Supporting Information

Ellagic Acid-Modified Gold Nanoparticles to Combat Multi-Drug Resistant Bacterial Infections *In Vitro* and *In Vivo*

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MATERIALS AND METHODS

Chemicals and Materials. Tannic acid (TA), epigallocatechin gallate (EGCG), ellagic acid (EA), gallic acid (GA), arbutin (Arb), and chloroauric acid ($HAuCl_4$) were purchased from Macklin (Shanghai, China). Trizol and LIVE/DEAD bacterial staining kit were provided by Invitrogen (Shanghai, China). 2′,7′-Dichlorofluorescein diacetate (DCFH-DA), alkaline phosphatase (AKP/ALP) kit, *β*-galactosidase activity detection kit, bicinchoninic acid (BCA) protein quantification kit, Gram staining kit and 4% tissue fixative (paraformaldehyde) were purchased from Solarbio (Beijing, China). Phosphate buffered saline (PBS, 5 mM K_2HPO_4, 5 mM KH_2PO_4, 150 mM NaCl, pH 7.4) was provided by Coolaber (Beijing, China). Crystal violet was purchased from Macklin (Shanghai, China). All chemicals were used without further purification. Ultrapure water (18.2 MΩ) was used throughout the experiments.

Preparation of Polyphenolic AuNPs. Gold nanoparticles (AuNPs) were synthesized through the reduction of $HAuCl_4$ solution using plant polyphenols. Briefly, an aqueous solution of $HAuCl_4$ (45 mL of 0.01% w/v) in distilled water was boiled at 100 ^oC in a round bottom flask. Ellagic acid (EA) (2 mg/mL, 250 μL) was added dropwise to the $HAuCl_4$ solution and the mixture was kept boiling for 10 min. The colloidal solution was stirred for 35 min at room temperature and the AuNPs were dialyzed (molecular weight cutoff, 1500 Da) in deionized water for 48 h. AuNPs were collected by centrifugation (7000 g, 20 min), washed in ultrapure water for three times, and dried for 6 h at 60 °C. The EA-AuNPs formed were stored at 4 °C. The synthesis of AuNPs with other plant polyphenols, tannic acid (TA-AuNPs),

epigallocatechin gallate (EGCG- AuNPs), gallic acid (GA- AuNPs), and arbutin (Arb- AuNPs) was the same as described for EA-AuNPs.

Characterizations of Polyphenolic AuNPs. Diameters and zeta potentials of five polyphenolic AuNP samples (20 μg/mL in deionized water) were measured using the Malvern Zetasizer Nano ZS. Transmission electron microscopy (TEM, FEI Talos 200s, Thermo Fisher Scientific, USA) was used to characterize the morphology, and high-angle annular dark-field emission TEM (HAADF-TEM) was used to produce a resolution image. TEM-EDS was used to measure the elemental composition of the EA-AuNPs in deionized water. The intensity of the fluorescence was converted into grey values and calculated using Image J. The absorbance of the polyphenol AuNPs (20 μg/mL in deionized water) was recorded with a UV–vis spectrophotometer (Shimadzu, UV-2600, Japan) over the range of 400–800 nm. X-ray photoelectron spectroscopy (XPS, Kratos Analytical Ltd., Axis Ultra DLD, Germany) was used to measure the element composition and chemical states of EA-AuNPs. For the evaluation of the stability of the EA-AuNPs, they were stored in water at 4 ^{o}C up to 7 days. Diameters and zeta potentials of EA-AuNPs were measured at on day 1, 3, 5 and 7 using the Malvern Zetasizer Nano ZS. Diameters and zeta potentials were measured in triplicate.

Microbial Strains Growth Conditions and Harvesting. Bioluminescent *Staphylococcus aureus* Xen36 (PerkinElmer Inc., Waltham, MA, USA) and *S. aureus* ATCC12600 (American Type Culture Collection, USA) were cultured in 10 mL tryptone soya broth (TSB) at 37 ^{o}C for 24 h. For *S. aureus* Xen36 kanamycin (100 μg/mL) was added. The preculture was transferred to 200 mL TSB and grown for another 18 h. Bacterial cultures were harvested by centrifugation for 5 min at 5000 *g*, washed twice with PBS, and re-suspended in PBS. The bacterial

concentration was determined in a Bürker-Türk counting chamber. *Escherichia coli* Xen14 and *E. coli* DH5α were cultured in Luria-Bertani broth (LB), and for *E. coli* Xen14 kanamycin (30 μg/mL) was added. Culturing and harvesting were done as described for *S. aureus*.

The multi-drug resistant ESKAPE strains (*Acinetobacter baumannii* 2349, *S. aureus* 6114, *Enterobacter faecium* 1762, *Klebsiella pneumoniae* 6696, *Pseudomonas aeruginosa* 3086, *Enterobacter* 3737) were isolated from patients from the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China. In the hospital the ESKAPE strains were identified and tested on their multi-drug resistance.[1,2] The Gram-positive bacteria were cultured in TSB, and Gram-negative bacteria were cultured in LB. Culturing and harvesting were done as described above.

In Vitro Antimicrobial Efficacy of EA-AuNPs on Planktonic Bacteria.

The Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC). MIC and MBC of five polyphenolic AuNPs for *S. aureus* Xen36 were determined. 100 μL suspension of polyphenolic AuNPs (100 μg/mL) was serially diluted in TSB medium in a 96-wells plate, after which 100 μL *S. aureus* Xen36 (2×10^5 bacteria/mL) in TSB was added and incubated for 24 h at 37 °C. After 24 h, MIC values were taken as the lowest concentration not showing any visible growth. Next, 10 μL of each clear suspension in the 96-well plate was plated on TSB agar. After 24 h incubation at 37 °C, the MBC value was determined as the minimal concentration not showing any colonies on the agar plate. MIC and MBC values were measured in triplicate.

The MIC and MBC of EA-AuNPs were also determined for the ESKAPE panel pathogens and two *E. coli* strains. Gram-positive bacteria were cultured in TSB medium, and Gram-negative bacteria were cultured in LB medium (see **Table 1**).

Bacterial Morphology by Scanning Electron Microscopy (SEM). A silicon wafer (0.4 cm × 0.4 cm) was placed at the bottom of one well of a 96-well plate. Then, EA-AuNPs (100 μL) with different concentrations (0, 1.56, 3.12, and 6.25 μg/mL) in PBS was added to the 96-well plate, and an equal volume (100 μL) of *S. aureus* Xen36 or *E. coli* Xen14 (2×10^7 bacteria/mL) in PBS was added and left for 3 h at 37 °C. PBS was used as a control. After 3 h, the silicon wafer was gently rinsed with sterile water to remove unattached bacteria, and bacteria on the silicon wafer were fixed with 4% paraformaldehyde for 15 min. After fixation, the bacteria on the silicon wafer were dehydrated in a series of ethanol solutions (30-100%) and dried in air. The attached bacteria on the silicon wafer surfaces were observed using a Field Emission Scanning Electron Microscope (SU8010, Hitachi, Japan) with an accelerating voltage of 5.0 kV. SEM-EDS was used to detect the elemental content of carbon, nitrogen, oxygen, and gold in *S. aureus* Xen36 exposed to EA-AuNPs.

Killing Efficacy of EA-AuNPs. EA-AuNPs (6.25 μg/mL, 100 μL) in PBS was added to a 96-well plate, and an equal volume (100 μL) of *S. aureus* Xen36 (2×10^7 bacteria/mL) in PBS was added and left for 3 h at 37 °C. PBS was used as a control. After 3 h, *S. aureus* Xen36 was stained with LIVE/DEAD stain (1 μM SYTO9 and 1 μM propidium iodide) for 20 min in the dark, green fluorescent stained bacteria are alive, red fluorescent bacteria are cell wall damaged. After 20 min, the stain was removed, and the bacteria were immersed in PBS for imaging using confocal laser scanning microscopy (CLSM, Nikon, A1, Japan). An argon ion

laser at 488 nm was used to excite SYTO9 (green fluorescent), and a HeNe laser at 543 nm was used to excite propidium iodide (red fluorescent), collecting fluorescence at 500−535 nm (SYTO9) and 583−688 nm (propidium iodide).

Bacterial Cell Wall Permeability. S. aureus Xen36 in PBS (2 mL, 2 × 10^7 bacteria/mL) was mixed with different concentrations of EA-AuNPs (0, 3.12, 6.25, and 12.5 μg/mL, 2 mL) in a 15 mL sterile centrifuge tube. After 3 h incubation at 37 °C, these suspensions were centrifuged at 7000 *g* for 10 min, and the supernatants were collected for further analyses. The release of intracellular alkaline phosphatase (AKP/ALPs) was determined using an AKP/ALP kit (Solarbio, Beijing, China). AKP/ALP uses *p*-nitrophenyl phosphate as a phosphatase substrate and turns from colorless to yellow (λ_{max} = 510 nm) when dephosphorylated by AKP/ALP. The absorbance at 510 nm was measured after 60 min at room temperature, and the activity was calculated using a calibration curve. Similarly, the *β*-galactosidase activity in the supernatant was tested via a *β*-galactosidase (β-GAL) activity assay kit (Solarbio, Beijing, China). *β*-galactosidase decomposed *p*-nitrobenzene-β-D-galactopyranoside into *p*-nitrophenol, and the activity of *β*-galactosidase was calculated by measuring its absorbance at 400 nm and using a calibration curve. All experiments were performed in triplicate.

Intracellular ROS Generation. The intracellular reactive oxygen species (ROS) generation by EA-AuNPs was determined by adding 5 mL of the fluorescent ROS probe (20 μM), DCFH-DA in PBS to 5 mL *S. aureus* Xen36 (2 × 10^7 bacteria/mL) in PBS. After incubation for 30 min in the dark with shaking, the bacteria were centrifuged and washed twice with PBS to remove DCFH-DA that was not internalized by bacteria. The bacteria were suspended and diluted in PBS to 10^7 bacteria/mL. An aliquot of 50 μL of the bacterial suspension was put in

a 96-well plate and 50 μL of different concentrations of EA-AuNPs (0, 3.12, 6.25, and 12.5 μg/mL) were added to the bacterial suspension. After incubation at 37 °C for 3 h, the fluorescence intensity (*Ex*=488 nm, *Em*=525 nm) was recorded on a Varioskan LUX microplate reader (ThermoFisher, USA), and the ROS generation was calculated using a calibration curve.

Biofilm Eradication of EA-AuNPs *In Vitro*. Bacterial biofilms were grown in 96-well plates as described before.[3,4] Briefly, 100 μL *S. aureus* Xen36 (10^8 bacteria/mL) in TSB was added to a 96-well plate and kept for 1 h at 37 °C to allow the bacteria to settle and adhere to the bottom of the well plate. The bacterial suspension was then removed, and the well was washed once with 100 μL PBS. A total of 200 μL growth medium was added to each well, and bacteria were incubated at 37 °C for 24 h. The growth medium was removed, and the biofilms were washed once with 100 μL PBS. Subsequently, the biofilms were exposed to 100 μL of different concentrations of EA-AuNPs (0, 1.56, 3.12, and 6.25 μg/mL) in PBS at pH 7.4 for 3 h, the EA-AuNPs suspension was removed, and the biofilm was washed with 100 μL PBS. The resulting biofilms were suspended in 100 μL PBS, diluted and spread on TSB agar plates, and incubated at 37 °C for 18 h for CFU counting. The experiment was done in triplicate with separately grown biofilms.

For analysis of *S. aureus* Xen36 biofilms with CLSM, they were grown in confocal dishes. Briefly, 1 mL *S. aureus* Xen36 (10^8 bacteria/mL) in TSB was added to a confocal dish and kept for 1 h at 37 °C to allow the bacteria to settle and adhere to the bottom of the confocal dish. The bacterial suspension was then removed, and the well was washed once with 1 mL PBS. A total of 1 mL growth medium was added to each confocal dish, and bacteria were incubated at

37 °C for 24 h. The growth medium was removed, and the biofilms were washed once with 1 mL PBS. Then, the biofilms were exposed to 1 mL of different concentrations of EA-AuNPs (0, 1.56, 3.12, and 6.25 μg/mL) in PBS at pH 7.4 for 3 h. The EA-AuNPs suspension was removed. The biofilms prior to and after exposure to EA-AuNPs were stained with LIVE/DEAD stain (1 μM SYTO9 and 1 μM propidium iodide) for 20 min in the dark. After 20 min, the stain was removed, and the biofilms were immersed in PBS and imaged using CLSM. Biofilm eradication with EA was done with the same procedure.

The biomass of the exposed biofilms to EA-AuNPs was determined with crystal violet staining. The biofilms grown as described above were gently rinsed with PBS and dried for 20 min in air. Next, 200 μL of crystal violet solution (1.0% w/v) was dropped on the biofilms. After 20 min, the biofilms were rinsed with PBS and dried for 10 min in air. To dissolve the crystal violet from the biofilm 200 μL of 90% ethanol was added. 100 μL of the stained solution was transferred to a fresh 96-well plate and the absorbance at 570 nm of each solution was recorded on a microplate reader (100-240 VAC, USA). The biomass was determined in triplicate.

***In Vitro* and *In Vivo* Biosafety of EA-AuNPs.**

Cells Growth Conditions. Mouse fibroblast cells (L929) were cultured in RPMI-1640 medium (ThermoFisher Scientific, Inc., Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS, Gibco, Shanghai, China), 100 U/mL penicillin (Genview, Beijing, China) and 100 μg/mL streptomycin (Solarbio, Beijing, China). The cell lines were incubated at 37 °C with 5% CO_2. Cells were detached from the cell-culture flask by trypsinization, collected by

centrifugation at 1000 rpm for 5 min, and re-suspended in fresh cell culture medium. The cell concentration was determined in a Bürker-Türk counting chamber.

Cytotoxicity Determination of EA-AuNPs. The L929 cell were seeded in 96-well plates (10^4 cells/well) and incubated for 24 h at 37 °C. The L929 cell were grown in RPMI-1640 medium with the additions. After 24 h, the growth medium was replaced with RPMI-1640 medium with the additions containing different concentrations of EA-AuNPs (0, 3.12, 6.25, 12.5, 25, 50, 100, and 200 μg/mL), different polyphenolic AuNPs (100 μg/mL), EA solution (100 μg/mL) and gentamicin (100 μg/mL). After incubation for another 24 h, the CCK-8 assay (Biosharp, Beijing, China) was used to determine the metabolic activity. Briefly, the RPMI-1640 medium was removed and 200 μL fresh RPMI-1640 medium and 20 μL CCK-8 dye were added to each well, and then the 96-well plate was kept in the incubator at 37 ℃ for 2 h. 200 μL of the solution was transferred to a fresh 96-well plate. The absorbance of each solution at 450 nm was recorded by a microplate reader (100-240 VAC, USA). The experiment was performed in triplicate. Only RPMI-1640 medium without L929 cells served as the negative control (OD_{RPMI}), and L929 cells exposed to RPMI-1640 medium as the positive control ($OD_{RPMI\ with\ cells}$). The optical density of each experimental group was compared with the control group. The relative cell viability (%) was calculated according to the following equation

$$\text{Cell viability (\%)} = (OD_{EA\text{-}AuNPs}-OD_{RPMI})/(OD_{RPMI\ with\ cells}-OD_{RPMI})\times 100\% \quad (1)$$

Hemolysis Test. Hemolytic effects of EA-AuNPs were evaluated using red blood cells (RBCs), obtained from healthy female ICR (CD-1) mice. The red blood cells (RBCs) were

collected through the mouse eye and separated from the fresh mouse blood (1 mL) by centrifugation at 3000 rpm for 5 min at 4 ℃, and washed three times with PBS and re-suspended in 10 mL PBS. Then, 500 μL of RBC suspension was mixed with 500 μL of an EA-AuNPs suspension with different concentrations up to 100 μg/mL and incubated for 3 h at 37 ℃. After centrifugation at 3000 rpm for 5 min, the absorbance of the supernatant was measured at 545 nm using a microplate reader (100-240 VAC, USA). RBCs exposed to 0.1% Triton X-100 served as the negative control, and exposure to PBS served as the positive control.

$$\text{Hemolysis (\%)} = (OD_{\text{EA-AuNPs}} - OD_{\text{Triton x-100}})/(OD_{\text{PBS}} - OD_{\text{Triton x-100}}) \times 100\% \quad (2)$$

Biosafety In Vivo in Mice. Female ICR (CD-1) mice (4–6 weeks, 22–24 g) were purchased from Zhejiang Vital River Laboratory Animal Technology Co., Ltd and were housed in a specific pathogen-free room. The animal experimental protocol was approved by the Institutional Animal Care and User Committee, Wenzhou Institute, University of Chinese Academy of Sciences (No. WIUCAS21071223).

The mice were randomly assigned into four groups of three animals each and received an intraperitoneal injection of 200 μL PBS, EA (100 μg/mL), EA-AuNPs (100 μg/mL), or gentamicin (100 μg/mL). After 24 h, just before sacrificing, blood was collected through the eye and left undisturbed for 30 min in collection tube containing anticoagulant EDTA, after which plasma was obtained by centrifugation at 500 g for determination of routine blood parameters. Meanwhile, the organs (heart, liver, spleen, lung, and kidney) were collected and processed for hematoxylin and eosin (H&E) staining for histopathological analysis. Briefly, organs were fixed in 10% formalin for 12 h, dehydrated with a series of ethanol (70%, 80%,

90%, 95%, 100%) for 30 min, embedded in paraffin wax, and sectioned into 4 μm slices and stained with H&E stain. Finally, the H&E tissue sections were analyzed using a light microscope.

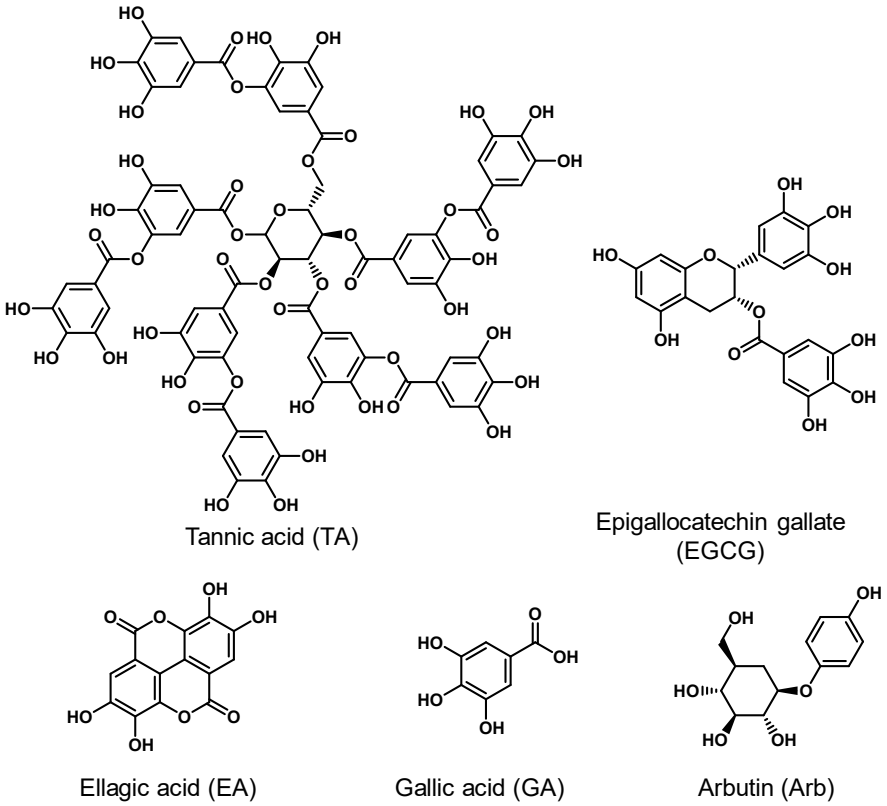
Mouse Acute Peritonitis Model for Evaluating the Killing of *S. aureus* by EA-AuNPs. The female ICR (CD-1) mice (4–6 weeks, 22–24 g) were used for *S. aureus* Xen36-infected peritonitis model by intraperitoneal administration of 0.3 mL of *S. aureus* Xen36 in PBS (5×10^8 CFU/mL). All experimental procedures have been subject to thorough review and approval by the Institutional Animal Care and Use Committee at the Wenzhou Institute, University of the Chinese Academy of Sciences (No. WIUCAS22031101), in accordance with the Administration Regulations governing the Affairs of Experimental Animals under the jurisdiction of the Ministry of Science and Technology in China. After 1-day post-infection, the animals were randomly assigned into four groups of six animals each, receiving an intraperitoneal injection of 200 μL PBS (negative control), gentamicin (100 μg/mL, positive control), EA (100 μg/mL), and EA-AuNPs (100 μg/mL). The biofilm growth of *S. aureus* Xen36 in mice was followed by bioluminescent intensity using a bio-optical imaging system (IVIS, 60 s, medium binning). Bioluminescent imaging was performed after treatment at 0 h, 6 h, 12 h, and 24 h. Then, blood was collected through the eye in collection tube containing anticoagulant EDTA for determining the bacterial concentration by colony-forming units (CFUs). Peritoneal lavage was performed by injecting 1.0 mL of PBS into the peritoneal cavity and massaging the abdomen and after the massage the mice were sacrificed. Subsequently, the abdomen was opened and 1.0 mL peritoneal fluid was taken from the abdominal cavity for determining the bacterial concentration by CFUs. The peritoneal fluid was serially diluted in

PBS and 10 μL of suspension in the 96-well plate was plated on a TSB agar plate. After 24 h at 37 ℃, colonies on the agar plate were counted.

Transcriptome Analysis in *S. aureus* after Exposure to EA-AuNPs and Gentamicin. *S. aureus* ATCC12600 (American Type Culture Collection, USA) was selected as a model strain to study the antimicrobial mechanism. *S. aureus* ATCC12600 was cultured in 100 mL TSB with 1.56 μg/mL (0.5 MIC) EA-AuNPs or 12.5 μg/mL (0.5 MIC) gentamicin at 37 ºC for 12 h. The bacteria were harvested by centrifugation for 5 min at 5000 g, and washed twice with PBS (sterile and enzyme-free, Macklin, China). Then, the bacteria were frozen in liquid nitrogen and ground quickly in a mortar. The total RNA of each sample was extracted using TRIzol Reagent Mini Kit (Qiagen). Next, the procedure of the mRNA purification and the sequence were determined using the Illumina HiSeq /Novaseq instrument, and data analysis was performed by a company (GENEWIZ, Shanghai, China). Briefly, the total RNA of each sample was quantified and qualified by Agilent 2100/2200 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA), NanoDrop (Thermo Fisher Scientific Inc.) and 1% agarose gel. mRNA was purified with Oligo(dT)-attached magnetic beads, and the purified mRNA was fragmented into small pieces with fragment buffer. Then, the mRNA sequence was determined using the Illumina HiSeq /Novaseq instrument.

Gene expression levels were calculated by RSEM software, and differential expression analysis was performed using DESeq2 (v1.4.5) with Q value ≤ 0.05. The significant levels of terms and pathways were corrected by Q value (Q value ≤ 0.05) by Bonferroni. The enrichment analysis of differently expressed genes was carried out using Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway.

Statistical Analysis. All data were expressed as means ± SD values. A one-way ANOVA was used to determine the statistical significance of differences between groups. GraphPad Prism 8 and Microsoft Excel 2019 software were used for data processing and statistical analysis (the $P < 0.05$ was considered statistically significant.).



Scheme S1. The molecular structure of the different polyphenols (TA, EGCG, EA, GA, Arb).

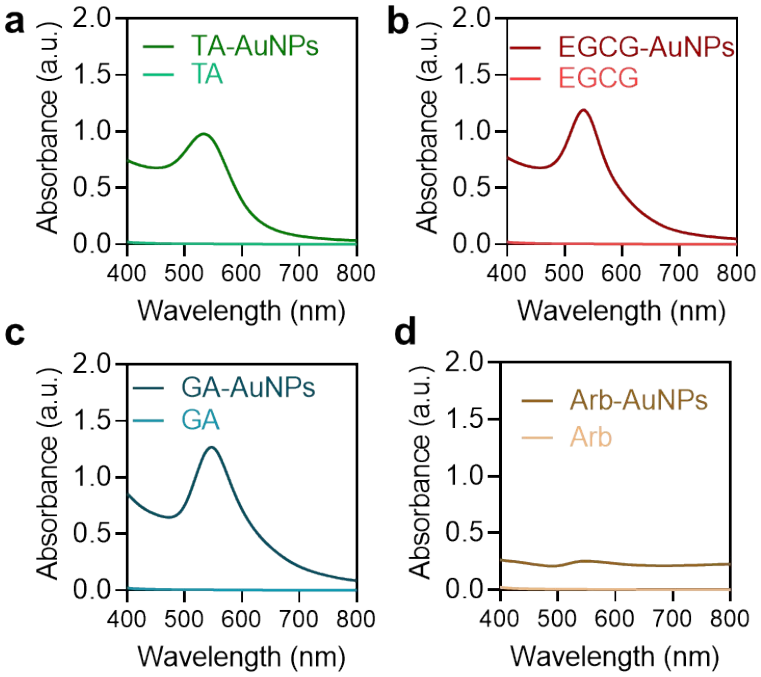


Figure S1. UV-vis absorption spectra of polyphenols (20 μg/mL) and polyphenolic AuNPs (20 μg/mL) in deionized water.

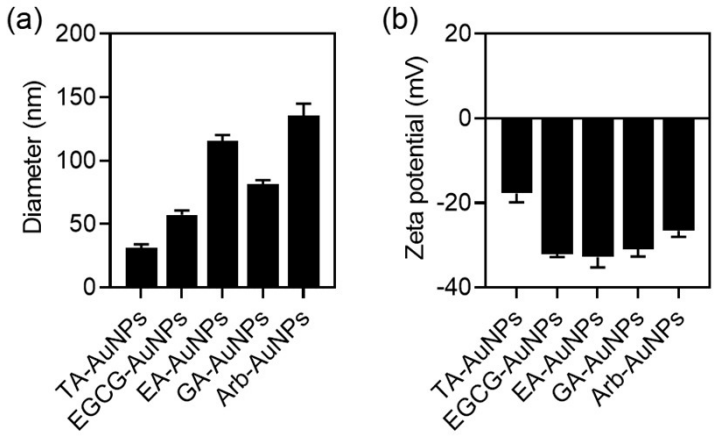


Figure S2. (**a**) Diameters of five polyphenolic AuNPs (TA-, EGCG-, EA-, GA-, Arb-) measured in PBS at pH 7.4 using the Malvern Zetasizer Nano ZS and (**b**) same as (**a**) but now for zeta potentials. Error bars were taken from three parallel experiments.

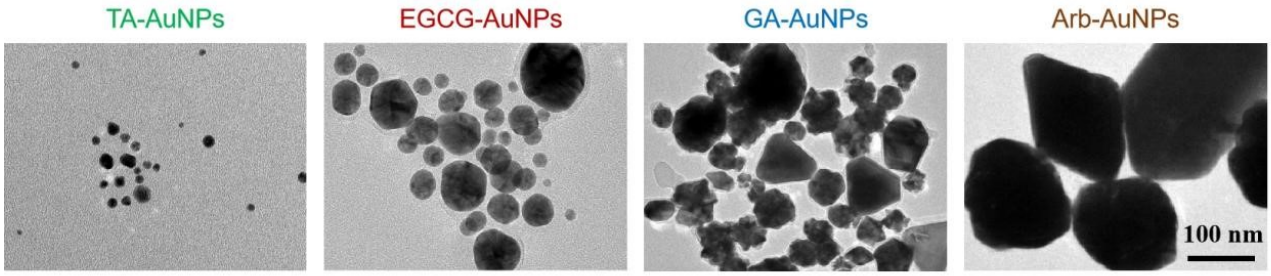


Figure S3. TEM images of polyphenolic AuNPs (TA-, EGCG-, GA-, Arb-, 100 μg/mL) in deionized water.

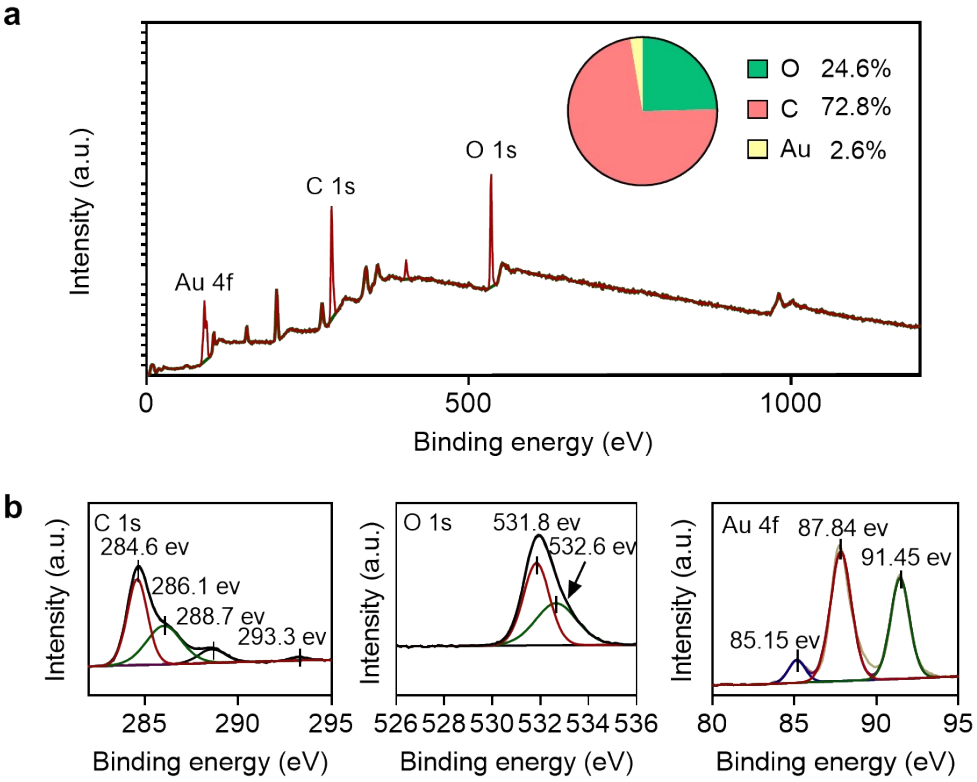


Figure S4. XPS wide scan and high resolution narrow scan spectra of EA-AuNPs. (**a**) Elemental composition of EA-AuNPs calculated from their corresponding XPS wide spectrum; (**b**) Elemental decompositions of C 1s , O 1s, and Au 4f XPS spectra of EA-AuNPs.

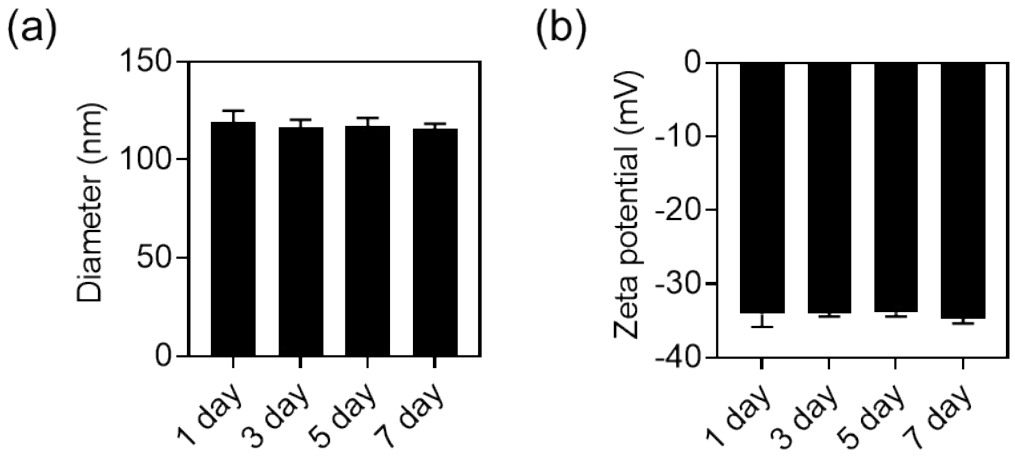


Figure S5. Stability evaluation of EA-AuNPs by storing them in water for different time points. (**a**) Diameters of EA-AuNPs at different time points; (**b**) same as (a) now for zeta potentials. Error bars were taken from three parallel experiments.

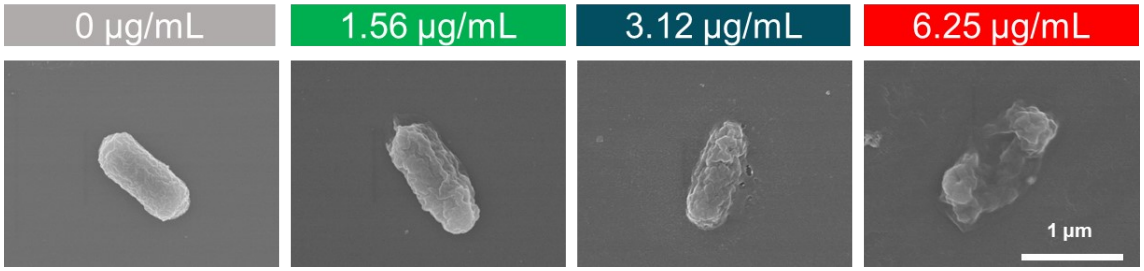


Figure S6. SEM images of *E. coli* Xen14 prior to and after exposure to different concentrations EA-AuNPs for 3 h in PBS (pH 7.4).

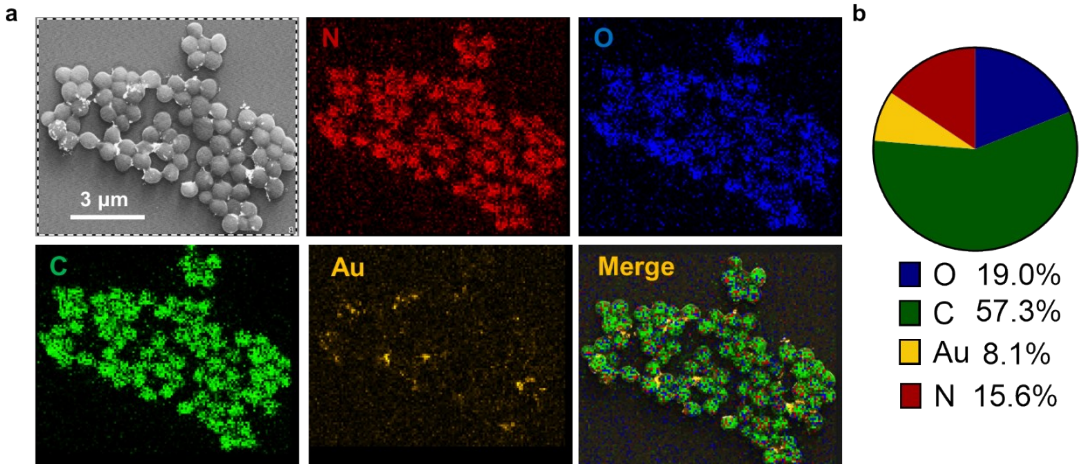


Figure S7. (**a**) SEM morphology and EDS images of *S. aureus* Xen36 after exposure for 3 h to EA-AuNPs (3.12 µg/mL); (**b**) elemental content of carbon, nitrogen, oxygen, and gold for the EA-AuNPs-incubated *S. aureus* Xen36.

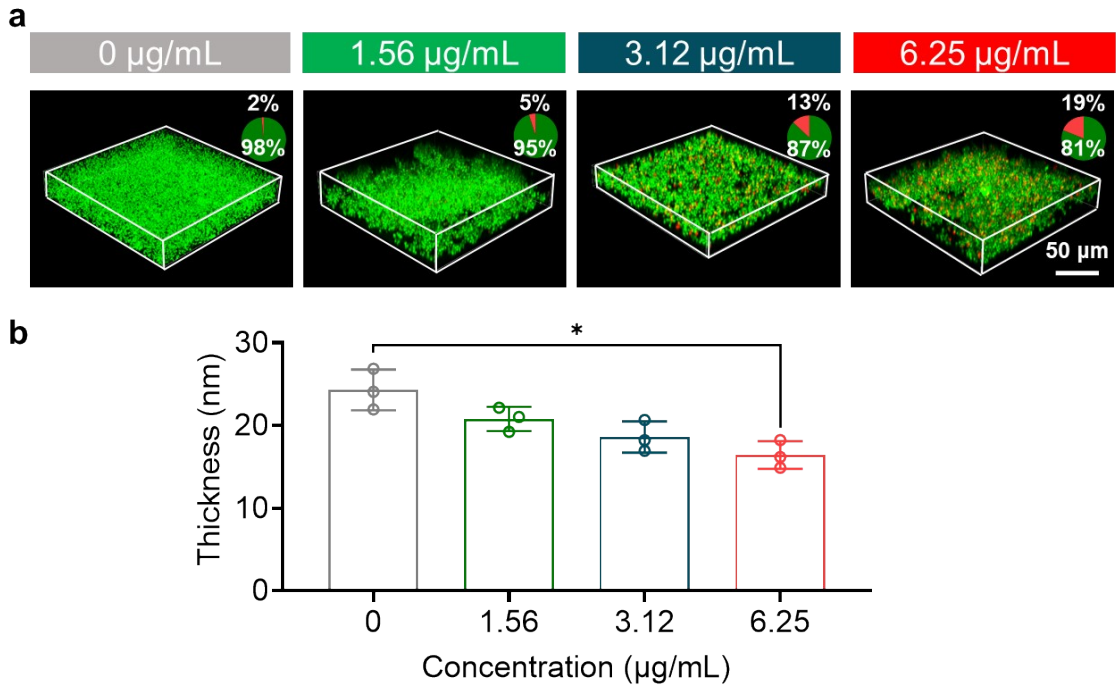


Figure S8. EA eradication of 24 h *S. aureus* Xen36 biofilm exposed for 3 h to different concentrations of EA in PBS. (**a**) Representative 3D CLSM images of live/dead stained *S. aureus* Xen36 biofilms, green fluorescent are live bacteria, red fluorescent cell wall damaged. The inset pie charts show the corresponding percentage of green/red fluorescence intensity in the biofilms; (**b**) Biofilm thickness, derived from 3D CLSM images as presented in a. *p < 0.05 indicate statistical significance (one-way ANOVA) over the differences indicated by the spanning bars.

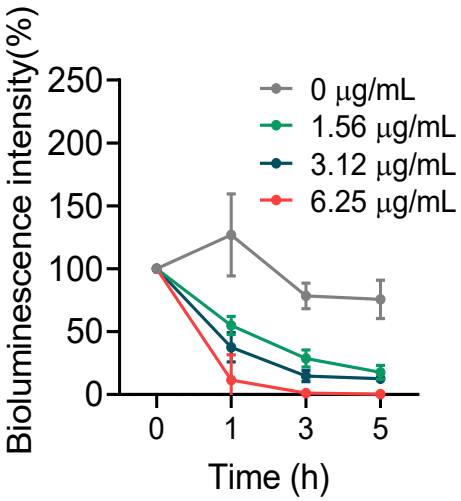


Figure S9. EA-AuNPs eradication of 24 h *S. aureus* Xen36 biofilm exposed to different concentrations of EA-AuNPs in PBS as a function of exposure time. The relative bioluminescence intensity of biofilms determined by IVIS. Error bars were taken from three parallel experiments.

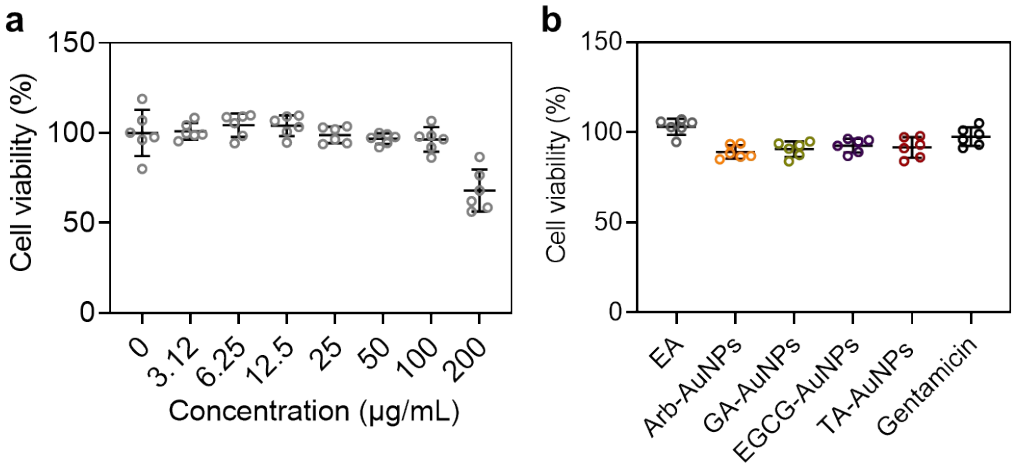


Figure S10. Cytotoxicity by exposing fibroblasts for 24 h to (**a**) different concentrations EA-AuNPs and (**b**) different polyphenolic AuNPs (100 μg/mL), EA (100 μg/mL) and gentamicin solution (100 μg/mL) in RPMI-1640 medium. Cell viability of L929 fibroblasts exposed to different materials was determined by measuring the metabolic activity. Cell viability is expressed relative to cells grown in RPMI-1640 medium (100%) without any materials.

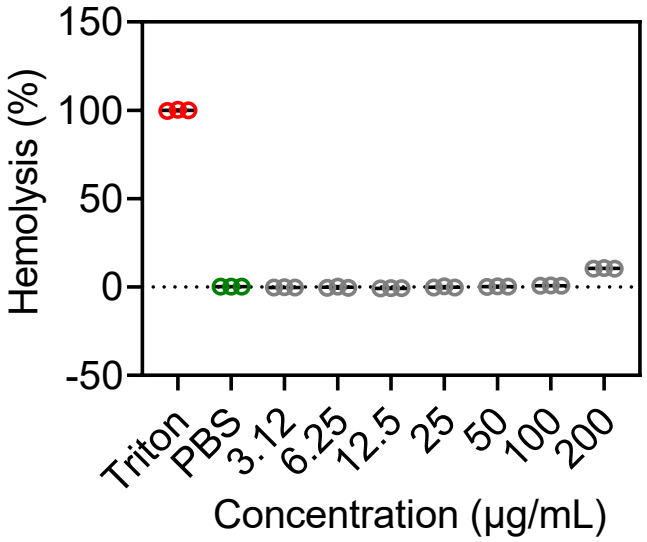


Figure S11. Hemolysis of red blood cells (RBCs) after treatments with different concentrations of EA-AuNPs. Hemolysis (0%) are RBCs not exposed to EA-AuNPs, but to PBS (positive control) and 0.1% Triton X-100, the negative control, is put on 100%.

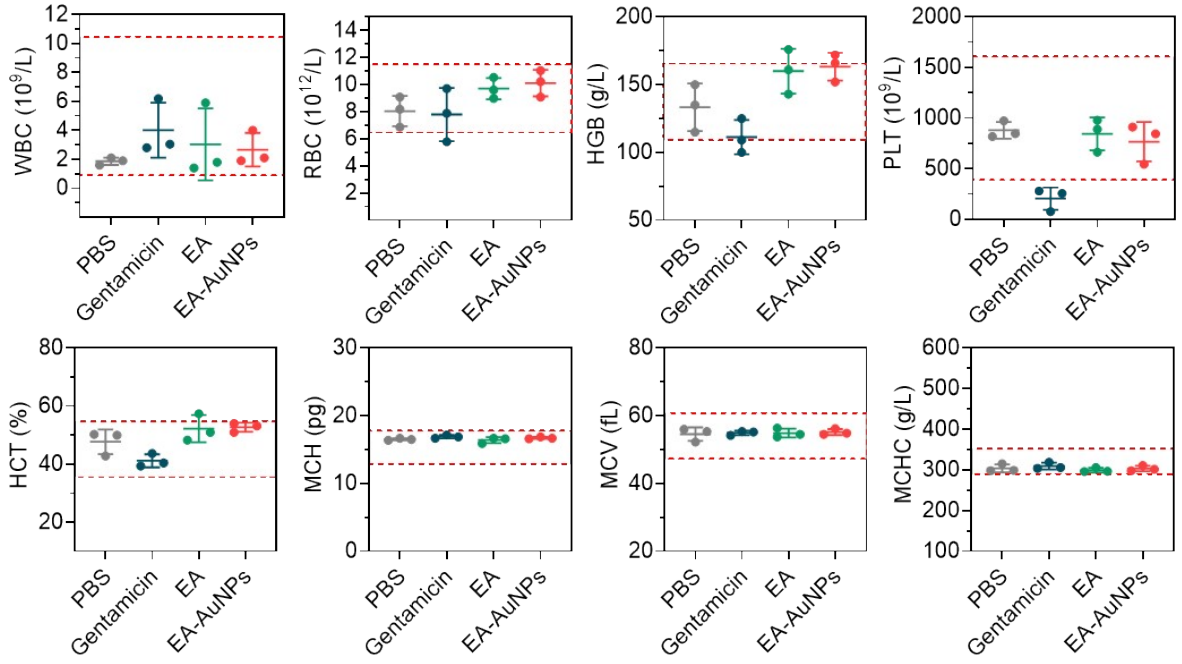


Figure S12. Blood parameters with healthy ranges indicated (dotted lines) in mice 24 h after an intraperitoneal injection with 200 μL PBS, gentamicin (100 μg/mL), EA (100 μg/mL) or EA-AuNPs (100 μg/mL). WBC: white blood cell; RBC: red blood cell; HGB: hemoglobin; PLT: platelet count; HCT: hematocrit; MCH: mean corpuscular hemoglobin; MCV: mean corpuscular volume; MCHC: mean corpuscular hemoglobin concentration. Error bars were taken from three mice.

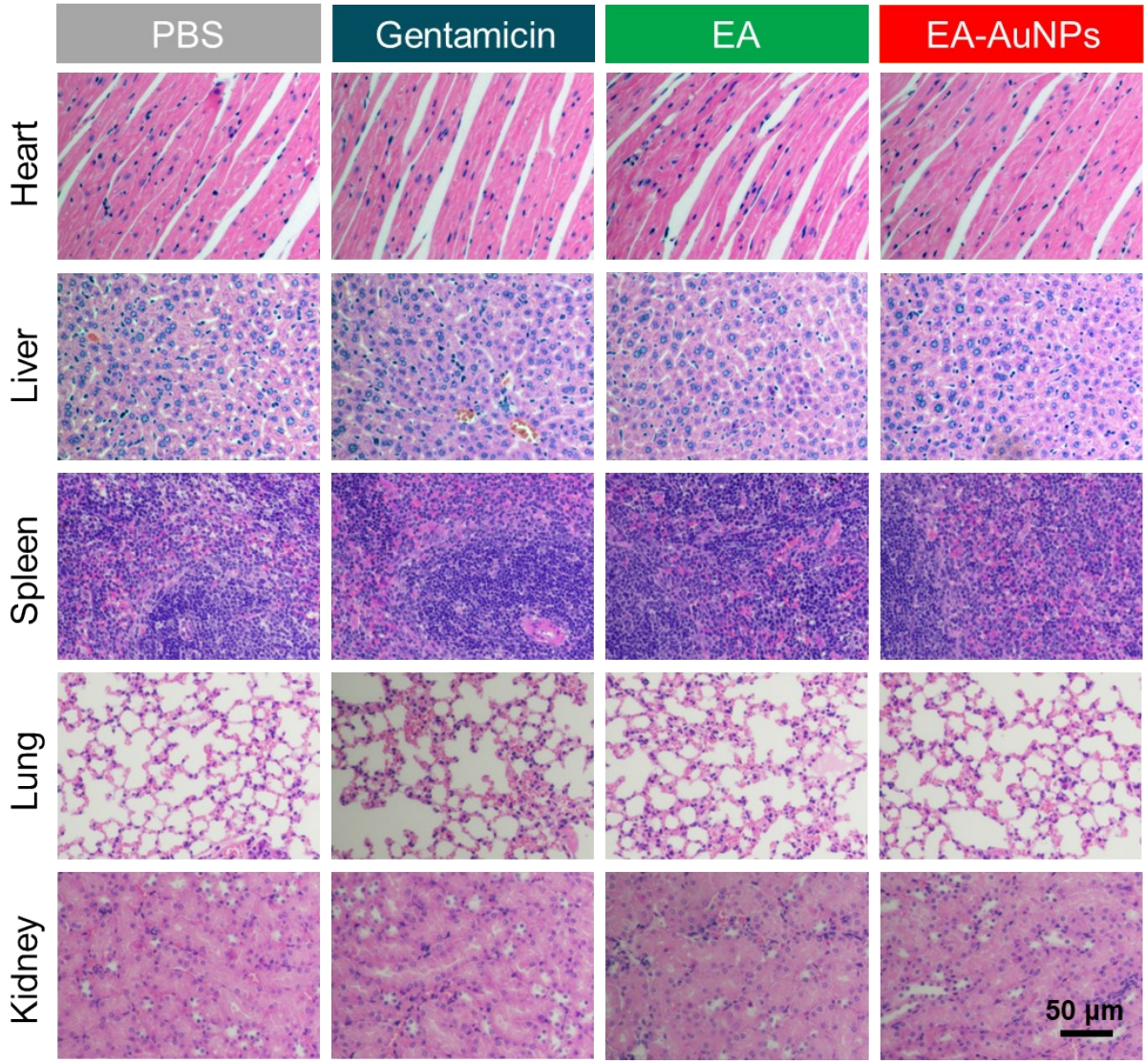


Figure S13. Histology analysis of heart, liver, spleen, lung, and kidney after intraperitoneal injection with 200 μL PBS, EA (100 μg/mL), gentamicin (100 μg/mL), and EA-AuNPs (100 μg/mL) in mice after 24 h.

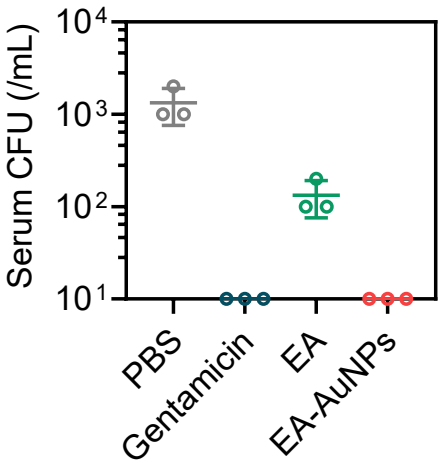


Figure S14. CFU of bacteria in blood in the peritonitis model induced by *S. aureus* Xen36 at 24 h post-treatment.

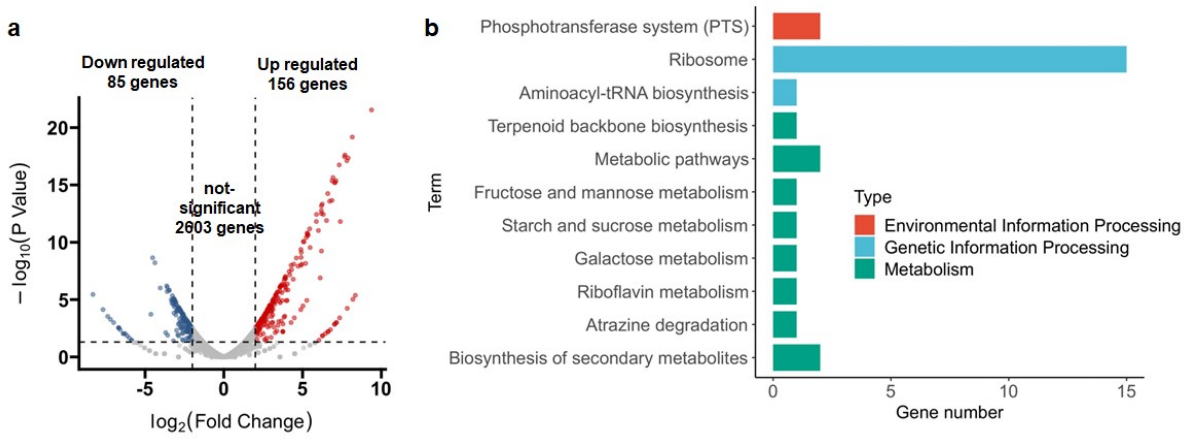


Figure S15. (**a**) Volcano plots of differentially expressed genes in *S. aureus* exposed to gentamicin compared to PBS; (**b**) The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analysis of differentially expressed genes between *S. aureus* exposed gentamicin and PBS.

Table S1. Minimal inhibitory concentration (MIC) and Minimal bactericidal concentration (MBC) of $HAuCl_4$, polyphenol, (TA, EGCG, EA, GA, or Arb)-AuNPs and gentamicin against *S. aureus* Xen36 in TSB.

Formulations	MIC (μg/mL)	MBC (μg/mL)
$HAuCl_4$	12.5	12.5
TA	＞50	＞50
EGCG	＞50	＞50
EA	＞50	＞50
GA	＞50	＞50
Arb	＞50	＞50
TA-AuNPs	25	25
EGCG-AuNPs	＞50	＞50
EA-AuNPs	3.12	3.12
GA-AuNPs	＞50	＞50
Arb-AuNPs	25	25
Gentamicin	12.5	25

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