Supporting Information

Copper-Doped Cherry Blossom Carbon Dots with Peroxidase-Like Activity for Antibacterial Applications

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1. Supplemental Methods

1.1 Electron Spin Resonance

Electron Spin Resonance (ESR) is a detection technique that employs molecular probes to capture target products. In this method, DMPO, a commonly used trapping agent, can react with hydroxyl radicals (•OH) to form a complex that generates characteristic ESR signals (DMPO-OH) when subjected to an applied magnetic field. The peak-to-peak height of the resulting ESR spectrum is indicative of the concentration of •OH radicals. In the experiment outlined, the concentration of DMPO utilized was 100 mM.

1.2 Bacterial culture

E. coli and *S. aureus* were individually inoculated into Luria Bertani (LB) broth, containing 25 g/L of the medium, and Tryptone Soy Broth (TSB), containing 30 g/L, respectively. These cultures were then placed into a shaking incubator set at 37.0°C with a shaking speed of 220 rpm for a duration of 12 hours. Subsequently, 100 µL of each bacterial culture was transferred to a 96-well plate to measure the optical density (OD) at a wavelength of 600 nm. The bacterial concentration was determined from the OD_{600} readings and then adjusted to the desired concentration by dilution with the respective growth medium. The diluted cultures were subsequently stored at a temperature of 4°C.

1.3 Cell culture

Mouse fibroblast cells (L929) were procured from Sangon Biotech Co., Ltd. These L929 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) and 1% Penicillin/Streptomycin (PS). The incubation was conducted at a temperature of 37°C within an atmosphere containing 5% CO_2, providing an optimal environment for cell growth and maintenance.

1.4 Exploration of antibacterial concentrations

E. coli (10^8 CFU mL^{-1}) was mixed separately with different concentrations of H_2O_2 (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} M) and Cu-CDs (15.625, 31.25, 62.5, 125, 250, 500 µg mL^{-1}) for 0.5 h. Next, 50 µL of the mixture was evenly spread onto the surface of an agar dish and incubated at 37°C for 12 hours.

1.5 Antibacterial Ring Test

During the experimental procedure, the optical density of the pure bacterial culture is meticulously adjusted to an OD_{600} of 0.1, equating to a concentration of 10^8 CFU mL^{-1}. This suspension is uniformly spread on the agar plate to ensure an even distribution of bacteria. Following this, paper discs with a radius of 2.5 mm, which have been saturated with a solution of carbon dots and allowed to dry, are carefully placed on the agar. Post a 12-hour incubation period, the radius of the formed inhibition zone is meticulously measured, providing a quantitative assessment of the antibacterial effect.

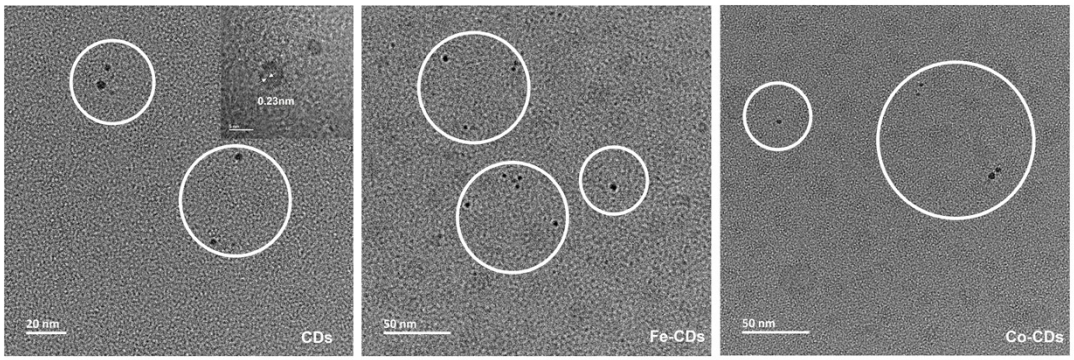

Fig. S1 TEM, HRTEM images of CDs and TEM images of Fe-CDs, Co-CDs.

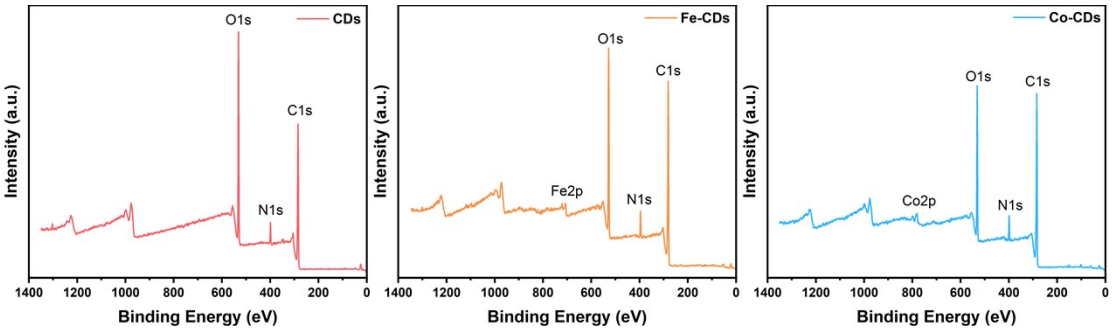

Fig. S2 XPS full spectrum of CDs, Fe-CDs and Co-CDs.

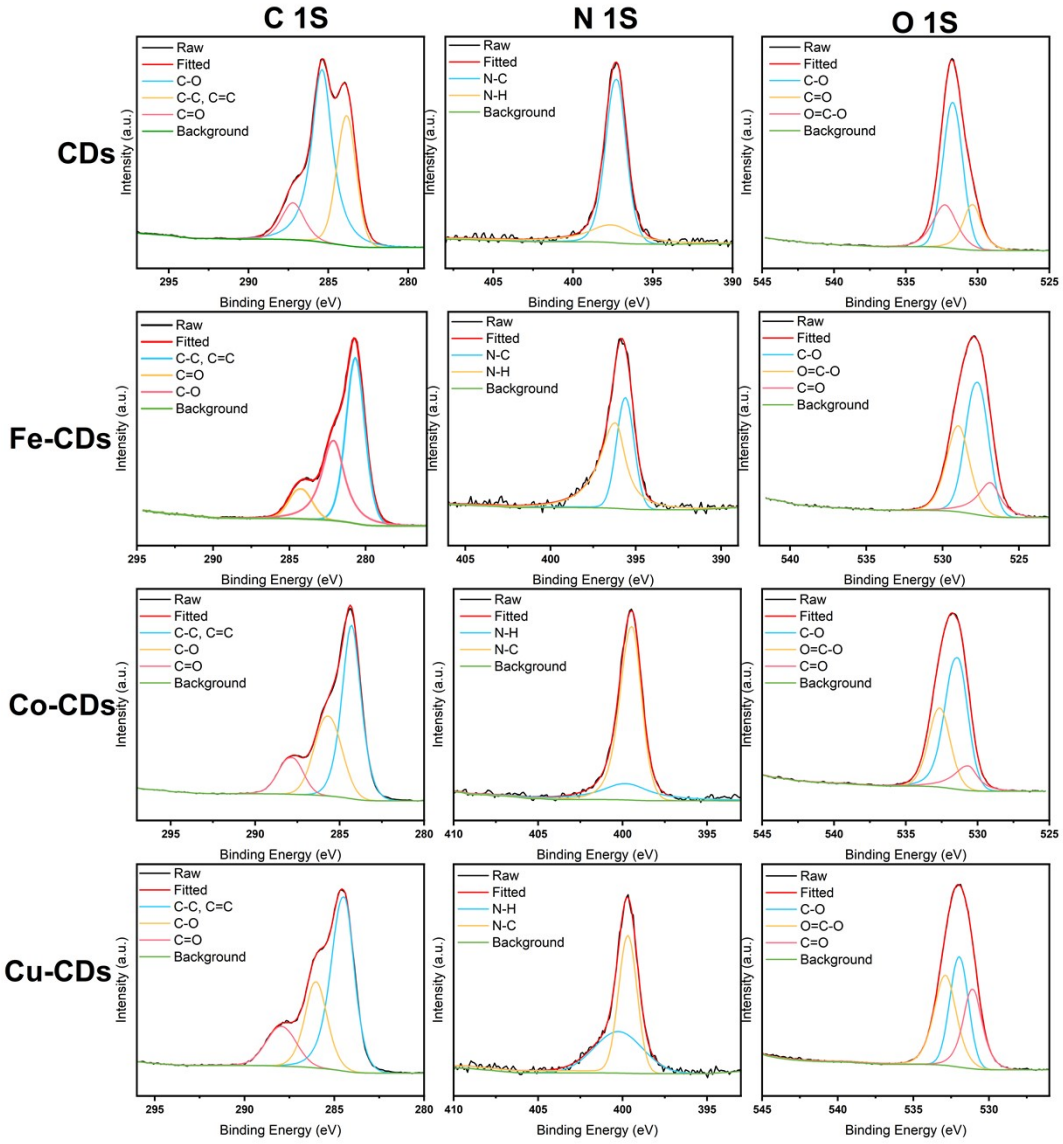

Fig. S3 High-resolution XPS spectrum of C 1s, N 1s, and O 1s for all CDs.

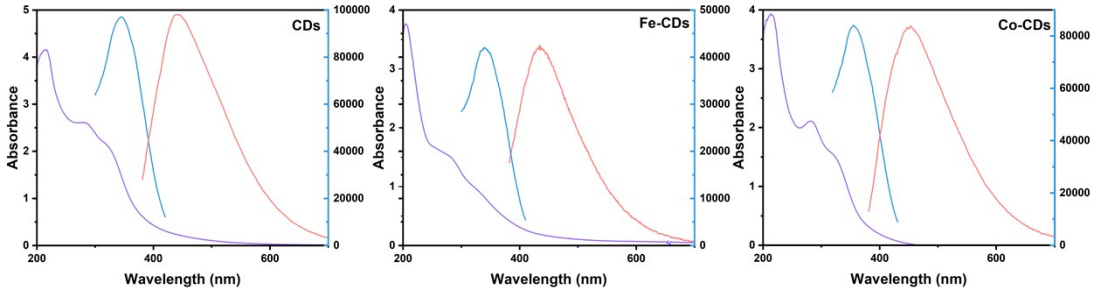

Fig. S4 UV–Vis absorption spectra, the emission spectra and the excitation spectra XPS full spectrum of CDs, Fe-CDs and Co-CDs.

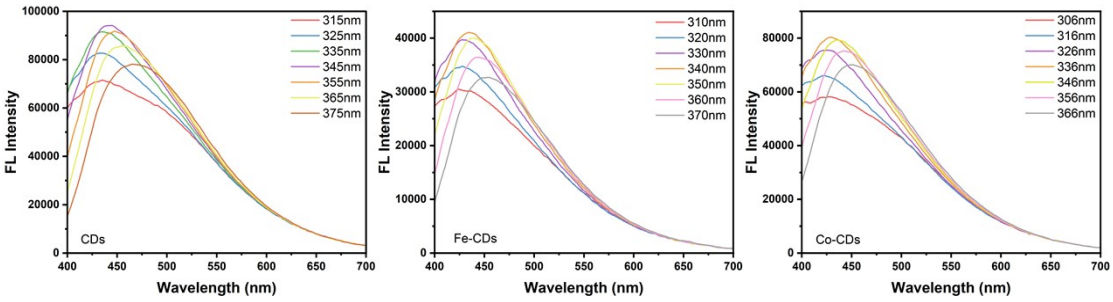

Fig. S5 Fluorescence spectra under different excitation wavelength of CDs, Fe-CDs and Co-CDs.

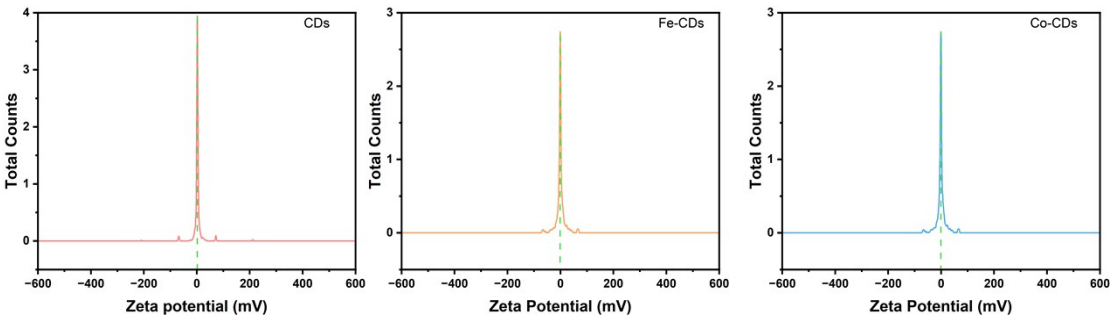

Fig. S6 Zeta potential distribution of CDs, Fe-CDs and Co-CDs.

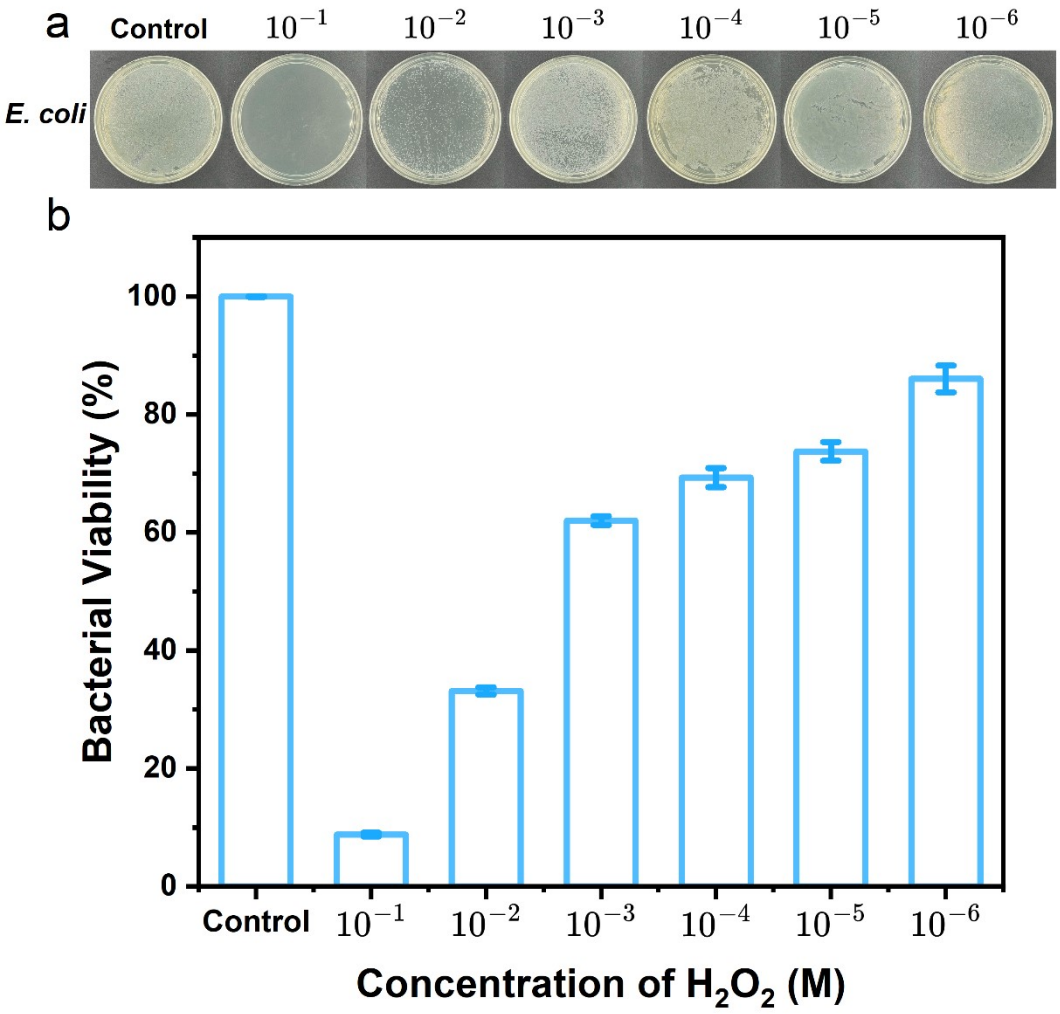

Fig. S7 The MIC of H_2O_2 to *E. coli*. (a) The images of agar plate of bacterial colonies of *E. coli* with concentrations of H_2O_2. (b) The bacterial viability of *E. coli* with different concentrations of H_2O_2. Data are presented as mean ± SD (n = 3), ns: $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

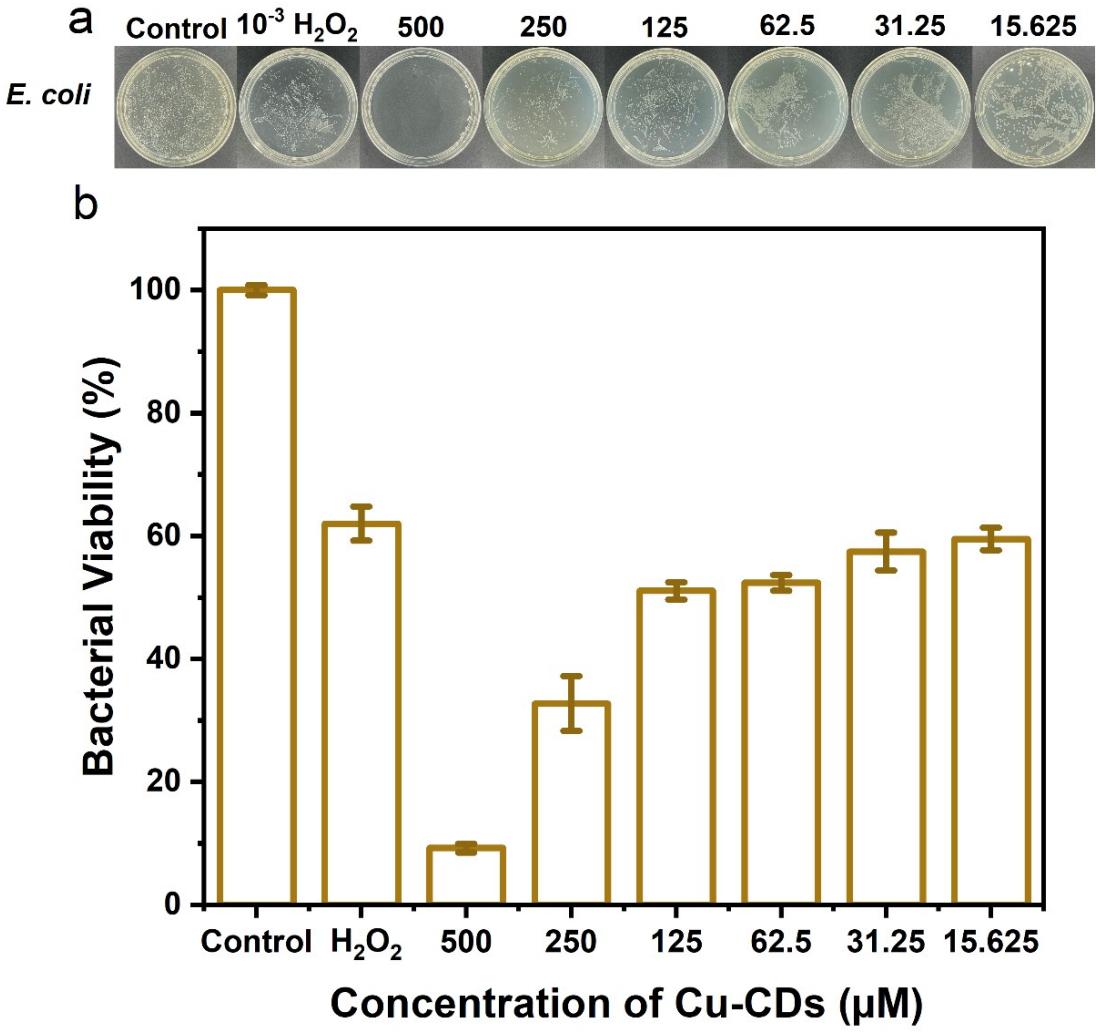

Fig. S8 The MIC of Cu-CDs with H_2O_2 to *E. coli*. (a) The images of agar plate of bacterial colonies of *E. coli* with concentrations of H_2O_2. (b) The bacterial viability of *E. coli* with different concentrations (μg mL^{-1}) of Cu-CDs. Data are presented as mean ± SD (n = 3), ns: $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

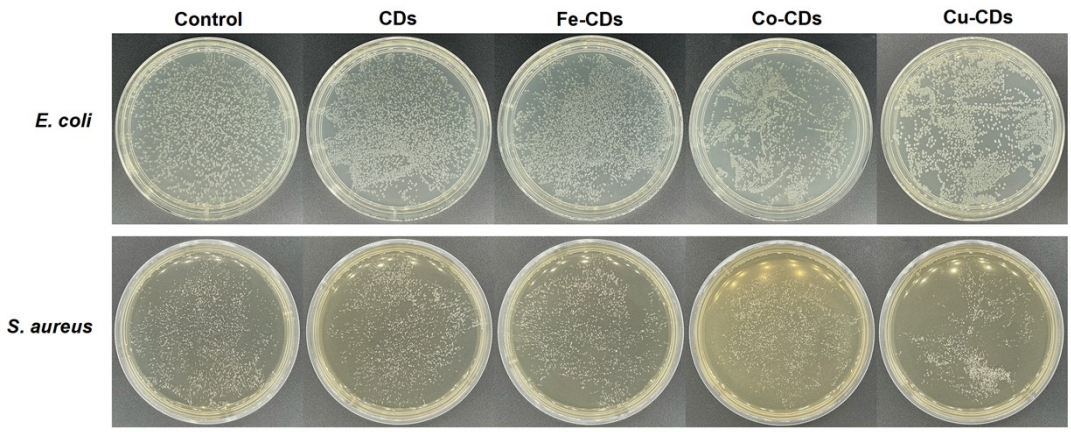

Fig. S9 Images of *E. coli* and *S. aureus* treated with various CDs nanozymes without H_2O_2.

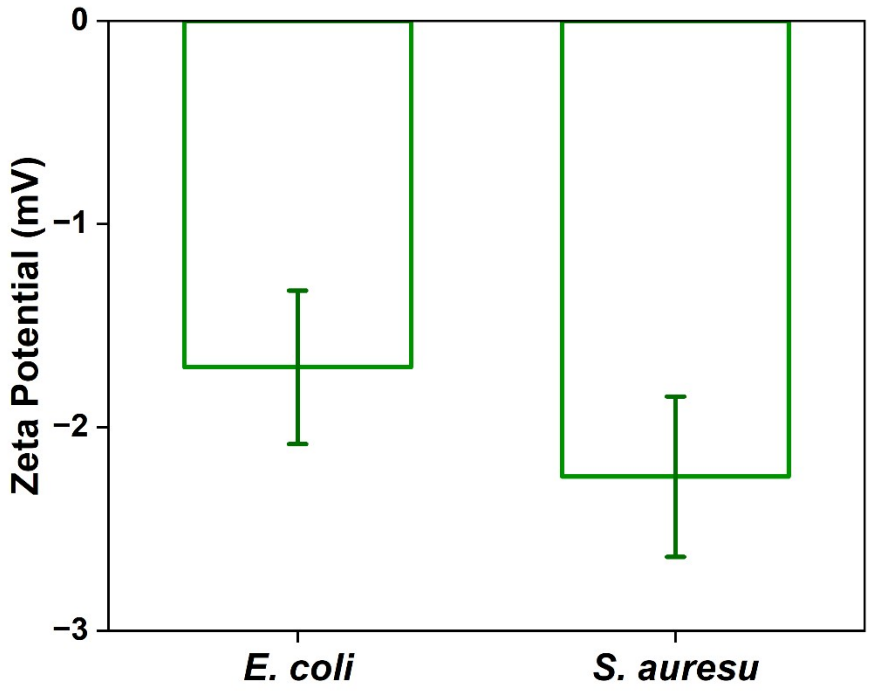

Fig. S10 Zeta potential of *E. coli* and *S. aureus* in the corresponding liquid culture medium.

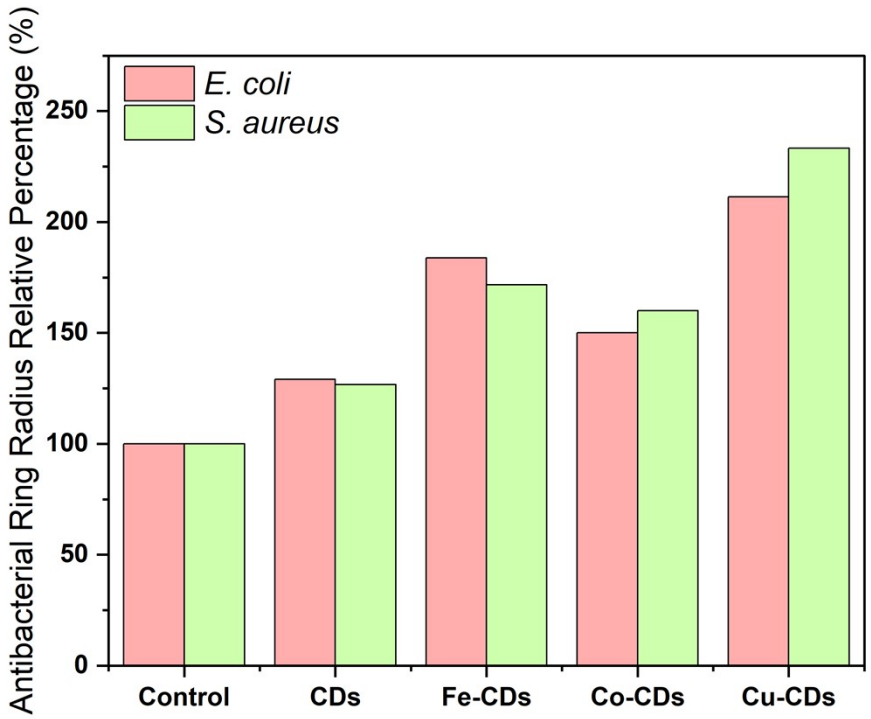

Fig. S11 Antibacterial ring radius relative percentage of CDs, Fe-CDs, Co-CDs, and Cu-CDs against *E. coli* and *S. aureus*.

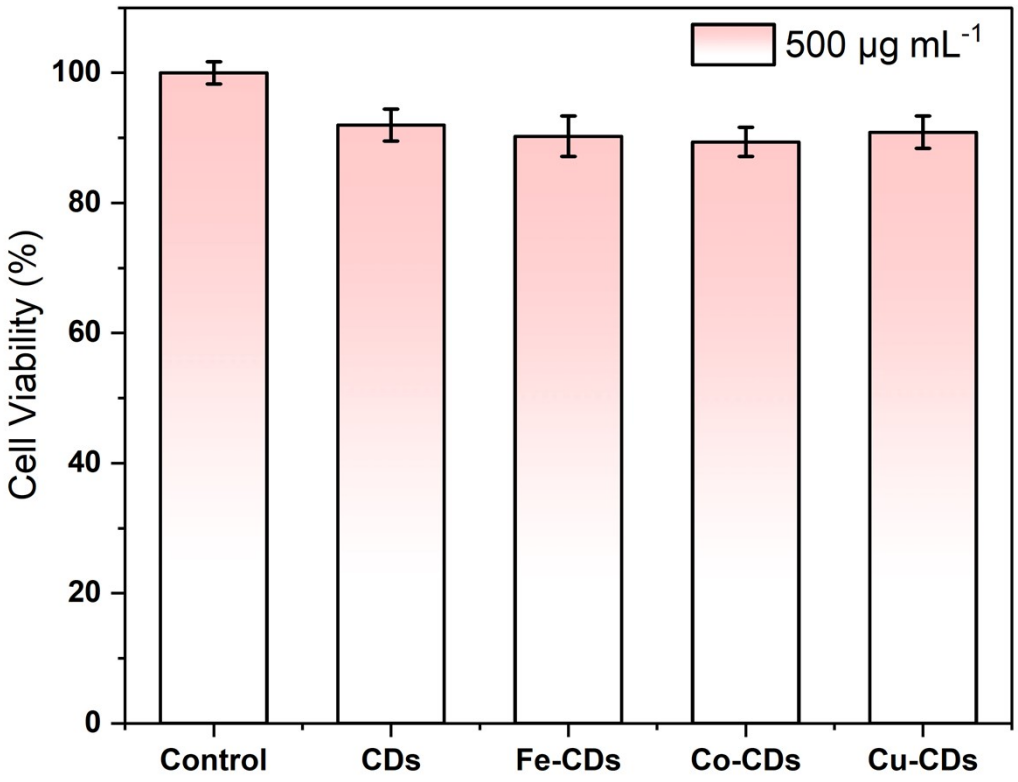

Fig. S12 Cell viability of HSF cells in response to CDs at concentration of 500 μg mL^{-1}.