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Preparing and Antimicrobial Activity of Hydrogel with Biosynthesized Silver Nanoparticles Using Carex Meyeriana Kunth

Qihui Shen, Henan Zhu, Hongli Zhou, Tienan Jiang and Yan Liu

Department of Chemistry and Pharmaceutical Engineering, Jilin Institute of Chemical Technology, Jilin, China

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Corresponding Author:
Yan Liu
Department of Chemistry and Pharmaceutical Engineering, Jilin Institute of Chemical Technology, Jilin, China
Email: ly@jlict.edu.cn

Abstract: In this study, a simple and environmentally stable method was developed to synthesize silver Nanoparticles (AgNPs). In addition, carex meyeriana kunth was used as a reducing and stabilizing agent with good bacterial inhibitory effects against *Escherichia coli (E. coli)* and *Bacillus subtilis* at very low doses. AgNPs were added to the Polyvinyl Alcohol (PVA) solution as an antibacterial agent and AgNPs-loaded PVA hydrogel (gel) was formed using the freeze-thaw method. The hydrogel containing AgNPs had a powerful antibacterial effect and the PVA/Ag hydrogel was tested as a trauma dressing, which is a promising composite material for development.

Keywords: Green Synthesis, AgNPs, Antimicrobial, Hydrogel

Introduction

The skin is the body's protective barrier to the outside world and it is critical to allow the barrier to healing after a wound appears (Mohammadpour *et al*., 2021). Many wound dressings have been developed to promote wound healing, ideal for absorbing or releasing water and preventing infection (Purna and Babu, 2000). Traditional materials for dressing preparation are hydrocolloids and foams. Hydrogel is now widely used in wound dressing (Dibazar *et al*., 2022). Hydrogel as a dressing has good mechanical properties and oxygen permeability and keeps the wound surface moist (Yu and Ober, 2003). Hydrogel removal leaves no residue and is virtually painless. This is because the hydrogel surface is moist and will not pull on the wound (Huang *et al*., 2023). Currently, patients greatly accept hydrogel wound dressings (Liu *et al*., 2023). However, problems such as wound inflammation and bacterial infections occur. Hydrogels alone do not have bactericidal properties, so it is important to develop hydrogel nanocomposites.

Silver is the most widely used antimicrobial agent because of its broad range of bactericidal properties and antimicrobial persistence, which is receiving much attention (Syed *et al*., 2019). AgNPs are reported to have a better bactericidal effect than positive silver (Bhattacharya and Mukherjee, 2008). Nanosilver can be combined with hydrophilic biocompatible polymeric materials and can be the basis for preparing antimicrobial materials (Varaprasad *et al*., 2011) for various medical applications, including antimicrobial coatings and wound dressings. Biosynthetic AgNPs are considered cost-effective, non-polluting, rapid, and easy to synthesize compared to chemical and physical methods (Almatroudi *et al*., 2020). Bioreduction has received a great deal of attention. At present, Tangerine Peer (Judy Azar and Mohebbi, 2013), Aloe Vera (Burange *et al*., 2021), Bate Vulgaris (Venugopal *et al*., 2017), Mikania micrantha leaves (Kale and Jagtap, 2018), Limon leaves (Kale and Jagtap, 2018), etc., have been used to synthesize AgNPs. We used carex meyeriana Kunth to synthesize AgNPs to continue the research and development of the green synthesis of AgNPs and their applications. There has been no research on the synthesis of AgNPs by carex meyeriana Kunth and their applications. Carex meyeriana Kunth is one of Northeast China's three treasures. It is recognized by various sources, low prices, safety and environmental protection, and rapid expansion. Carex meyeriana Kunth also offers deodorizing, meridian cleaning, tiredness relief, improved blood circulation, immunity, and other health advantages (Cheng *et al*., 2020). Research has shown that carex meyeriana Kunth mainly contains volatile oil and polysaccharide (Hu *et al*., 2018), which can scavenge active oxygen radicals and have a strong antioxidant effect.

Bacterial infection prevention is one of the most important properties of wound dressings (Brothers *et al*., 2015). AgNPs have excellent antibacterial properties. Therefore, the focus of this study was to successfully prepare carex meyeriana Kunth AgNPs and to successfully dope the AgNPs into polyvinyl alcohol gel. This was done for possible wound dressing applications and to measure the antibacterial properties of the prepared AgNPs and gel.

Materials and Methods

Materials

All compounds were of analytical quality and were utilized without being modified or purified in any way. PVA 2000 and silver nitrate ($AgNO_3$) were bought from Aladdin, whereas carex meyeriana Kunth was purchased from the market and crushed in the laboratory. All glass containers were cleansed with ultra-pureified water and thoroughly dried before usage.

The absorption spectrum of biosynthesized AgNPs was studied in a range of 250-550 nm by an Ultraviolet-visible Spectrophotometer (U-Vis) (Lambda 750, PerkinElmer). A Fourier-Transform Infrared spectroscopy (FTIR) spectrum of AgNPs was documented via FTIR (spectra one, perkinelmer) in a wavenumber range of 500-4000 cm^{-1} to find out the functional groups of the particles. The size and morphology of AgNPs were observed via Scanning Electron Microscopy (SEM) (JSM-7610F Plus) and Transmission Electron Microscopy (TEM) (TESCAN MIRA4). The zeta potential and particle size distribution were measured using Nano-ZS90 (Malvern).

Synthesis of AgNPs and AgNPs Loaded PVA Hydrogel

$AgNO_3$ (1 mmoL) was mixed with 100 mL of 2 g/L aqueous solution of the carex meyeriana Kunth powder and incubated at 80°C. This continued until the solution color changed to brown-yellow, indicating the production of AgNPs. The obtained solution was kept in the dark condition to avoid autoxidation of AgNPs.

A certain PVA amount was dissolved in distilled water at 90°C under stirring. Then, the AgNPs solution was added and stirred continuously for 30 min. The mixture was placed in a cell culture plate and allowed to settle to room temperature before being freeze-thawed three times at 18°C. The resulting PVA-AgNPs gel was kept from light and humidity.

Antibacterial Activity of AgNPs and AgNPs Loaded PVA Hydrogel

The antibacterial activity of AgNPs prepared from the carex meyeriana Kunth powder was measured using a disk diffusion method (Bavelaar *et al*., 2021) and the inhibition zone revealed an antibacterial effect. The antibacterial detection medium was autoclaved at 121°C for 15 min. The sterilized medium was poured into a 10 cm Petri plate at a depth of about 3 mm and cooled at room temperature. Then, 1×10^8 cfu/mL of the experimental strain was evenly applied to the solid medium with a micropipette, 20 μL of AgNPs was added dropwise on a filter paper sheet, dried and pasted on the medium and incubated at 37°C for 12 h. After incubation, the inhibition zone diameter was visible as a clear color less disk and was measured using a ruler. The experiments were carried out in triplicate.

A 1 cm diameter PVA/AgNPs gel and bacterial solution were simultaneously placed in a conical flask and incubated at 37°C for 6 h. 100 μL of the bacterial solution was taken and spread on the LB solid medium and incubated in an incubator at 37°C for 12 h. The strains' growth was examined and compared with that of the PVA gel. The bacteriostatic rate, *R*, could be calculated according to Eq. 1:

$$R\% = \frac{C_o - C_t}{C_n} \times 100\% \quad (1)$$

where, C_o and C_t represent the concentration of PVA gels and PVA/AgNPs gels, respectively, which were measured three times in each experiment.

Results and Discussion

Carex meyeriana Kunth contains phytochemicals such as polysaccharides and flavonoids, which can reduce Ag^+ to Ag^0. Carex meyeriana Kunth powder can be used to wrap AgNPs to prevent them from becoming too big. Moreover, no further lowering agents were required. Color changes indicate the production of AgNPs.

Characterization and Antibacterial Activity of AgNPs

The formation of AgNPs could be preliminarily judged according to the color change (Yadav *et al*., 2021). The color changed from light yellow to dark brown, showing the formation of AgNPs (Fig. 1a). The produced AgNPs were analyzed via UV-Vis in the wavelength range of 250-550 nm, with an absorption peak appearing at 433 nm (Fig. 1a) in the region of AgNPs' plasmon resonance peak (Sana and Dogiparthi, 2018). Thus, the characteristic peak of the synthesized product at 433nm can prove the formation of AgNPs.

The functional groups of the carex meyeriana Kunth powder and carex meyeriana Kunth AgNPs were analyzed via infrared spectrogram and the resolution of FTIR was between 400-4000 cm^{-1} to ensure the formation of AgNPs. The absorption band at 1631 cm^{-1} corresponds to C═O stretching vibration. The peak at 1056 cm^{-1} is related to C─O phenolic compounds. Compared with AgNPs (Trivedi *et al*., 2021; Fang *et al*., 2021), the band at 1416 cm^{-1} pertains to C─H bending, indicating the presence of proteins. There are more infrared absorption peaks of natural products in the carex meyeriana Kunth powder, which may be related to different groups, such as C═C tensile vibration (1614 cm^{-1}) and C─O tensile vibration (1116 cm^{-1}) (Fig. 1b) (Hu *et al*., 2019).

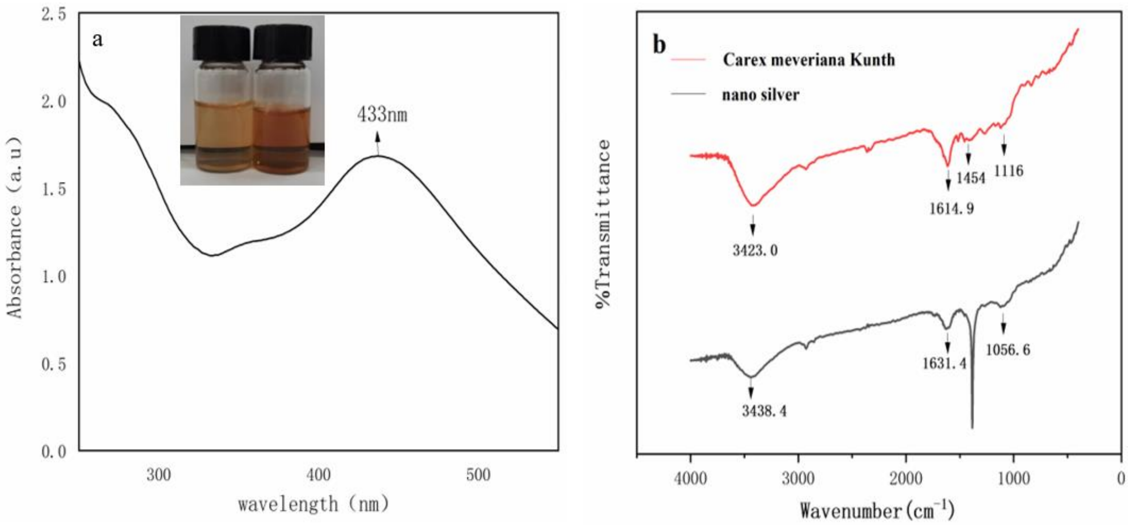


Fig. 1: UV-Vis absorption (a) and FTIR (b) spectra of the synthesized AgNPs

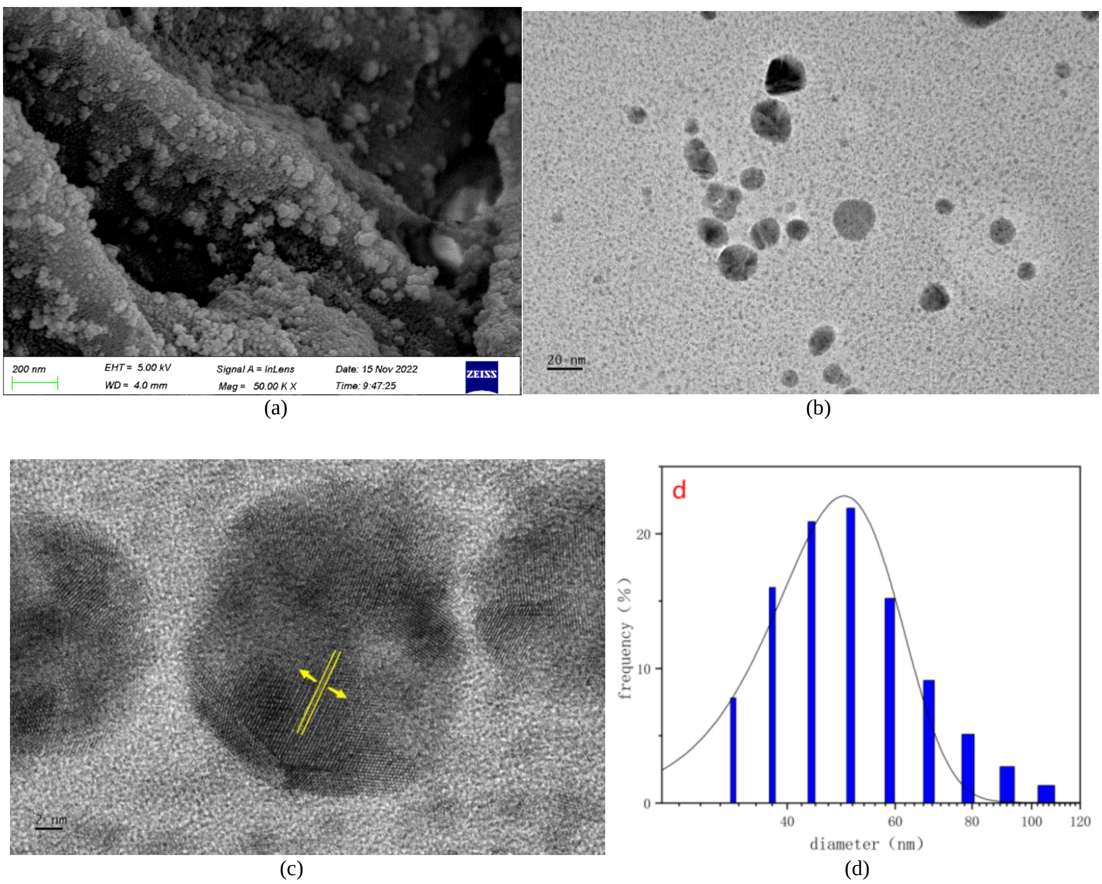


Fig. 2: SEM (a), TEM (b), HRTEM (c) images and particle size distribution (d) of AgNPs

TEM is the most suitable method to study the size and morphology of AgNPs (Ebrahiminezhad *et al*., 2017). The TEM results revealed that the AgNPs synthesized from carex meyeriana Kunth were mostly spherical in structure with sizes ranging from 37-122 nm (Fig. 2b). Smaller particle size indicates the effectiveness and quality of

AgNP (Hedberg *et al*., 2014; Ponsanti *et al*., 2020). The particle size distribution of AgNPs was studied via dynamic light scattering and the average particle size was 64.17 nm (Fig. 2d). SEM results showed that most of the synthesized AgNPs were spherical. The size distribution of the prepared AgNPs was found to be quite wide (Fig. 2a) and the particle size was uneven. This indicates that the plant-reduced nanoparticles were flexible in producing nanoparticles with different particle sizes and size distributions, with 25% of AgNP particles at 60 nm. The resulting AgNPs were not only dispersed in the solution but also attached to the carex meyeriana Kunth powder.

Zeta potential analysis was conducted to determine the extent of the electrostatic or charge repulsion/attraction between AgNPs. The zeta potential for the synthesized AgNPs was 20.3 mV (Fig. 3a), showing the stability of AgNPs. There were other ingredients in carex meyeriana Kunth, such as polysaccharides, flavonoids, alkaloids, etc., (Rizwana *et al*., 2021), which helped the formation of nanoparticles. Furthermore, the AgNP particles were separated and wrapped by themselves, reducing agglomeration and making the AgNP more stable (Das *et al*., 2013). Energy Dispersive Spectroscopy (EDS) elemental analysis of the AgNP solution revealed that various elements would create peaks with distinct atomic structures (Cvetkovikj *et al*., 2013). Metallic silver had a strong characteristic peak in the EDS spectrum (Fig. 3b).

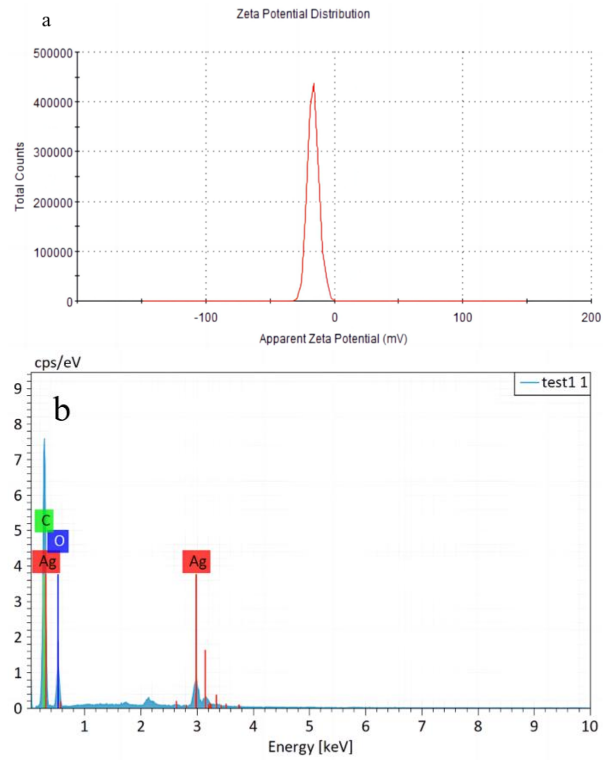


Fig. 3: Zeta potential (a) and EDX spectroscopy (b) of AgNPs

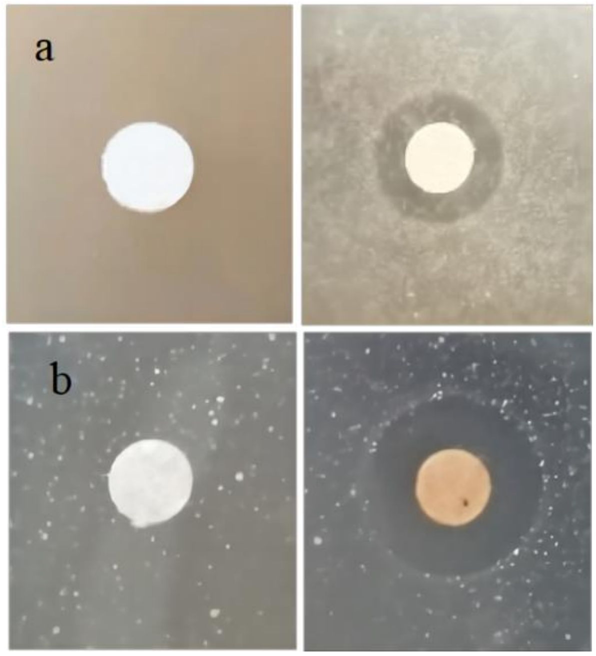


Fig. 4: The inhibition halos of *E. coli* (a) and *Bacillus subtilis* (b)

Green synthesis has become an important way of preparing AgNPs (Durán *et al*., 2010; Puišo *et al*., 2014; Ajitha *et al*., 2016; Ghiuță *et al*., 2018) and antibacterial activity has been one of its notable characteristics (Benakashani *et al*., 2016). Figure 4 depicts the antibacterial activity of AgNPs produced from carex meyeriana Kunth powder against gram positive and gram-negative bacteria. In contrast to the carex meyeriana kunth only sample, the inhibitory halos of AgNPs were visible. The means of the diameter of the inhibition halos for *E. coli* and *Bacillus subtilis* were determined to be about 10.48 ± 1 and 11.59±0.25 mm, respectively.

Characterization and Antibacterial Activity of AgNPs-Loaded PVA Hydrogel

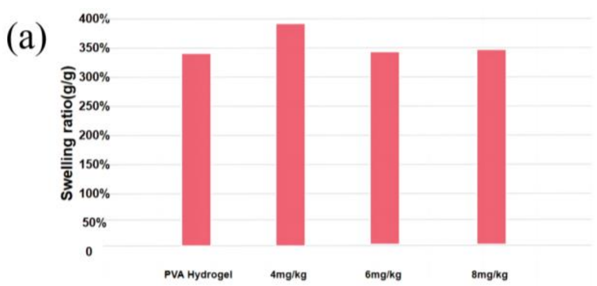
Fluid handling capacity is an important reference for gels as dressings in biomedicine. The biggest challenge in wound management is that wound exudate cannot be treated in time (Muluye *et al*., 2014). The wound dressing should have an absorption capacity to ensure that exudate can be absorbed in time to prevent bacterial infection caused by the exudate accumulation. Measuring the hydrogel liquid handling capacity was done based on dissolution. PVA is a hydrophilic polymer and PVA hydrogel has a high swelling ratio (Kumar and Münstedt, 2005). Since AgNPs are hardly hydrophilic (Bhowmick and Koul, 2016), the hydrophilicity of the AgNPs-loaded PVA hydrogel decreased as the AgNP content rose. However, since a small AgNP amount could achieve effective antibacterial activity, it had almost no effect. Figure 5(a), it was found that the swelling ratio was between 300 and 400%, showing good water absorption ability that could effectively absorb wound exudate.

The ideal wound dressing provides a moist environment (Chang *et al*., 2022). The moderate hydrophilicity of the gel will keep the wound moist, thus accelerating wound healing (Sabri *et al*., 2020). Figure 5(b-c) shows the water content and saturated water content of the gel for the test. The water content of the gel could reach about 90% and the saturated water content up to 94%. The inclusion of AgNPs had almost no effect on the water content. The data showed that the prepared AgNPs loaded with PVA hydrogel could keep the wound in a moist environment and facilitate wound healing.

Biological activity testing of AgNPs loaded PVA hydrogel was done by both co-culture and inhibition loop methods. In the test, the most common pathogenic bacteria (*E. coli*, *Bacillus subtilis*) were selected and co-cultured with hydrogel to determine the antibacterial activity of hydrogel (Fig. 6) (Jelena *et al*., 2022). The growth of colonies was more directly observed by plate counting (Chokesawatanakit *et al*., 2021). Table 1, the gel without AgNPs had no antibacterial activity, but the gel with silver displayed a concentration rose (Fig. 7). The antibacterial rates of AgNPs loaded PVA hydrogel against *Bacillus subtilis* and *E. coli* were 99.0% +0.11 and 99.19% +0.11, respectively.

Discussion

AgNPs with antibacterial activity were successfully prepared via a simple, convenient, and environmentally friendly method using carex meyeriana Kunth as a stabilizer and reducing agent. The created AgNPs were spherical with a diameter of around 60 nm. The antibacterial activity was investigated further and the results revealed that the prepared AgNPs exhibited good antibacterial ability even at very low silver concentrations.



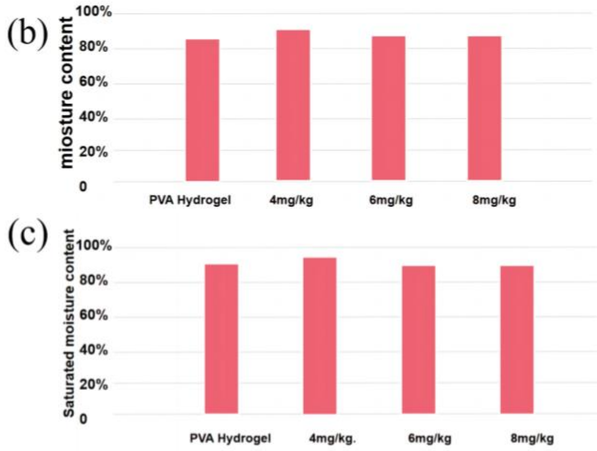


Fig. 5: The swelling rate (a), water content (b), and saturated water content (c) of AgNPs loaded PVA hydrogel

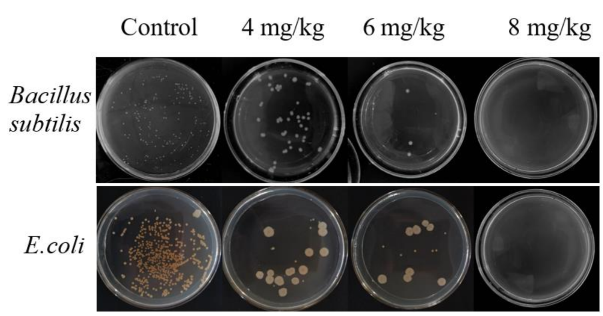


Fig. 6: The antibacterial activity of the gel containing different Ag concentrations

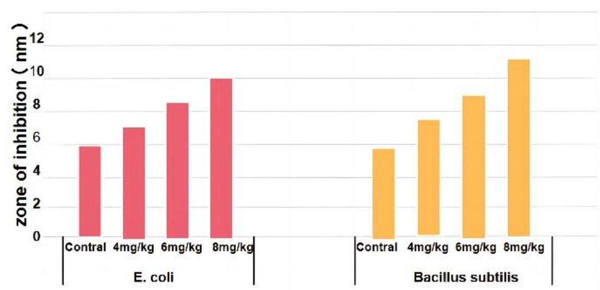


Fig. 7: The Graphic of the antibacterial effect from the gel containing different Ag concentrations

Table 1: Antibacterial activity of the gel containing different Ag concentrations

Concentration	Culture	Zone of inhibition in mm*
Control	*E. coli*	6.0000000
4 mg/kg		7.29±0.16
6 mg/kg		8.73±0.22
8 mg/kg		10.27±0.11
Control	*Bacillus subtilis*	6.0000000
4 mg/kg		7.44±0.18
6 mg/kg		9.01±0.07
8 mg/kg		11.39±0.15

Moreover, they effectively inhibited more than 90% of the bacteria with a sustained inhibition time of more than 80 h. In conclusion, the experiments presented a cost-effective and eco-friendly technique for synthesizing AgNPs, which may aid in developing potential biomedical applications. The water content and saturation water content of the prepared AgNPs loaded PVA hydrogel could reach 90 and 94%, respectively. The swelling rate could reach 300-400% and AgNPs loaded PVA hydrogel absorbed wound fluid and had the powerful antibacterial ability, fluid handling capacity, and antimicrobial activity in line with ideal wound dressing requirements.

Conclusion

This study presents a new idea for the phytoreduction method of AgNPs. In addition, it provides direction for future research in a wide range of biomedical and pharmaceutical applications. A hydrogel with antibacterial activity prepared by combining AgNPs and hydrogel could protect wound dehiscence, absorb exudate from wounds and provide a moist environment for wounds with good moisturizing properties. AgNPs loaded PVA hydrogel will bring long lasting development to the field of trauma, especially open infections. In addition, a positive exploration of the green antibacterial hydrogel preparation was performed.

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Author's Contributions

Qihui Shen, Hongli Zhou and Yan Liu: Guided the designed route and provided experimental guidance for this manuscript.

Henan Zhu and Tienan Jiang: The experiment was carried out and completed. Revised and proofread the thesis.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and that no ethical issues are involved.

Conflict of Interest

The authors declare that they have no competing interests. The corresponding author affirms that all of the authors have read and approved the manuscript.

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