were placed at room temperature and the composite gels were withdrawn every other day, and the bacteriostatic effect of the PVA/CTS/DAN/CuNP gels of different days was tested with the plate counting method to determine the stability of the PVA/CTS/DAN/CuNP gels. In addition, the PVA/CTS/DAN/CuNP gels were placed in PBS solutions with different pH values (4, 5, 6, 7, 8, 9, 10, and 11) overnight, and then the gels were fetched and carefully washed with water. The surface moisture was wiped dry, and the bacteriostatic ability of the PVA/CTS/DAN/CuNP gels was tested using the plate counting method in the solutions with different pH values to evaluate the influence of the bacteriostatic effect of the gels at different pH values and assess the stability of the composite.

2.6. Statistical Analysis

All experiments were repeated at least three times unless otherwise stated. SPSS 25.0 software was used for one-way analysis of variance (ANOVA).

3. Results and Discussion

3.1. Gel Characterization

3.1.1. FT-IR

The chemical functional groups in gel materials can be analyzed using Fourier transform infrared spectroscopy (FT-IR) [42]. The FT-IR spectra of PVA/CTS, CTS/DAN/CuNP, PVA/CTS/DAN, and PVA/CTS/DAN/CuNP composite hydrogels are presented in Figure 2. There was a strong broad peak near 3428 cm^{-1} which was assigned to the stretching vibration of hydrogen bonding [43,44]. Both PVA and CTS contain hydroxyl groups, and these hydroxyl groups could form intermolecular hydrogen bonds. The amino ($-\text{NH}_2$) and hydroxyl ($-\text{OH}$) groups of CTS might participate in the formation of hydrogen bonds, further adding to the complexity of hydrogen bonding. The peak at 2930 cm^{-1} was associated with the stretching vibration of the C-H bond. The large number of methylene units contained in CTS and PVA, and CTS also contains methyl units and the C-H stretching vibration of these alkyl chains usually occurred in the region of $2800\text{--}3000\text{ cm}^{-1}$, so the peak at 2930 cm^{-1} of the gel was assigned to the symmetric stretching vibration of the methylene groups [45,46]. The peaks at 1640 and 1577 cm^{-1} were assigned to the aromatic ring vibration of CTS. The CTS molecule contains an aromatic glucosamine ring, and the C=C bond stretching vibration of this ring appeared near 1640 cm^{-1} , while the vibrational peaks of the aromatic ring skeleton appeared near 1577 cm^{-1} , which reflected the characteristics of the vibration of the amino-glucose ring in the CTS molecule and is one of the CTS's one of the characteristic absorption peaks [47,48]. The peak at 1128 cm^{-1} was associated with the C-O bond in the gel, which might be due to the cross-linking reaction of the alcohol group in the PVA molecule with the hydroxyl or amino group in the CTS molecule, and the peaks near 1128 cm^{-1} were more pronounced for the PVA/CTS/DAN/CuNP gel and CTS/DAN/CuNP gel compared to the other two gels without the incorporation of copper nanoparticles, which might be due to the effect of the presence of copper nanoparticles on the intensity of the peaks [49].

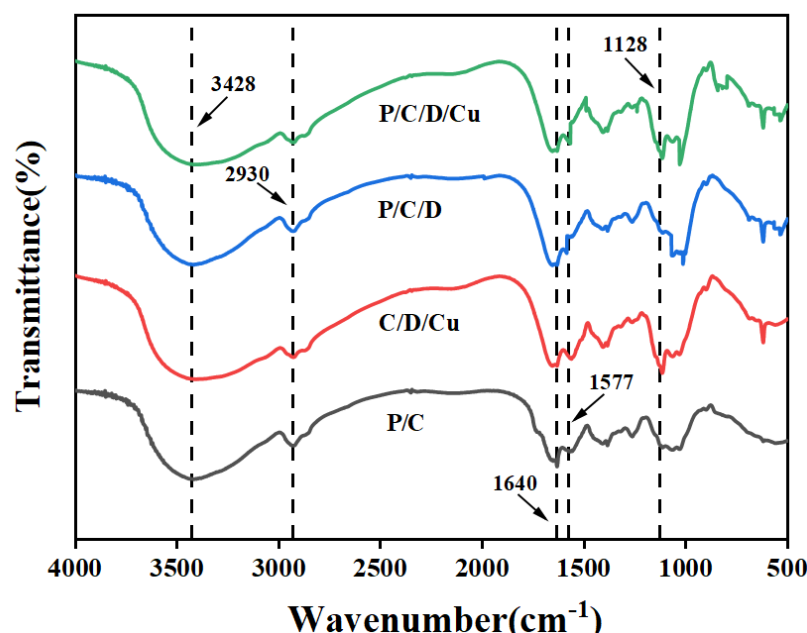


Figure 2. FTIR spectra of PVA/CTS (P/C) gel, CTS/DAN/CuNP (C/D/Cu) gel, PVA/CTS/DAN (P/C/D) gel and PVA/CTS/DAN/CuNP (P/C/D/Cu) gel.

3.1.2. SEM

The PVA/CTS/DAN/CuNP gel was measured using SEM, and the SEM image of the composite gel was shown in Figure 3. From the figure, it could be seen that the surface of the P/C gel was rough, while the surface of the CTS/DAN/CuNP gel was flatter, which

proved that the mixing of PVA and CTS made the network structure of the composite gel more complex than that of the single CTS gel [50]. The reduced surface roughness of the PVA/CTS/DAN gel samples compared to the PVA/CTS gels might be attributed to the fact that the flavonoids and polyphenols contained in the dandelion extracts could act as effective cross-linking agents to promote cross-linking between the polymer chains to form a tighter cross-linking network. From the comparison of Figure 3f,h, it could be seen that the surface of the gel became rougher after the addition of nano-copper; this was probably due to the fact that the nano-copper agglomerated and formed aggregates during the gel formation process, and these aggregates made the surface of the gel form an inhomogeneous roughened structure, which meant that the gel's specific surface area increased, which helped to improve the adsorption performance of the gel [51,52]. These results confirmed the successful preparation of PVA/CTS/DAN/CuNP composite gels.

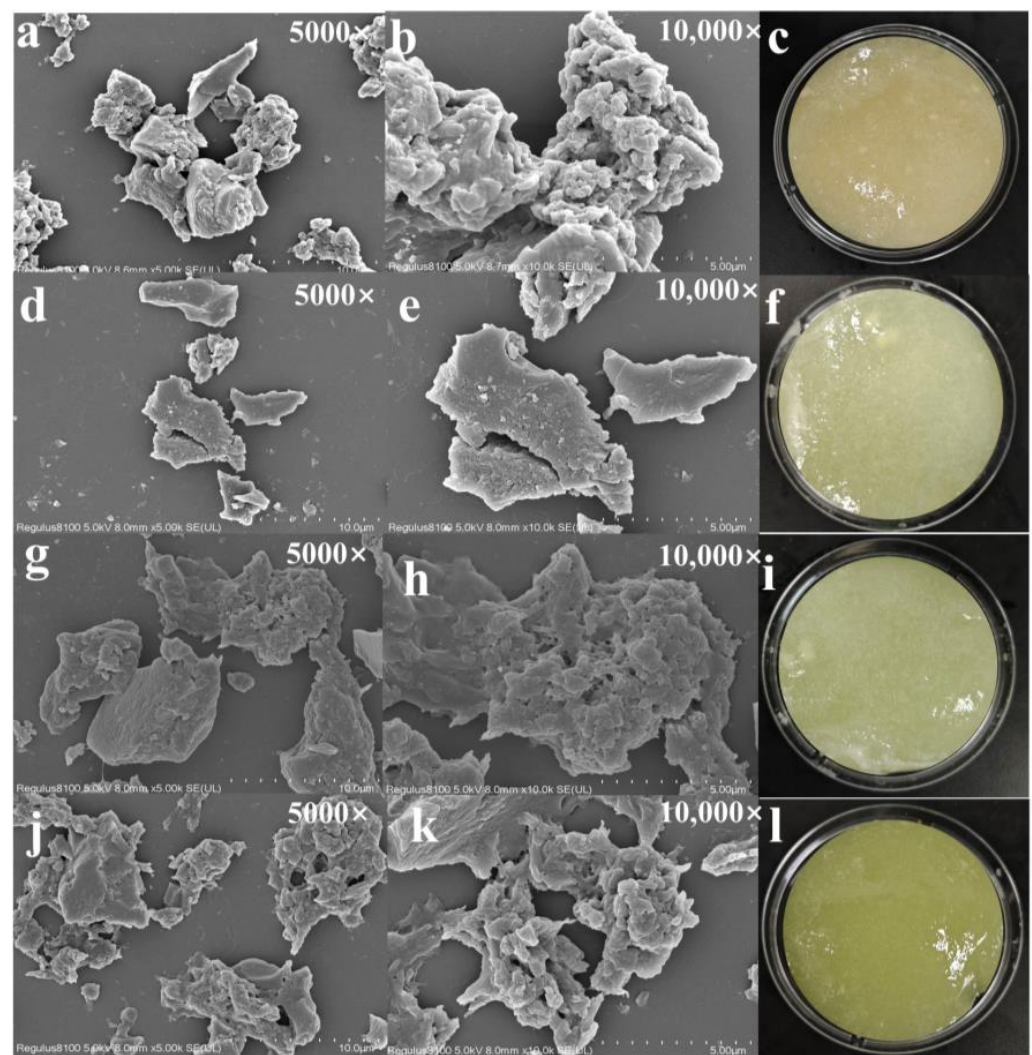


Figure 3. SEM images of PVA/CTS (a,b), CTS/DAN/CuNP (d,e), PVA/CTS/DAN (g,h), and PVA/CTS/DAN/CuNP (j,k); Pictures of PVA/CTS (c), CTS/DAN/CuNP (f), PVA/CTS/DAN (i), and PVA/CTS/DAN CuNP (l).

3.1.3. XRD

The crystal structures of the composite gels were analyzed using XRD, and the results are shown in Figure 4. The four composite gels exhibited characteristic diffraction peaks near $2\theta = 19.5^\circ$, which was related to the semi-crystalline state of the composite gels forming polymers; in addition, the position of the peaks was not significantly affected by the

addition of copper nanoparticles [53]. The PVA/CTS/DAN/CuNP and CTS/DAN/CuNP gels with the addition of CuNPs had diffraction peaks near 44° , 50° , and 74° compared to the other two, and the diffraction peak positions corresponded to the crystallographic planes of (111), (200), and (220), respectively; therefore, these peaks may correspond to the crystal structure of copper nanoparticles [54]. The results showed that CuNPs were successfully introduced within the composite gel.

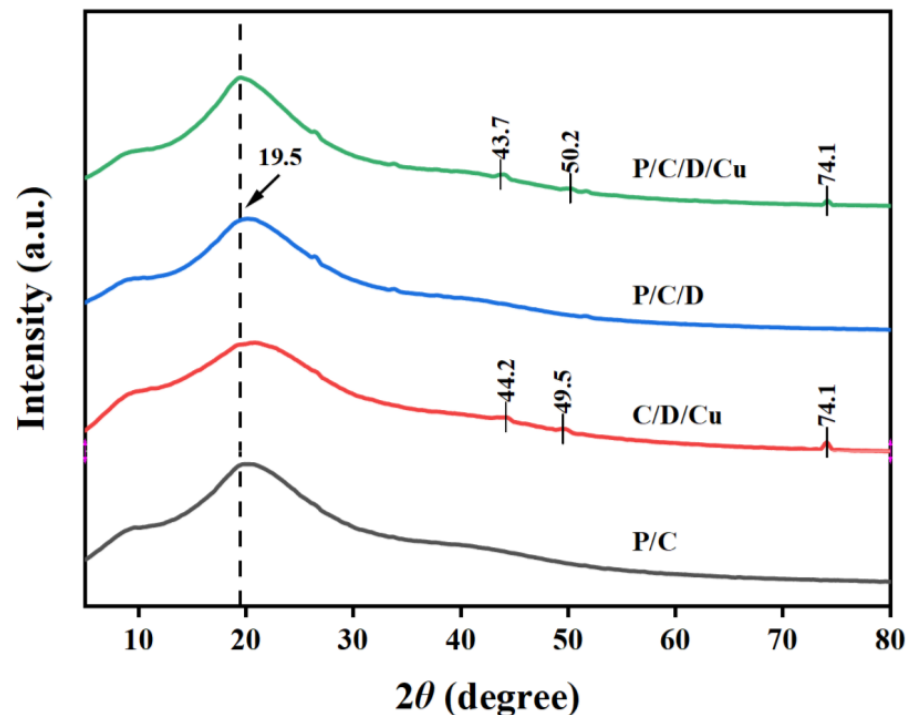


Figure 4. X-ray diffraction spectra of PVA/CTS (P/C) gels, CTS/DAN/CuNP (C/D/Cu) gels, PVA/CTS/DAN (P/C/D) gels, and PVA/CTS/DAN/CuNP (P/C/D/Cu) gels.

In a concise summary, CuNPs and PVA/CTS/DAN/CuNP composite gel were successfully prepared based on the analysis with FT-IR, SEM and XRD. To well understand the surface, pore, and structure properties and gel components, other techniques including BET, EDX, and TEM can be implemented in the future.

3.2. Antibacterial Activity

3.2.1. Antibacterial Activity of Composites

The antibacterial activity of three bacteria, *E. coli*, *P. aeruginosa*, and *S. aureus*, was investigated by agar diffusion method on four types of gels (PVA/CTS, CTS/DAN/CuNP, PVA/CTS/DAN, PVA/CTS/DAN/CuNP gels), and the results are displayed in Figure 5a. The PVA/CTS gel material manifested the worst inhibitory effect on *E. coli*, *S. aureus*, and *P. aeruginosa*, with the diameters of the inhibitory zone being 17, 15, and 16 mm, respectively. Upon adding CuNPs, the diameters of the inhibition zone of CTS/DAN/CuNP gel and PVA/CTS/DAN/CuNP gel materials on *E. coli*, *S. aureus*, and *P. aeruginosa* increased, and the inhibitory effect was obviously strengthened. Among them, the PVA/CTS/DAN/CuNP gels showed a significant inhibitory effect on all three test bacteria ($p < 0.05$), with the diameter of the inhibitory zone being 24 mm (for *E. coli*), 21 mm (for *S. aureus*), and 23 mm (for *P. aeruginosa*). Meanwhile, the inhibitory ability of CTS/DAN/CuNP gel declined due to the absence of PVA, with diameters of 21 mm (for *E. coli*), 20 mm (for *S. aureus*), and 20 mm (for *P. aeruginosa*), respectively. The bacteriostatic activity of the PVA/CTS/DAN gel with the addition of dandelion extract was enhanced compared to the PVA/CTS gel,

and the diameters of the inhibition zone against *E. coli*, *S. aureus*, and *P. aeruginosa* were 20 mm, 18 mm, and 18 mm, respectively.

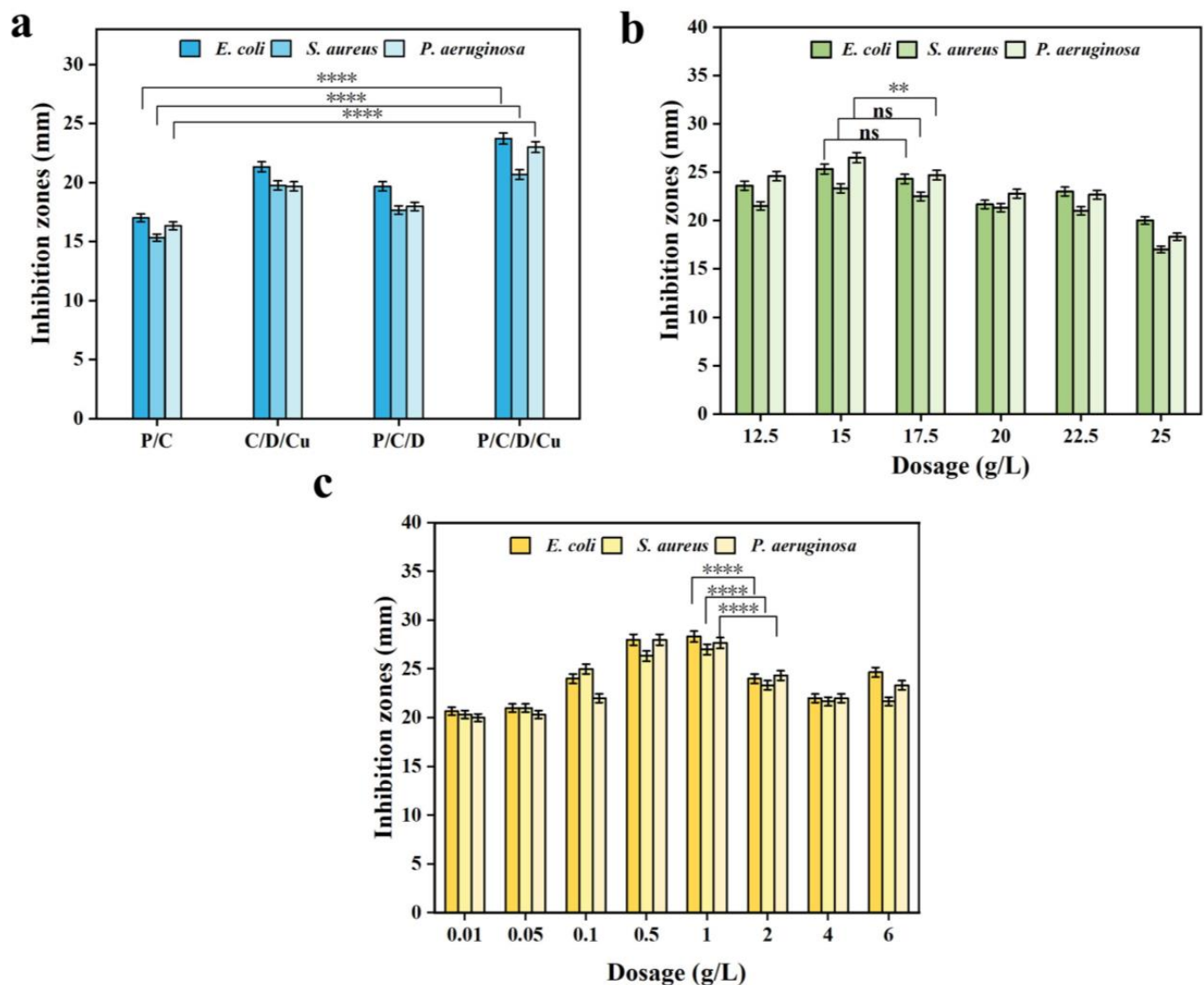


Figure 5. Antibacterial effect of composite gels prepared with different materials [PVA/CTS (P/C), CTS/DAN/CuNP (C/D/Cu), PVA/CTS/DAN (P/C/D) and PVA/CTS/DAN/CuNP (P/C/D/Cu)] (a); CTS dosage (12.5, 15, 17.5, 20, 22.5, and 25 g/L) (b); and CuNP dosage (0.01, 0.05, 0.1, 0.5, 1, 2, 4, and 6 g/L) (c) (**** denotes $p < 0.0001$; ** denotes $p < 0.0$; ns denotes no statistical significance).

3.2.2. Effect of the CTS and CuNP Loading on the Inhibitory Activity of PVA/CTS/DAN/CuNP Gels

The effects of CTS dosage on the antibacterial activity of the gel are showcased in Figure 5b. The antibacterial effect of the gel on *E. coli*, *S. aureus*, and *P. aeruginosa* manifested a trend of increasing and then decreasing with the increase of the CTS content of the gel. The PVA/CTS/DAN/CuNP gel showed the highest bacterial inhibitory activity against bacteria when the dosage of CTS was 15 g/L, which was significantly higher than the other concentrations ($p < 0.05$), and the diameters of the inhibition zone on *E. coli*, *S. aureus*, and *P. aeruginosa* were 25, 23, and 27 mm. However, the increase in CTS content did not linearly improve the bacteriostatic effect of the gel, and a decreasing trend in the bacteriostatic effect of the gel was observed as the CTS content continued to increase. CTS had a certain synergistic antimicrobial effect, so the increase in the CTS content was able to improve the bacteriostatic activity of the gel. The PVA:CTS ratio in the gel affected the cross-linking

state of PVA/CTS/DAN/CuNP gel, changed the internal pore structure of the gel, and also influenced the bacteriostatic activity of the gel [55]. From the above results, it could be found that the optimal addition of CTS in the composite gel was 15 g/L.

As showcased in Figure 5c, with the increase of the content of CuNPs within the gel, the inhibitory effect of the gel was gradually enhanced, and the inhibitory effect of the gel on bacteria reached the strongest when the concentration of CuNPs was 0.5–1.0 g/L; the circle of inhibition was the largest and significantly higher than the other concentrations of the gel ($p < 0.05$). However, when the content of CuNPs reaches a certain level, excessive copper nanoparticles might affect the porosity of the gel, and these changes could influence the distribution and release of CuNPs in the gel, thus impacting the bacterial inhibition effect of the gel.

E. coli, *S. aureus*, and *P. aeruginosa* are three different bacteria, they differ in their biological characteristics, cellular structure, and susceptibility to antimicrobial agents. *P. aeruginosa* is able to form biofilms, which provide additional protection, and inhibition of *P. aeruginosa* usually requires disruption of the outer membrane, inhibition of biofilm formation, or targeting of specific metabolic pathways, which may lead to instability in the inhibitory effect of the gel against *P. aeruginosa*. It was found that the inhibitory effect on *P. aeruginosa* could be affected when the gel composition was changed or when the CTS content and the CuNP content in the gel were changed. Although the inhibition of *P. aeruginosa* by the different components of the gel was unstable, the gel inhibited *E. coli* and *P. aeruginosa* generally better than *S. aureus*, and it was proved that the inhibition of gram-negative bacteria by the gel was more effective. This might be due to the fact that the cell walls of gram-negative bacteria are thinner as compared to gram-positive bacteria, which makes them more susceptible to cell wall disruption [36].

3.3. Reusability and Stability of the Composite Gel

The antibacterial effect of the composite gel for different days was showcased in Figure 6a, the inhibitory activity of the gel against bacteria was 100% in the first eight days. After the eighth day, there was a significant decrease in the inhibitory effect of the gel ($p < 0.05$). The inhibitory activity of the composite gel weakened, but the inhibitory activity of the gel still remained above 90% on the 14th day, implying that the composite gel could maintain stable antibacterial activity for a longer period. The pH value of the aqueous solution had a certain influence on the antibacterial activity of the gel, as displayed in Figure 6b. The antibacterial activity of the composite gel in acidic solution remained high. Especially at pH values of 4 and 5, the antibacterial rate of the gel reached 100%, significantly higher ($p < 0.05$) than the inhibition rate of the gel in solution at other pHs. The antibacterial rate of the gel gradually decreased with the increase in the pH value of the aqueous solution. CTS is a polysaccharide with amino groups, and the amino groups in its molecules can be easily protonated under acidic conditions to form a positive charge, which manifests a higher antimicrobial effect [32]. The above results illustrated that the composite gel was highly applicable to the environment, and could be used in an acidic environment with a long shelf life.

Excellent antibacterial composite gel materials should not only have high antibacterial activity but also maintain good recycling performance. As showcased in Figure 6c, the bacteriostatic rate of the gel remained above 90% after six repetitions of bacteriostatic inhibition in water, and the bacteriostatic inhibition rate at the sixth repetition weakened by no more than 3% compared with the highest bacteriostatic inhibition rate, and the results manifested that the composite gel could be reused. However, the antibacterial effect of the gel was significantly reduced ($p < 0.05$) after the seventh experimental repetition, which might be because the active ingredients in the composite gel began to decompose due to repeated use, and the effective bacteriostatic substances were consumed [56].

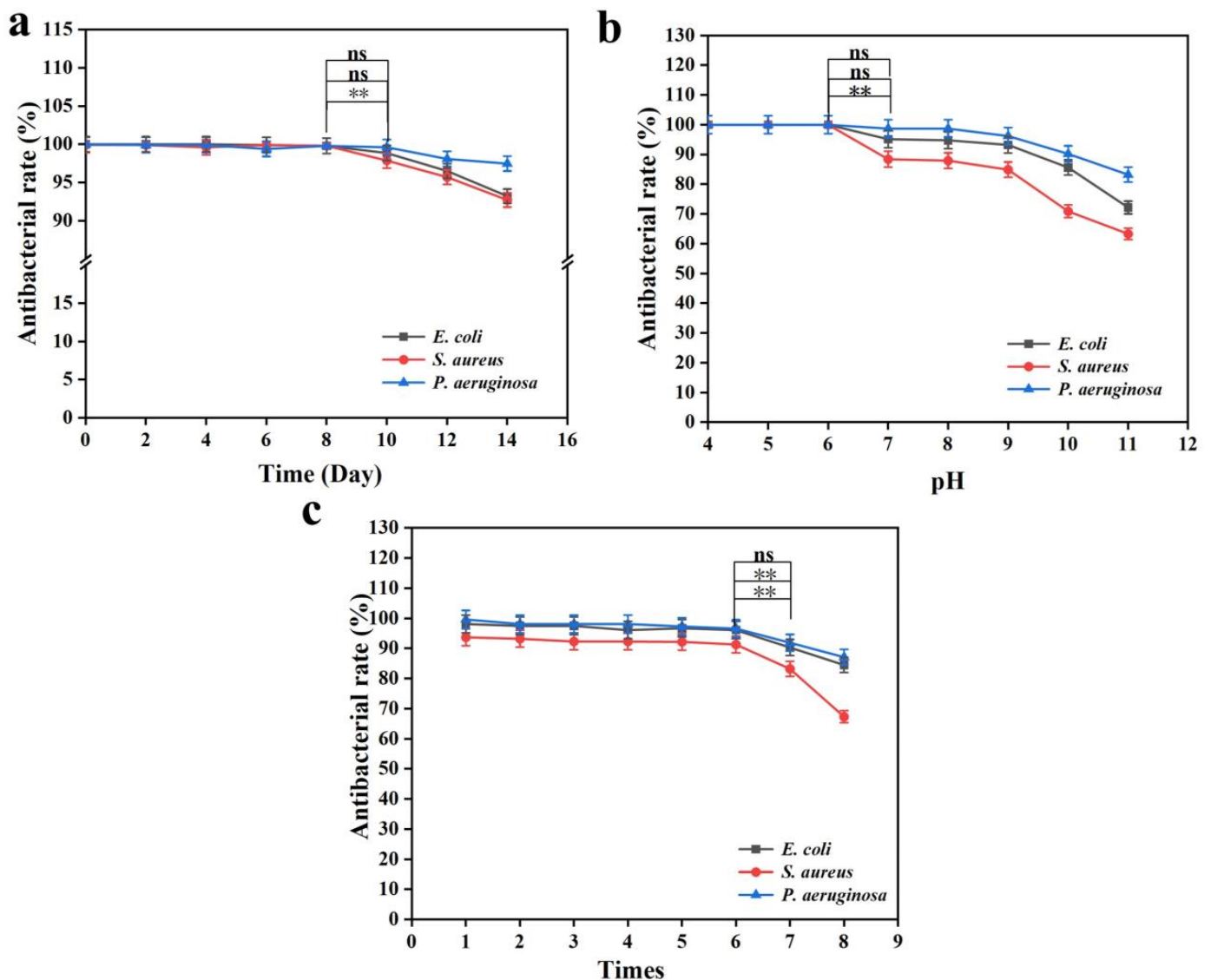


Figure 6. Bacterial inhibition in different days of preparation (0, 2, 4, 6, 8, 10, 12, and 14 days) (a); different pH solutions (4, 5, 6, 7, 8, 9, 10, and 11) (b); and repeated use of gel (1, 2, 3, 4, 5, 6, 7, and 8 times) (c). (** denotes $p < 0.01$; ns denotes no statistical significance).

3.4. Proposed Mechanism of Bacterial Inhibition

The antimicrobial mechanism of PVA/CTS/DAN/CuNP composite gel is displayed in Figure 7. The cation formed by CTS dissolved in water gives itself good bacteriostatic activity, so the PVA/CTS gel without added CuNPs also has some bacteriostatic activity [57,58]. CTS containing a large number of amines and hydroxyls has some synergistic antimicrobial effects. It can dissociate into positively charged cations in an aqueous solution, and the bacteria are negatively charged, which leads to electrostatic interactions between chitosan and bacteria, thus disrupting the bacterial cell membrane and leading to bacterial death [36,59]. The incorporation of CuNPs showed better bacteriostatic effects, mainly due to the CuNPs entering the cell interior, and then generating reactive oxygen species (ROS), which can bind to DNA molecules and disrupt the helical structure, cause the denaturation of proteins and rupture of enzymes, and inhibit the growth of the bacteria, ultimately leading to the death of the bacteria [60–66].

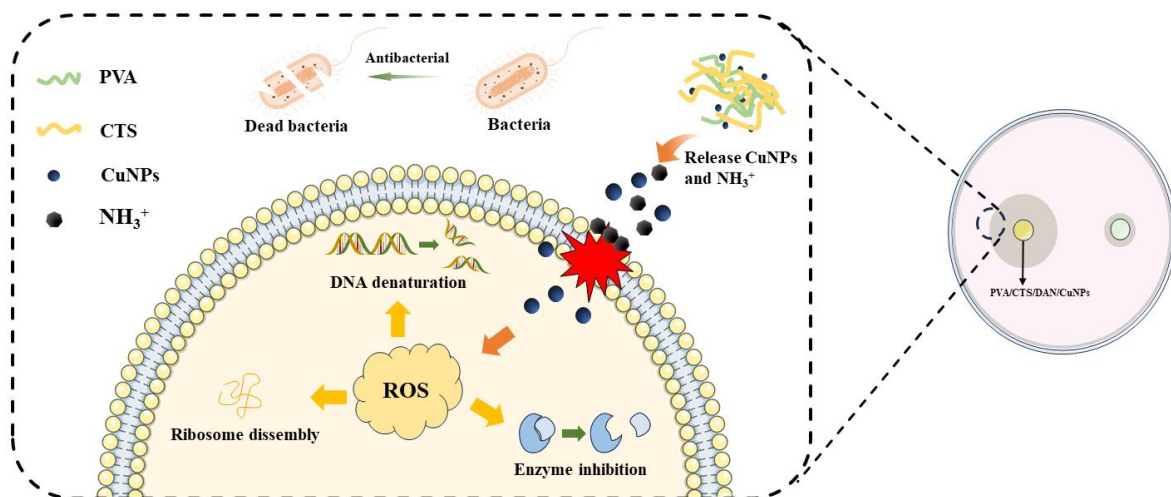


Figure 7. The antibacterial mechanism of PVA/CTS/DAN/CuNP composite gels.

4. Conclusions

Microbiological contamination of water bodies is a global problem, and these microbiological contaminations not only lead to waterborne diseases but also damage water ecosystems and affect the sustainable use of water resources. Therefore, it is particularly important to develop an antibacterial gel with excellent antibacterial properties, green, and low cost. PVA is a highly water-soluble molecular polymer with good biocompatibility and can be degraded under certain conditions to reduce environmental pollution. CTS, as a natural polysaccharide extracted from the shells of marine organisms, possesses certain antimicrobial properties in its own right. CuNPs have highly efficient antimicrobial properties and were prepared by using dandelion extracts with green reduction of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ to reduce chemical pollution and meet the requirements of environmental protection. Therefore, a PVA/CTS/DAN/CuNP gel was designed to inhibit microbial growth. In this study, the antibacterial effect of PVA/CTS/DAN/CuNP gel was analyzed, and the results showed that the composite had good inhibitory ability against *E. coli*, *S. aureus*, and *P. aeruginosa*. In addition, the results of the reusability and stability of the gel showed that the antimicrobial activity of the gel could reach more than 90% in the first six replicates, and the inhibitory rate still remained above 90% after 14 days of preparation. Thus PVA/CTS/DAN/CuNP gels had good reusability and stability. In conclusion, a novel PVA/CTS/DAN/CuNP composite gel was prepared in this study, which has potential application in inhibiting bacterial growth in the water body.

Author Contributions: Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing—original draft: M.H. and T.Z.; Supervision, writing—review and editing: Y.H. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data is contained within the article.

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