Eastern Lubber Grasshopper Extract-Inspired Silver Nanoparticles Selectively Inhibit Methicillin-Resistant *Staphylococcus aureus*

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Supplementary figures and captions

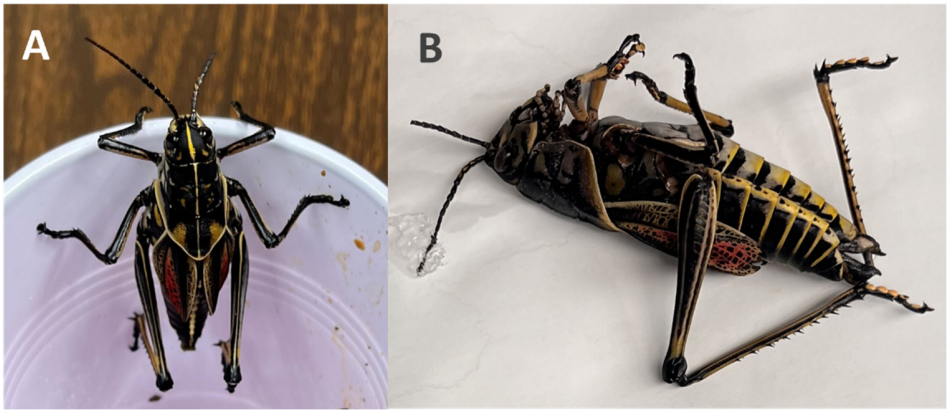

Figure S1. A female Eastern lubber grasshopper (ELG) (scientific name: *Romalea microptera*): (A) Top-down view of the ELG: The Eastern lubber grasshopper (*Romalea microptera*) is a large grasshopper, distinguished by its vivid and striking coloration. The ELG has a distinctive coloration pattern, featuring a primarily black body with vibrant yellow, orange, and red markings on the wings. The forewings (tegmina) are short. (B) Lateral view of the ELG: The head is large with prominent compound eyes and short, robust antennae. The thorax is broad and shield-like, adorned with a colorful pattern. The abdomen is similarly colorful and stout. The legs are thick and strong, with the hind legs adapted more for walking than jumping.

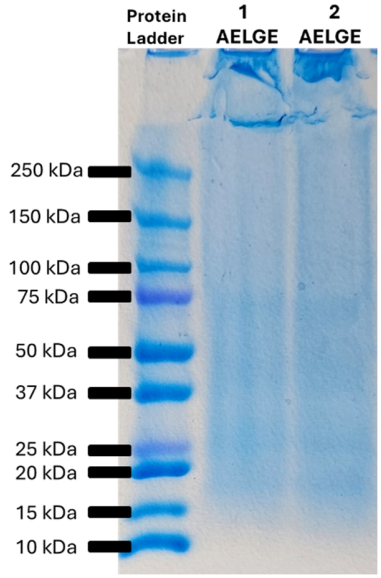

Figure S2. Proteins in Aqueous Eastern lubber grasshopper (ELG) extract (AELGE): The same AELGE sample was run on an SDS polyacrylamide gel (lanes 1 and 2). The protein bands were unclear, and most of the proteins in the AELGE sample failed to penetrate the SDS polyacrylamide gel, remaining at the top of the wells. A possible explanation is that the AELGE proteins are mostly glycoproteins, which may have difficulty penetrating the SDS gel.

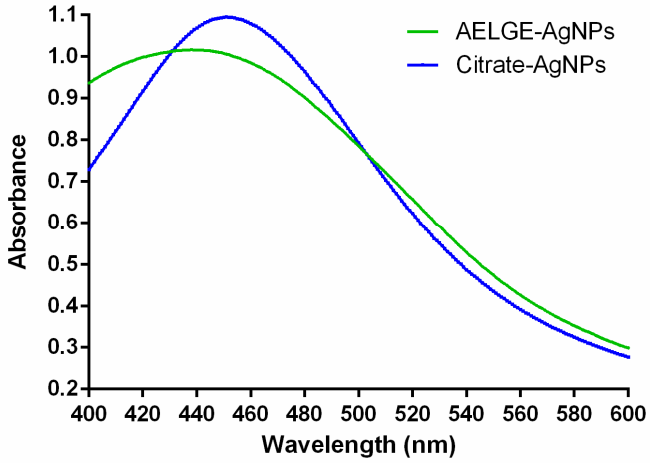

Figure S3. UV-Vis spectra of AELGE-AgNPs and Citrate-AgNPs: The broad peak in the UV-Vis spectrum of AELGE-AgNPs, located around 440 nm within the 400–600 nm range, suggests an average particle size of approximately 40–50 nm. Similarly, the broad peak of Citrate-AgNPs, centered around 455 nm, indicates an average size of approximately 60 nm (Paramelle et al., 2014).

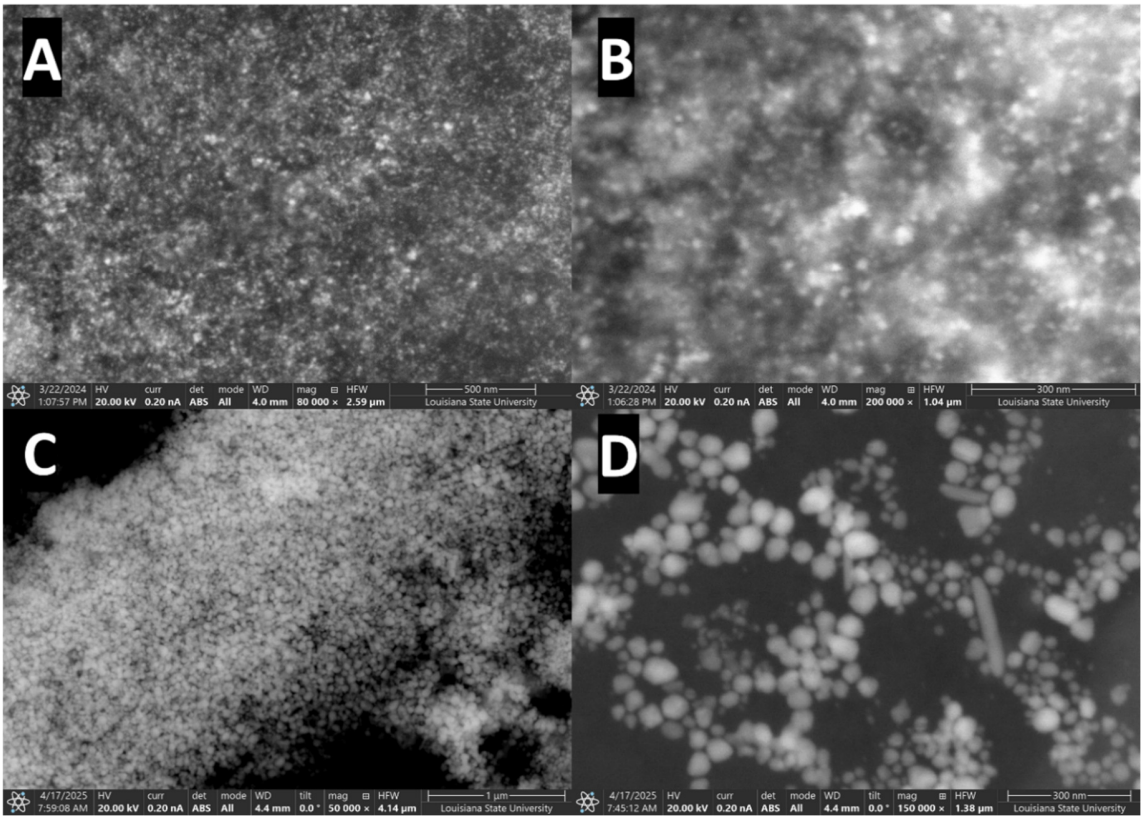

Figure S4. Scanning Electron Microscope (SEM) images of AELGE-AgNPs and Citrate-AgNPs: (A) Fairly uniform-sized AELGE-AgNPs are observed in the SEM images. (B) AELGE-AgNPs are shown at a greater magnification in the SEM image. (C) Fairly uniform-sized citrate-AgNPs are observed in the SEM images as well. (D) Citrate-AgNPs are shown at a greater magnification in the SEM image.

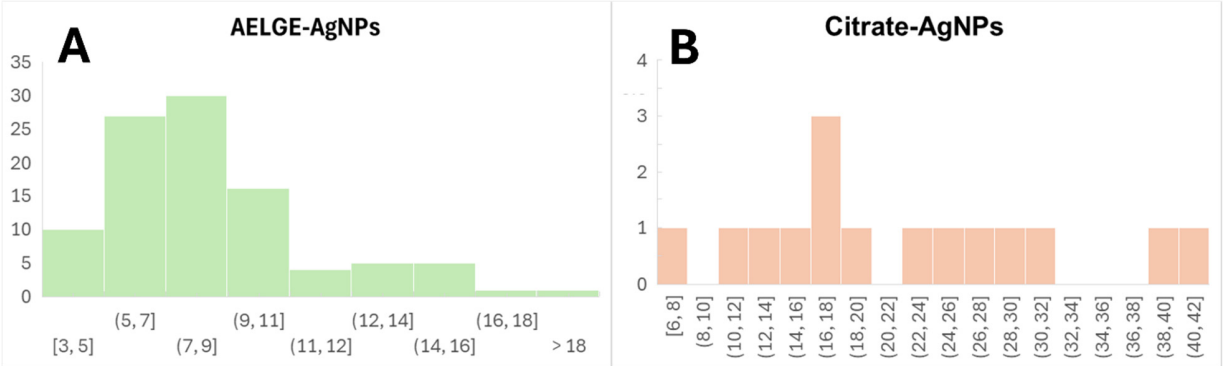

Figure S5. Histogram of particle sizes for AELGE-AgNPs and Citrate-AgNPs observed in the TEM images in Figure 3C and 3D: (A) AELGE-AgNPs exhibit fairly uniform and small sizes, mostly ranging from 3 to 11 nm, as shown in Figure 3C. (B) Citrate-AgNPs display a broader size distribution, ranging from 6 to 42 nm, as seen in Figure 3D.

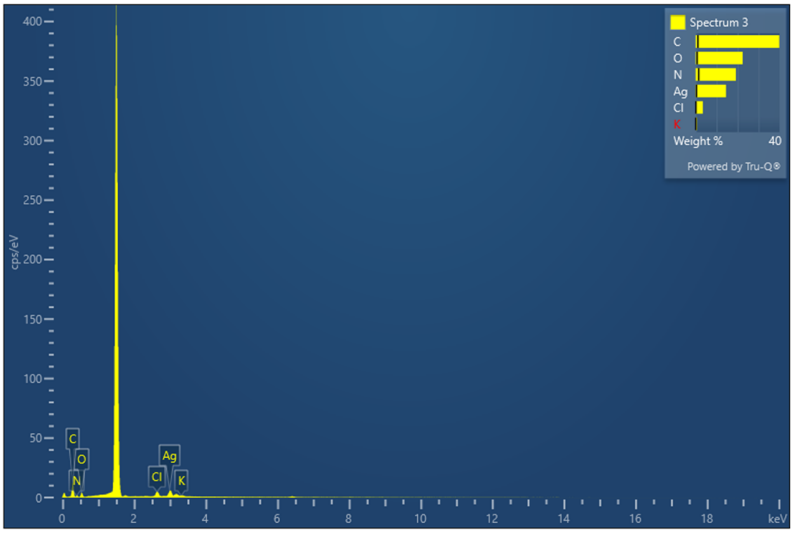

Figure S6. EDX Data of AELGE-AgNPs: The AELGE-AgNPs were both synthesized and capped with biological molecules, incorporating elements such as C, N, and O. This suggests that the biological molecules in the Eastern lubber grasshopper extract include proteins, polypeptides, and other typical organic compounds containing nitrogen and oxygen. (Note: an aluminum signal was detected because of aluminum tape under the samples.)

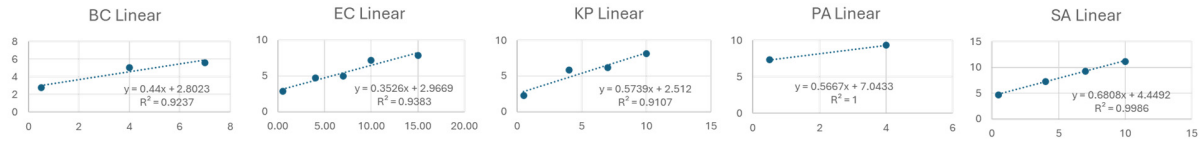

Figure S7. Estimated free Ag^+ ion ZOI by mathematical calculations with average ZOI (mm) (y axis) and applied Ag^+ ion concentration (mM) (x axis): ZOI values at lower Ag^+ ion concentrations display a good linear pattern. A linear graph was created for each bacterium using as many data points as possible from the power function graph, ensuring that the R^2 value exceeded 0.9000 to indicate a high level of confidence.

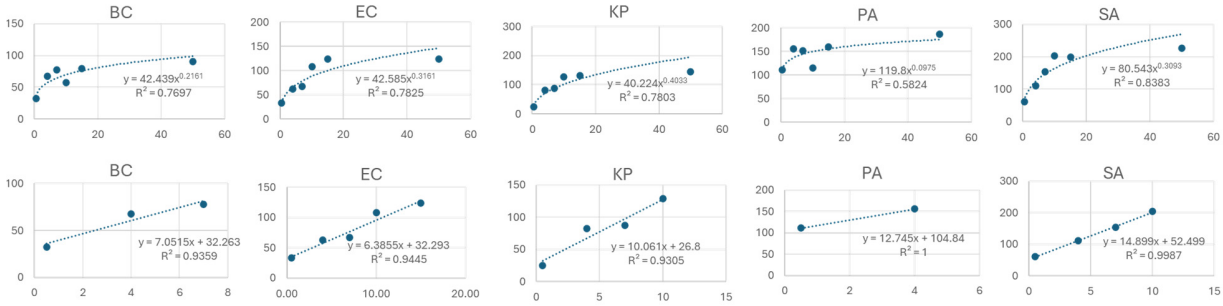

Figure S8. Estimated AELGE-AgNP ZOI area (ZOIA) Enhancement compared to free Ag^+ ions by mathematical calculations with ZOIA (mm^2) (y axis) and applied Ag^+ ion concentration (mM) (x axis): The assumption is that the ZOI area (ZOIA) is proportional to the number of bacterial cells killed, which were initially placed on the petri dish for growth. Therefore, ZOIA can indicate how effectively AELGE-AgNPs inhibit the growth of cultured bacteria on the petri dish.

(Top row): ZOIA was measured by $Total\ inhibited\ circular\ area\ of\ Ag^{+}ion\ solution = \pi(r = \ radius\ of\ the\ estimated\ inhibited\ area\ in\ circle)^2 - (6mm\ disc\ area = \pi(3mm, 0.5 * 6mm)^2)$. The relationship between ZOIA (mm^2) (y axis) and applied Ag^+ ion concentration (mM) (x axis) is shown as a power function graph for each bacterium tested in the disk diffusion assay. **(Bottom row)**: ZOIA values at lower Ag^+ ion concentrations also displayed a good linear pattern. A linear graph was created for each bacterium using as many data points as possible from the power function graph, ensuring that the R^2 value exceeded 0.9000 to indicate a high level of confidence.

Assumptions before calculations: Fifty microliters (50 µl) of Ag^+ ion solution at 50 mM was added to 300 µl of AELGE solution to form AELGE-AgNPs in the mixture. One milliliter (1 ml) of distilled water was then added to 350 µl of the AELGE-AgNP solution before testing in the disk diffusion assay. Therefore, the amount of silver in the AELGE-AgNP solution is equivalent to the amount of silver in the Ag^+ ion solution at 1.85 mM for an equal volume. Silver acts as the primary agent to kill bacteria by generating reactive oxygen species (ROS), which cause oxidative stress and damage to bacterial cellular components.

Calculation: $Initial\ conc.\ 50\ mM\ \times \frac{50\mu l = initial\ volume}{1350\mu l = final\ volume} = final\ conc. \approx 1.85\ mM$

Estimated ZOIA of Ag^+ ion at 1.85 mM: as a x value was calculated for each bacteria following the (bottom row) linear equation **(in ZOIA graph)** for each bacterium (y axis = ZOIA in nm^3 and $x = Ag^+$ ions concentration in mM)

BC: $\left(7.0515\frac{mm^2}{mM} \times 1.85mM\right) + 32.263\ mm^2 = 45.31\ mm^2$

EC: $\left(6.3855\frac{mm^2}{mM} \times 1.85mM\right) + 32.293\ mm^2 = 44.11\ mm^2$

KP: $\left(10.061\frac{mm^2}{mM} \times 1.85mM\right) + 26.8\ mm^2 = 45.41\ mm^2$

PA: $\left(12.745\frac{mm^2}{mM} \times 1.85mM\right) + 104.84\ mm^2 = 128.42\ mm^2$

SA: $\left(14.899\frac{mm^2}{mM} \times 1.85mM\right) + 52.499\ mm^2 = 80.06\ mm^2$

ZOIA of AELGE-AgNPs: $Total\ inhibited\ circular\ area\ of\ AELGE - AgNP\ solution = \pi(r = \ radius\ of\ the\ total\ inhibited\ area\ in\ circle)^2 - (6mm\ disc\ area\ 28.27\ mm^2 = \pi(3mm, 0.5 * 6mm)^2)$.

BC: $\pi(\frac{11.6425mm\ (diameter\ of\ tatal\ inhibited\ circular\ area)}{2})^2 - 28.27\ mm^2 = 78.19\ mm^2$

EC: $\pi(\frac{11.9475mm\ (diameter\ of\ tatal\ inhibited\ circular\ area)}{2})^2 - 28.27\ mm^2 = 83.84\ mm^2$

KP: $\pi(\frac{10.4925mm\ (diameter\ of\ tatal\ inhibited\ circular\ area)}{2})^2 - 28.27\ mm^2 = 58.19\ mm^2$

PA: $\pi(\frac{10.04mm\ (diameter\ of\ tatal\ inhibited\ circular\ area)}{2})^2 - 28.27\ mm^2 = 50.90\ mm^2$

SA: $\pi(\frac{15.155mm\ (diameter\ of\ tatal\ inhibited\ circular\ area)}{2})^2 - 28.27\ mm^2 = \mathbf{152.12\ mm^2}$

ZOIA of AELGE-AgNPs compared to ZOIA of free Ag^+ ions:

BC: $78.19\ mm^2 - 45.31\ mm^2 = 32.88\ mm^2$

EC: $83.84\ mm^2 - 44.11\ mm^2 = 39.73\ mm^2$
KP: $58.19\ mm^2 - 45.41\ mm^2 = 12.78\ mm^2$
PA: $50.90\ mm^2 - 128.42\ mm^2 = -77.52\ mm^2$
SA: $\mathbf{152.12\ mm^2} - 80.06\ mm^2 = \mathbf{72.06\ mm^2}$

References

1. Paramelle D, Sadovoy A, Gorelik S, et al (2014) A rapid method to estimate the concentration of citrate capped silver nanoparticles from UV-visible light spectra. Analyst 139:4855–4861. https://doi.org/10.1039/c4an00978a