

Supporting Information

Green Synthesis of Silver Nanoparticles using *Phyllanthus emblica* Extract: Investigation of Antibacterial Activity and Biocompatibility *in vivo*

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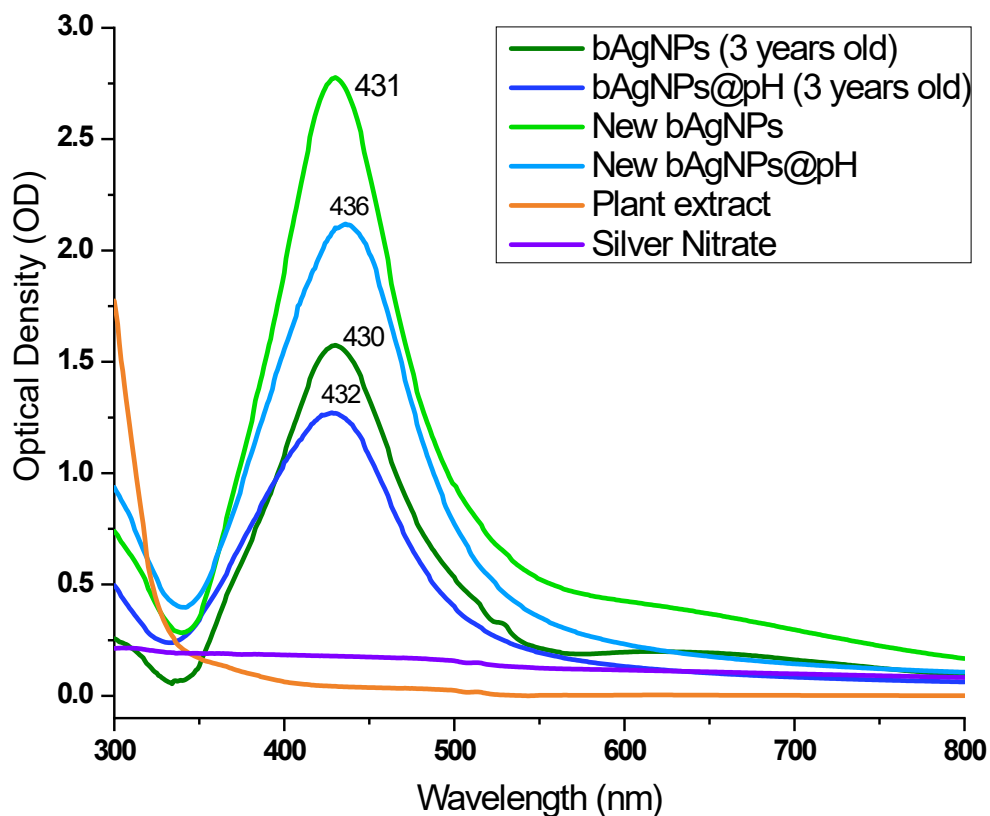


FIGURE S1 | Observation of nanoparticles stability. Ultraviolet-visible spectra of silver nanoparticles synthesized using *Phyllanthus emblica* flesh extract. High-intensity surface plasmon resonance bands are visible at 431 and 436 nm for newly synthesized bAgNPs (without controlled pH) and bAgNPs@pH (controlled pH), respectively. The high-intensity surface plasmon resonance bands for previously (i.e., 3 years ago) synthesized bAgNPs (without controlled pH) and bAgNPs@pH (controlled pH) are also visible at 430 and 432 nm, respectively. There is no discernible differences in the absorption spectra of nanoparticles synthesized at different times, indicating that both the as-synthesized bAgNPs and bAgNPs@pH are highly stable.

TABLE S1. Hydrodynamic Size, Polydispersity index (PDI), Zeta potentials of biogenic AgNPs

Biogenic AgNPs	Hydrodynamic Size (nm)	PDI	Zeta potential (mV)
bAgNPs@pH	116.8 ± 72.9 (86.1%)	0.566	-24.4 ± 2.2
	13.2 ± 4.7 (13.9%)		
bAgNPs	366.4 ± 148.2 (93.2%)	0.214	-24.2 ± 0.9
	94.7 ± 17.2 (6.7%)		

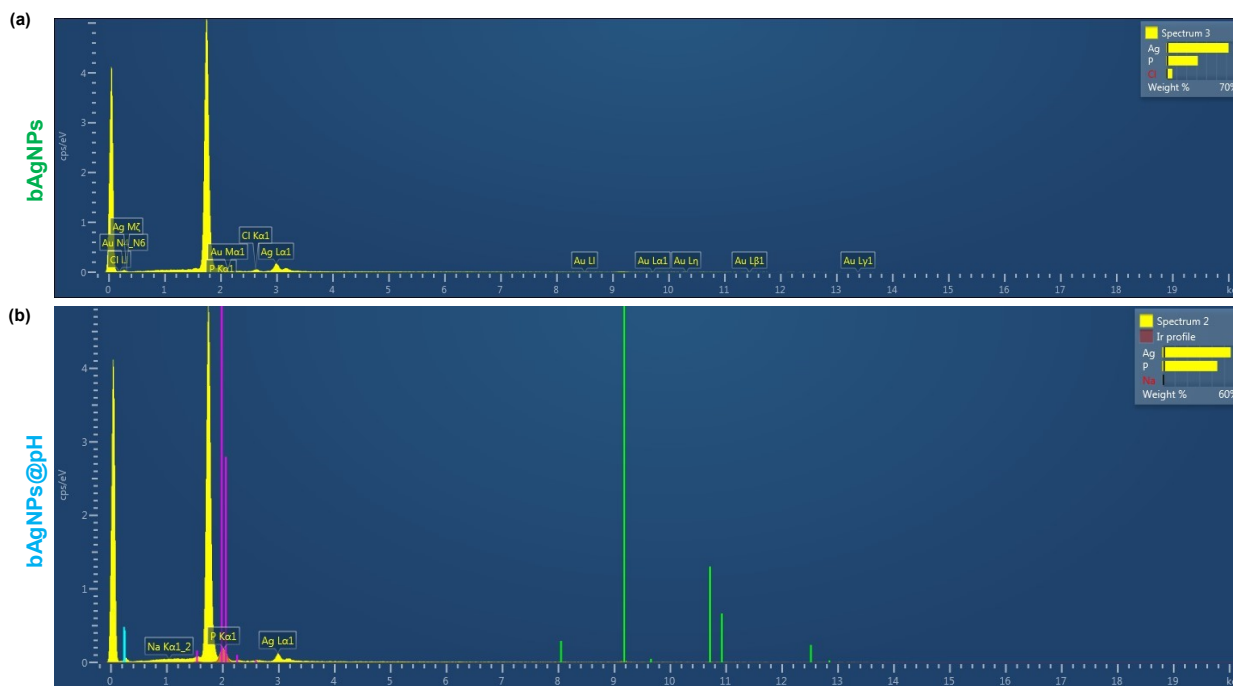


FIGURE S2 | Energy dispersive X-ray (EDS) spectra of bAgNPs **(a)**, and bAgNPs@pH **(b)**. Peaks at ~3 keV indicate the presence of Ag in the biogenic nanoparticles.

TABLE S2. Antibacterial Activity (*i.e.*, MIC Values and the ZOI) of bAgNPs

Name of strains	Zone of inhibition (mm)				MIC ((μ g)		Pathogenicity
	bAgNPs (60 μ g)	bAgNPs@pH (60 μ g)	Ethanollic plant extract (60 μ g)	AgNO ₃	bAgNPs	bAgNPs @pH	
<i>B. subtilis</i>	13 \pm 1	14.67 \pm 0.25	6.33 \pm 0.58	8.0 \pm 0.0	0.50	0.50	Nonpathogenic
<i>S. typhi</i>	15.67 \pm 0.30	15.33 \pm 0.58	7.0 \pm 0	7.0 \pm 0.55	0.50	0.50	Pathogenic
<i>H. Alvei</i>	23.67 \pm 0.58	25.67 \pm 0.58	10.33 \pm 0.5	7.0 \pm 0.0	0.25	0.25	Pathogenic
<i>EPEC</i>	18.33 \pm 0.58	20 \pm 1	9.33 \pm 0.58	7.0 \pm 0.0	0.25	0.25	Pathogenic
<i>E. coli DH5α</i>	13.67 \pm 0.58	15 \pm 1	6.67 \pm 0.58	9.0 \pm 0.33	0.50	0.50	Nonpathogenic
<i>V. cholerae</i>	18.33 \pm 0.58	20.33 \pm 0.58	7.5 \pm 0.58	8.5 \pm 0.0	0.25	0.25	Pathogenic
<i>S. aureus</i>	17 \pm 1	22.25 \pm 0.75	9.67 \pm 0.58	9.0 \pm 0.0	0.50	0.125	Pathogenic

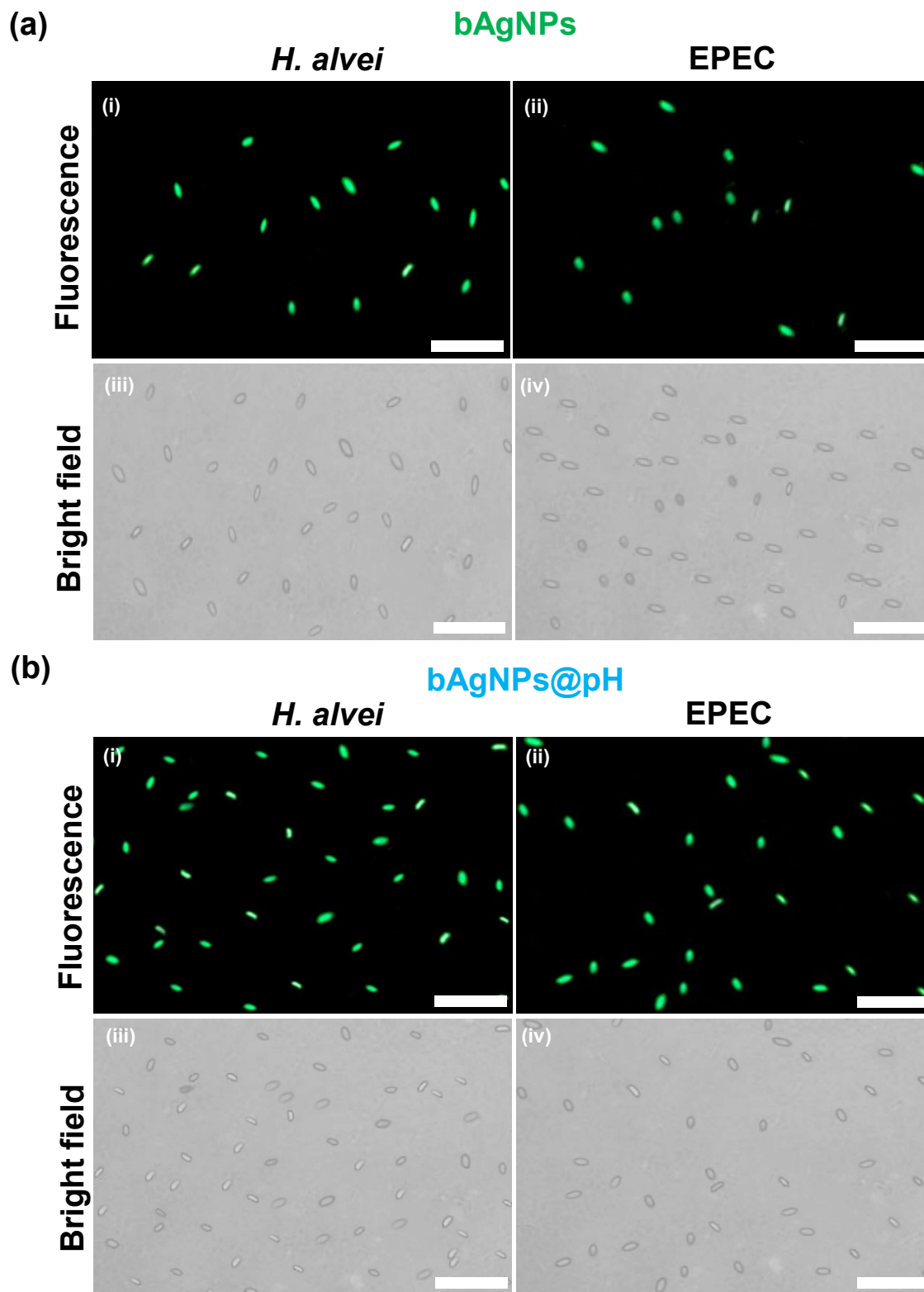


FIGURE S3 | (a) CellTox Green assay shows that bAgNPs bring about bacterial [*H. alvei* (i) and EPEC (ii)] cell wall damage. Figure (iii) and (iv) are bright field images of them, respectively. (b)

CellTox Green assay shows that bAgNPs@pH bring about bacterial [*H. alvei* (i) and EPEC (ii)] cell wall damage. Figure (iii) and (iv) are bright field images of them, respectively. Scale bar: 20 μm .

Table S3: Zone of inhibition (mm) in different doses of nanoparticles and at different time points

Name of Bacteria	bAgNPs			bAgNPs@pH			Plant extract 60 μg
	Hours	30 μg	60 μg	Hours	30 μg	60 μg	
<i>Bacillus subtilis</i>	16	12.5 \pm 0.58	13 \pm 1	16	12.67 \pm 0.58	14.67 \pm 0.25	6.33 \pm 0.58
	20	11.33 \pm 0.58	12 \pm 1	20	12.25 \pm 0.25	14 \pm 1	6.67 \pm 0.25
	24	10.25 \pm 1.25	11 \pm 1	24	11 \pm 1	12 \pm 1	6.33 \pm 0.58
<i>E. coli (DH5a)</i>	16	12.33 \pm 0.58	13.67 \pm 0.58	16	13.67 \pm 0.58	15 \pm 1	6.67 \pm 0.58
	20	11.67 \pm 0.58	13.33 \pm 0.58	20	13.00 \pm 0.25	14.33 \pm 0.58	6.33 \pm 0.58
	24	10.33 \pm 0.58	11 \pm 1	24	10.67 \pm 0.58	11.67 \pm 0.58	6.0 \pm 0.58
<i>V. cholera</i>	16	16.67 \pm 1.15	18.33 \pm 0.58	16	19 \pm 1	20.33 \pm 0.58	7.5 \pm 0.58
	20	15.67 \pm 0.58	15.33 \pm 0.58	20	17.33 \pm 0.58	18.0 \pm 0.58	7.0 \pm 0.58
	24	13.33 \pm 0.58	12.67 \pm 0.58	24	15.33 \pm 0.58	16.33 \pm 0.58	7.0 \pm 0.58
<i>S. aureus</i>	16	14.75 \pm 0.75	17 \pm 1	16	21.5 \pm 0.5	22.25 \pm 0.75	9.67 \pm 0.58
	20	14 \pm 1	15.75 \pm 1.25	20	16.5 \pm 1.5	17.75 \pm 0.25	9.33 \pm 0.25
	24	8.5 \pm 0.5	11.75 \pm 0.25	24	10.5 \pm 0.5	11.75 \pm 0.25	8.67 \pm 0.58
<i>Salmonella typhi</i>	16	13.67 \pm 0.58	15.67 \pm 0.58	16	13.33 \pm 0.58	15.33 \pm 0.58	7.0 \pm 0
	20	12.33 \pm 0.58	14.67 \pm 0.58	20	12.33 \pm 0.58	14.33 \pm 0.58	7.0 \pm 0
	24	10.33 \pm 0.58	13 \pm 1.73	24	10 \pm 1	11.67 \pm 1.15	6.33 \pm 0.58
<i>Hafnia alvei</i>	16	22 \pm 1	23.67 \pm 0.58	16	22 \pm 1	25.67 \pm 0.58	10.33 \pm 0.5
	20	21.67 \pm 0.58	22.33 \pm 0.58	20	21.33 \pm 0.58	24 \pm 1	10.0 \pm 0
	24	18.33 \pm 0.58	19.33 \pm 0.58	24	18 \pm 1	19.67 \pm 0.58	8.67 \pm 0.58
<i>E. coli (EPEC)</i>	16	17 \pm 0.58	18.33 \pm 0.58	16	17.67 \pm 0.58	20 \pm 1	9.33 \pm 0.58
	20	16.67 \pm 0.58	17.33 \pm 0.58	20	16.33 \pm 0.58	19.33 \pm 0.58	9.0 \pm 0.58
	24	14.33 \pm 0.58	14.67 \pm 0.58	24	14 \pm 1	15.67 \pm 0.58	8.67 \pm 0.58

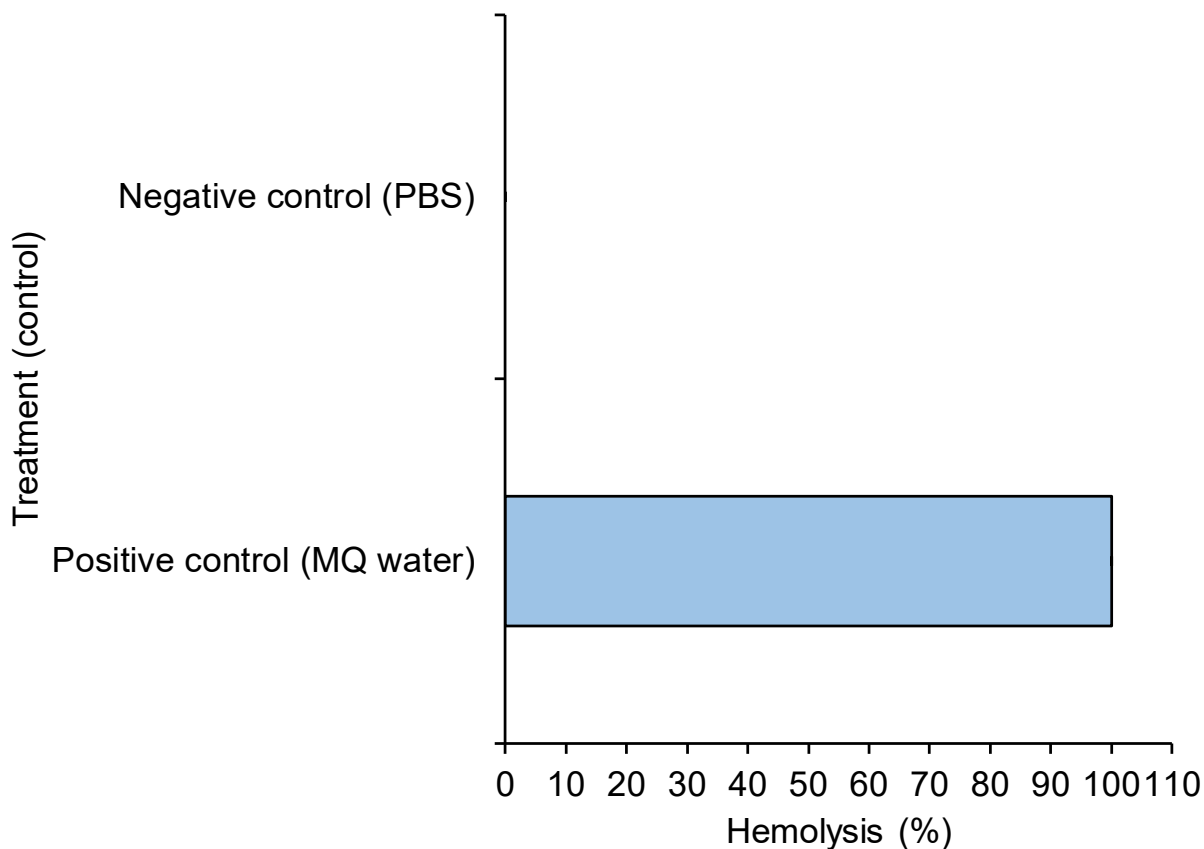


FIGURE S4 | Control experiment for hemolysis was performed using Mili Q water (MQ) as positive control, Phosphate buffer saline (PBS) as negative control.

Table S4: Zone of inhibition of standard drugs used for the antibacterial assays at 16 hours.

Name of Bacteria	Povidone iodine		Ciprofloxacin	
	Hours	100 µg	Hours	5 µg
<i>Bacillus subtilis</i>	16	12.5 ± 1.0	16	28.5 ± 1.0
<i>E. coli (DH5α)</i>	16	13.5 ± 1.5	16	29 ± 0.5
<i>V. cholera</i>	16	14.29 ± 0.5	16	30 ± 1.0
<i>S. aureus</i>	16	15 ± 0.25	16	31.58 ± 0.25
<i>Salmonella typhi</i>	16	14.5 ± 1.5	16	30.43 ± 0.75
<i>Hafnia alvei</i>	16	15 ± 1.0	16	29 ± 1.0
<i>E. coli (EPEC)</i>	16	12.5 ± 0.5	16	23 ± 0.50

Table S5: The normal reference range of liver and kidney function biomarker ¹.

Name of tests	Normal reference range of Wistar rat	
ALT (Alanine aminotrasferase)	13-56	U/L
AST (Aspartate aminotrasferase)	34-109	U/L
γ-GT (Gamma-glutamyl transpeptidase)	0.5-5.3	U/L
CREAT (Creatinine)	0.2-0.7	mg/dl

References

1. Loeb, WF and Quimby, FW. 1999. The Clinical Chemistry of Laboratory Animals, 2nd ed. Philadelphia: Taylor & Francis USA