Supporting information

Remarkable effect of jacalin in diminishing the protein corona interference in the antibacterial activity of pectin capped copper sulfide nanoparticles

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#equally contributed

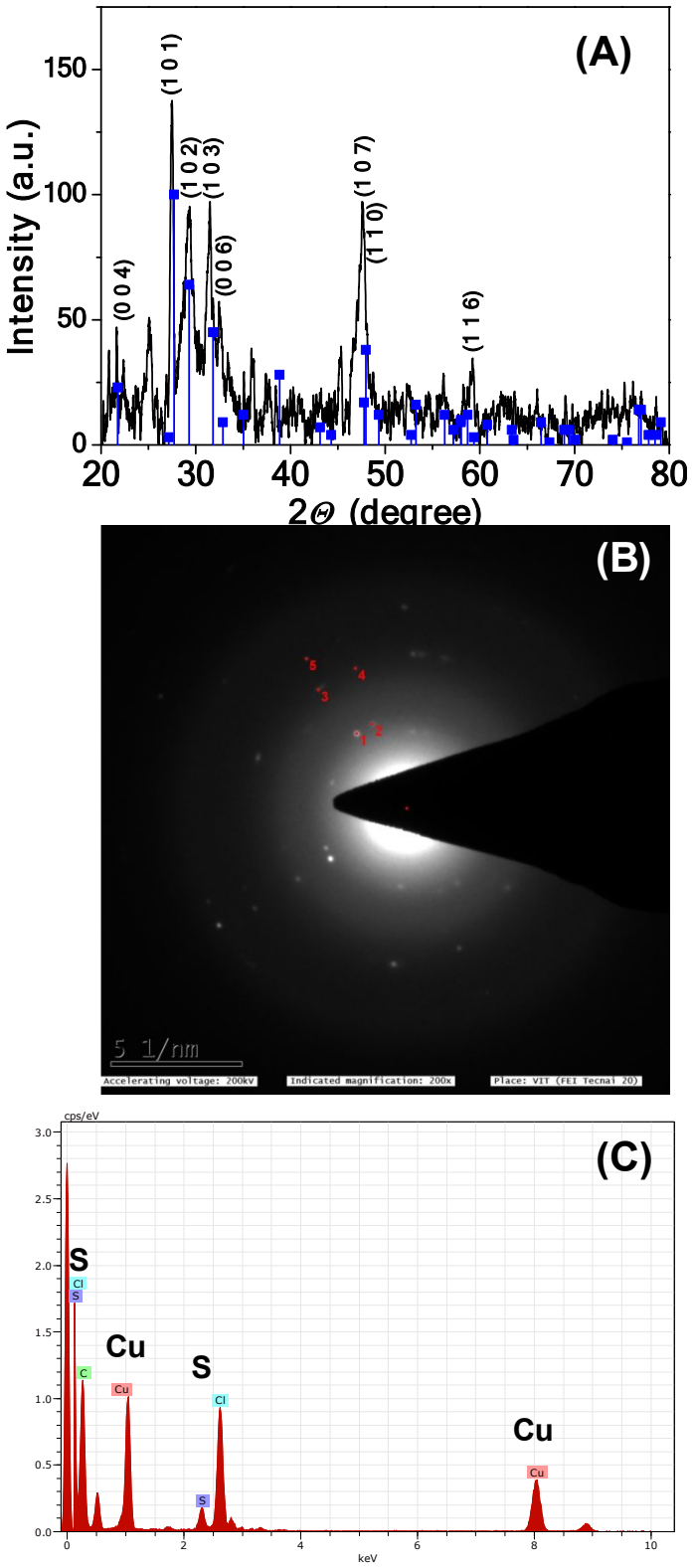


Figure S1: (A) Powder XRD pattern of pCuS NPs. Blue lines corresponds to standard values of CuS (Covellite JCPDS – 74-1234), (B) Transmission electron microscopy image of pCuS NPs. (B) SAED pattern of pCuS NPs. (C) EDAX data confirms that the NPs is composed of Cu and S.

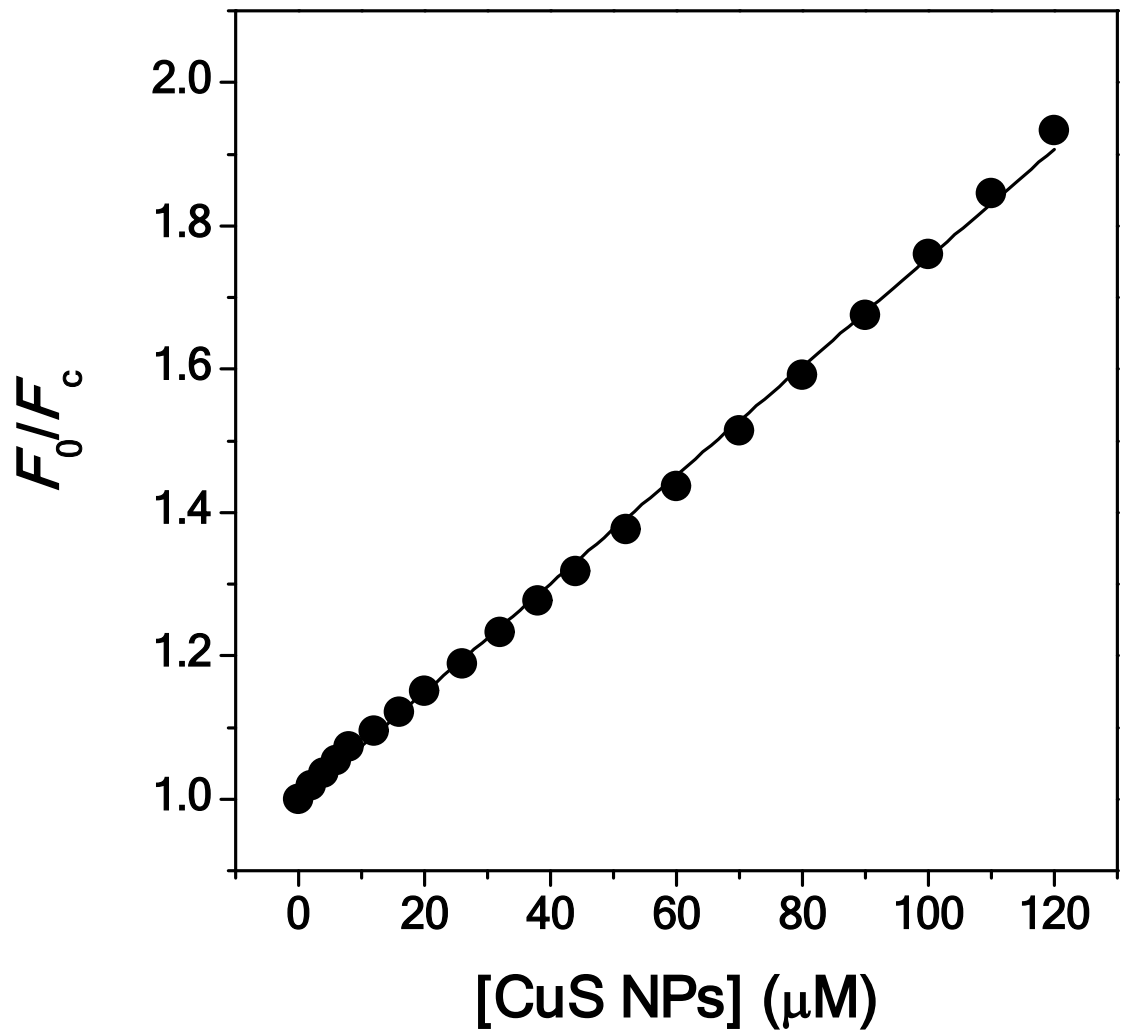


Figure S2: Stern–Volmer plot of BSA fluorescence quenched by pCuS.

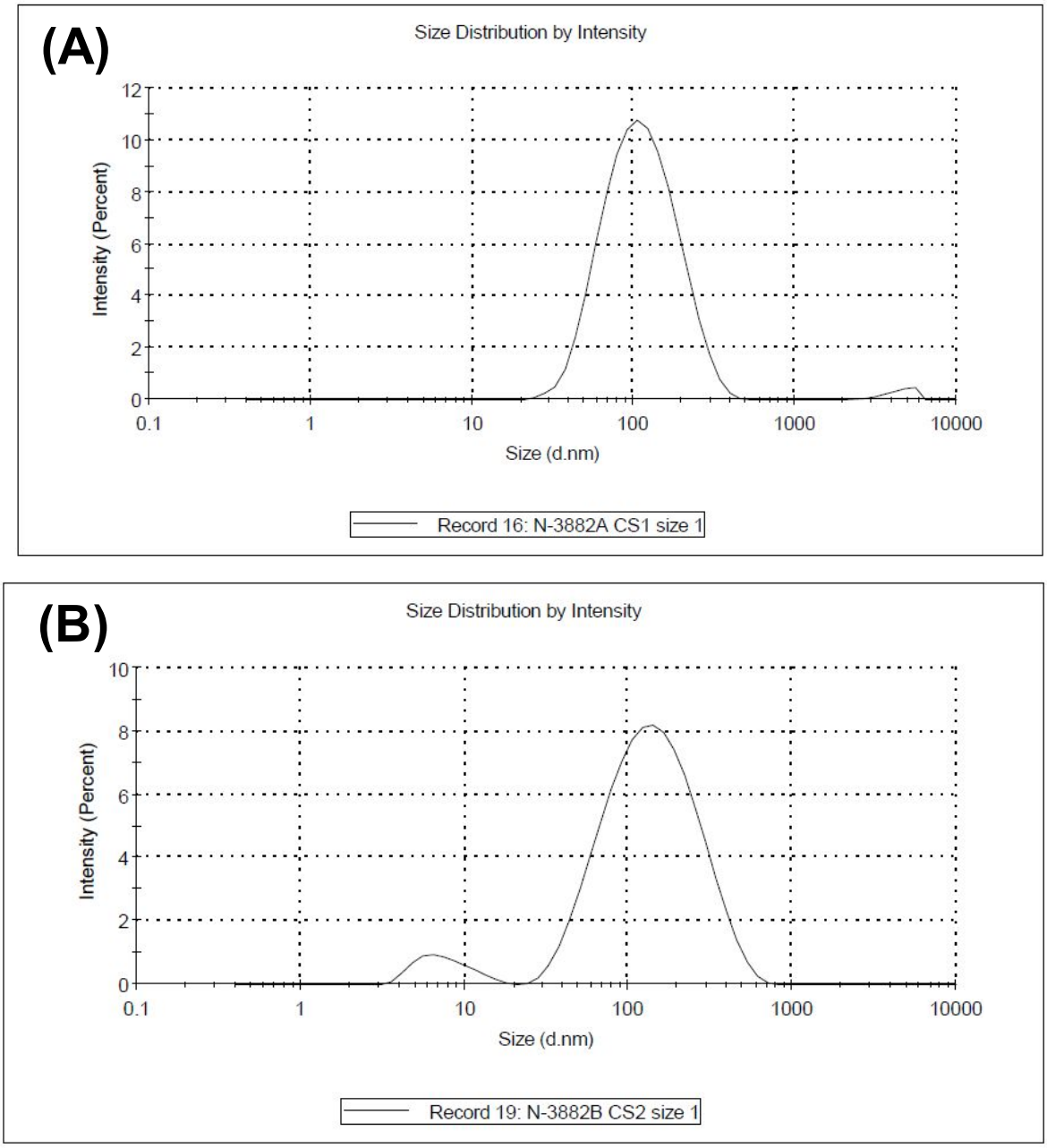
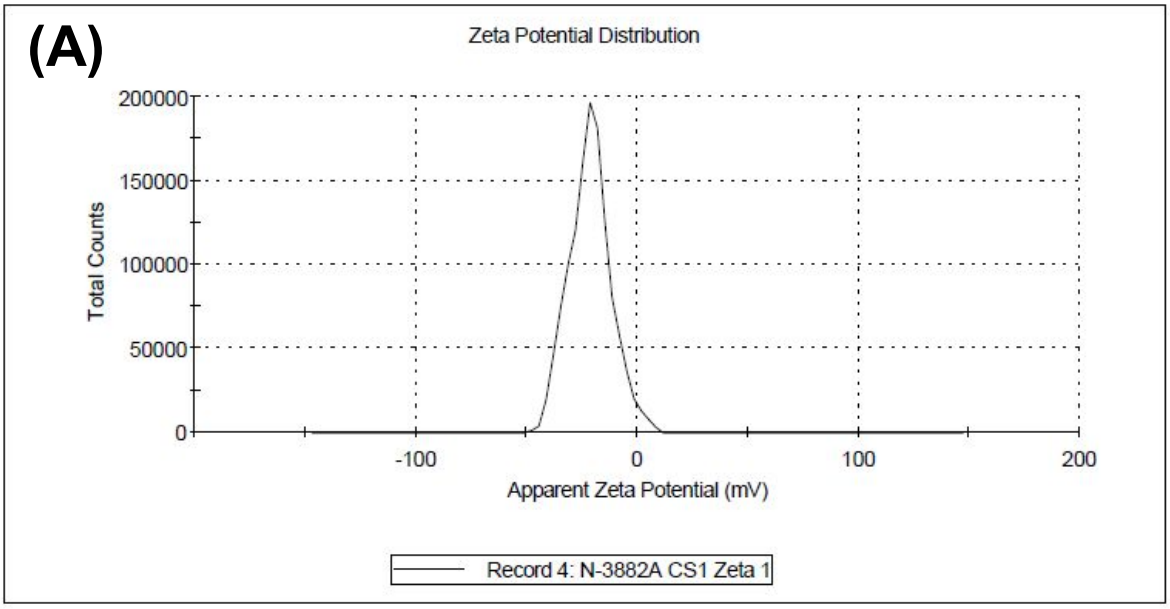


Figure S3: Hydrodynamic diameter of the NPs was analyzed by particle size analyzer. (A) pCuS, (B) BSA + pCuS.



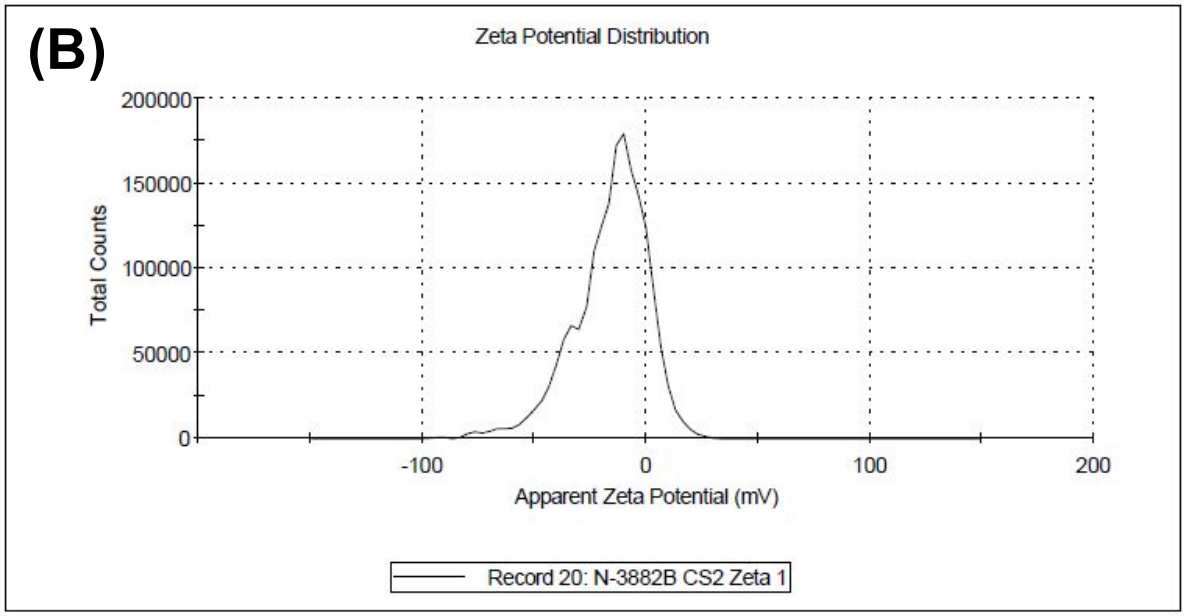


Figure S4: Zeta potential of (A) pCuS, (B) BSA + pCuS.

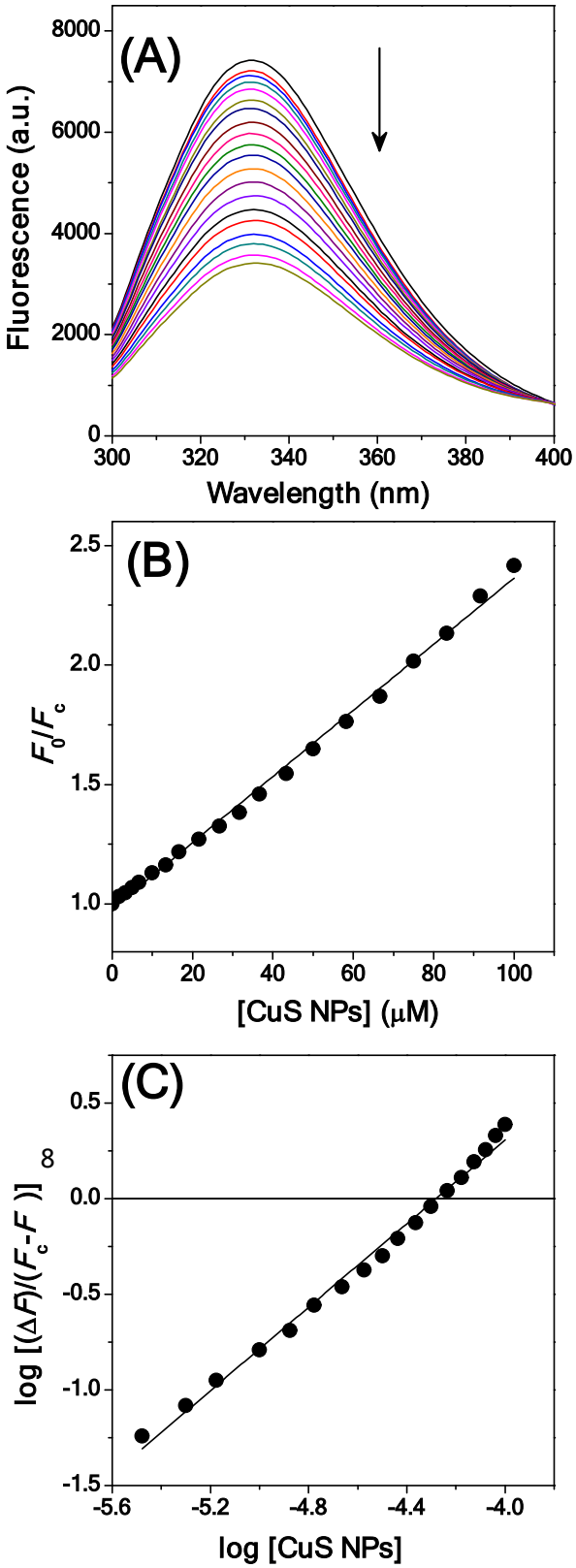


Figure S5: Fluorescence titration. (A) Addition of pCuS to jacalin quenches the intrinsic fluorescence. Excitation wavelength = 280 nm. (B) Stern–Volmer plot of jacalin fluorescence quenched by pCuS (C) Double logarithmic plot for the interaction pCuS with jacalin. The abscissa intercept of this plot yielded the pK_a value of the jacalin-pCuS interaction.

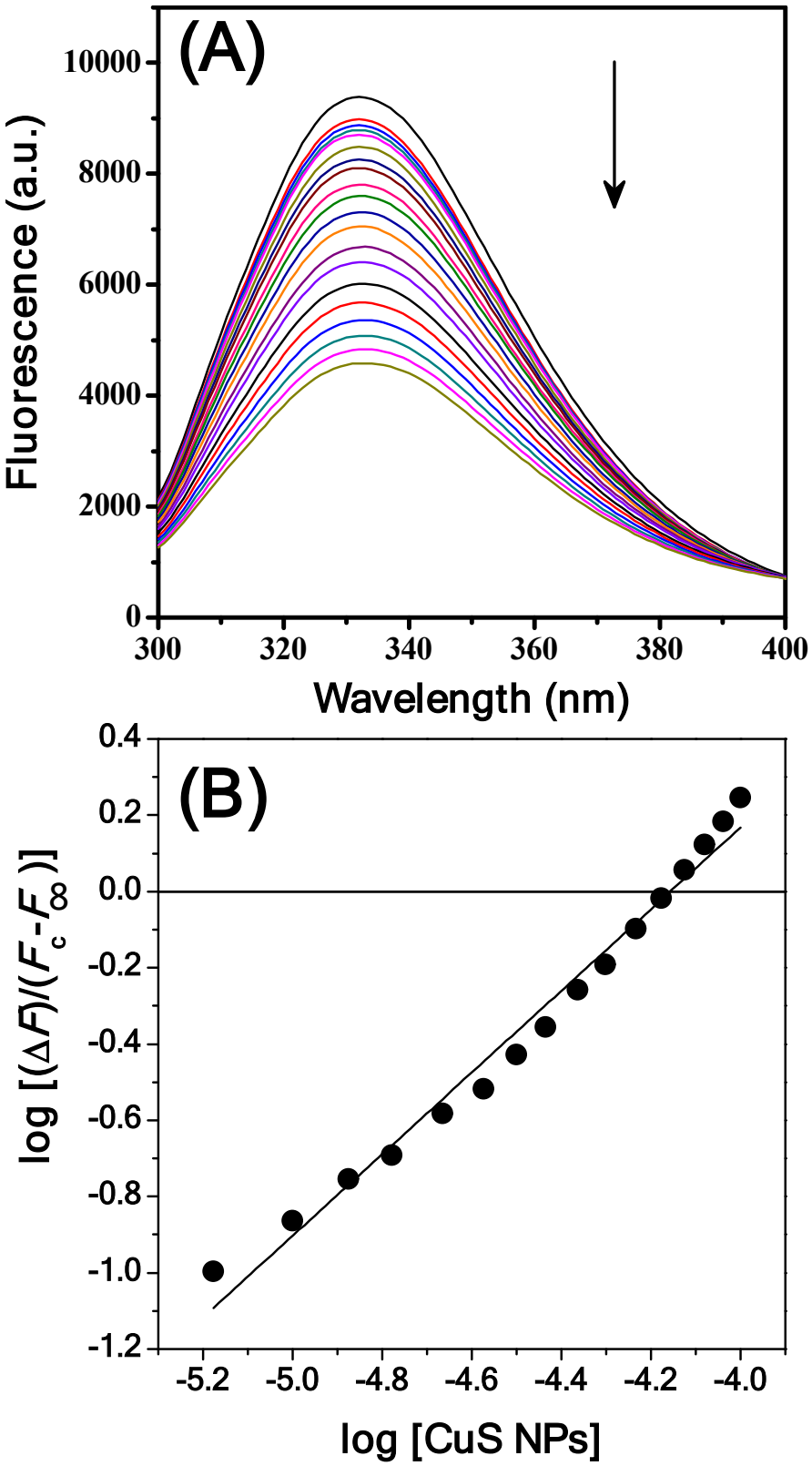


Figure S6: Interaction of jacalin with pCuS in the presence of 100 mM galactose. (A) Fluorescence titration shows the addition of pCuS to jacalin + 100 mM galactose quenches the intrinsic fluorescence, indicating binding. (B) Double logarithmic plot for the interaction pCuS with jacalin + 100 mM galactose.

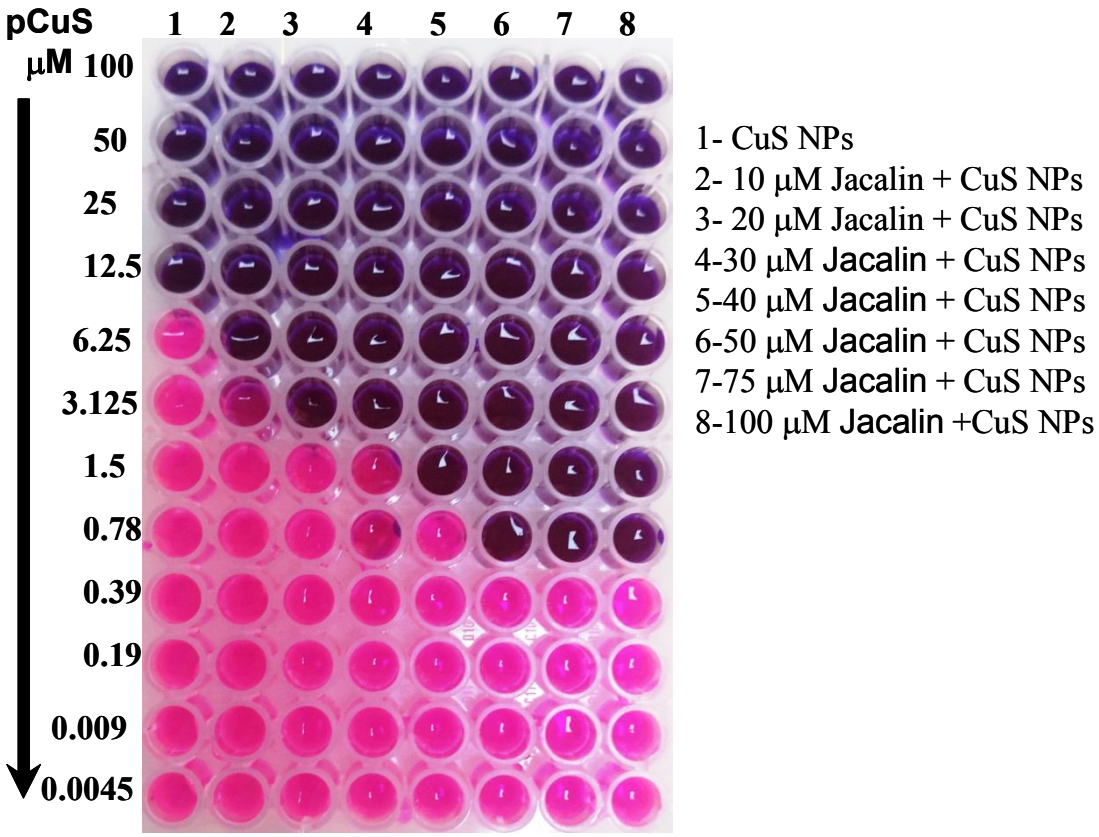


Figure S7: Effect of jacalin on the MIC of pCuS. *E. coli* cells were exposed to various concentrations of pCuS in the presence of fixed concentration of jacalin. The concentration of jacalin increases from Lane 2 to Lane 8. It is noted that the MIC of pCuS decreases significantly in the presence of jacalin. The lowest MIC (0.78 μM) was obtained from 50 μM of Jacalin. It is noted that above 50 μM Jacalin, MIC does not improved. Thus, 50 μM Jacalin was selected for the study.

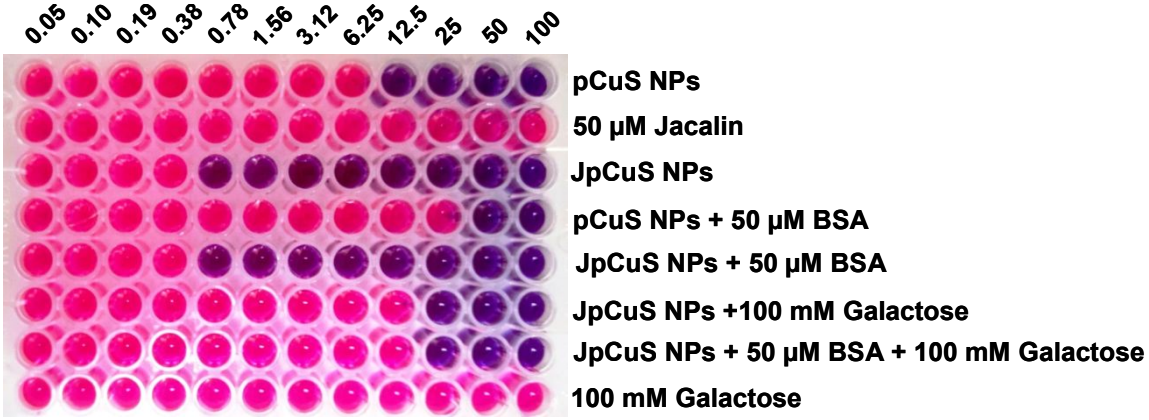


Figure S8: Representative REMA. *E. coli* cells were treated with various combinations of pCuS. It is noted that jacalin binding reduces the MIC from 12.5 µM to 0.78 µM. At the same time, BSA binding increases the MIC from 12.5 µM to 50 µM, which suggests interference from BSA in the antimicrobial activity of pCuS. However, addition of BSA to JpCuS shows no interference. Blocking the sugar binding site of jacalin with galactose hampered the antimicrobial activity of JpCuS. Noteworthy, jacalin and galactose does not show any antimicrobial activity.

Table S1: MIC determined at various combinations of pCuS. It is noted that the interference from BSA was diminished by functionalization of NPs with Jacalin. At the same time, blocking the sugar binding site of jacalin could hamper the activity.

bacteria	MIC (µM)							
	pCuS	Jacalin	JpCuS	pCuS + 50 µM BSA	JpCuS + 50 µM BSA	JpCuS + 100 mM Galactose	JpCuS + 100 mM Galactose + 50 µM BSA	100 mM Galactose
E. coli	12.5	ND	0.78	50	0.78	25	25	ND
P. aeruginosa	12.5	ND	0.78	50	0.78	25	25	ND
B. subtilis	12.5	ND	0.78	50	0.78	25	25	ND
S. aureus	12.5	ND	0.78	50	0.78	25	25	ND

ND – not determined

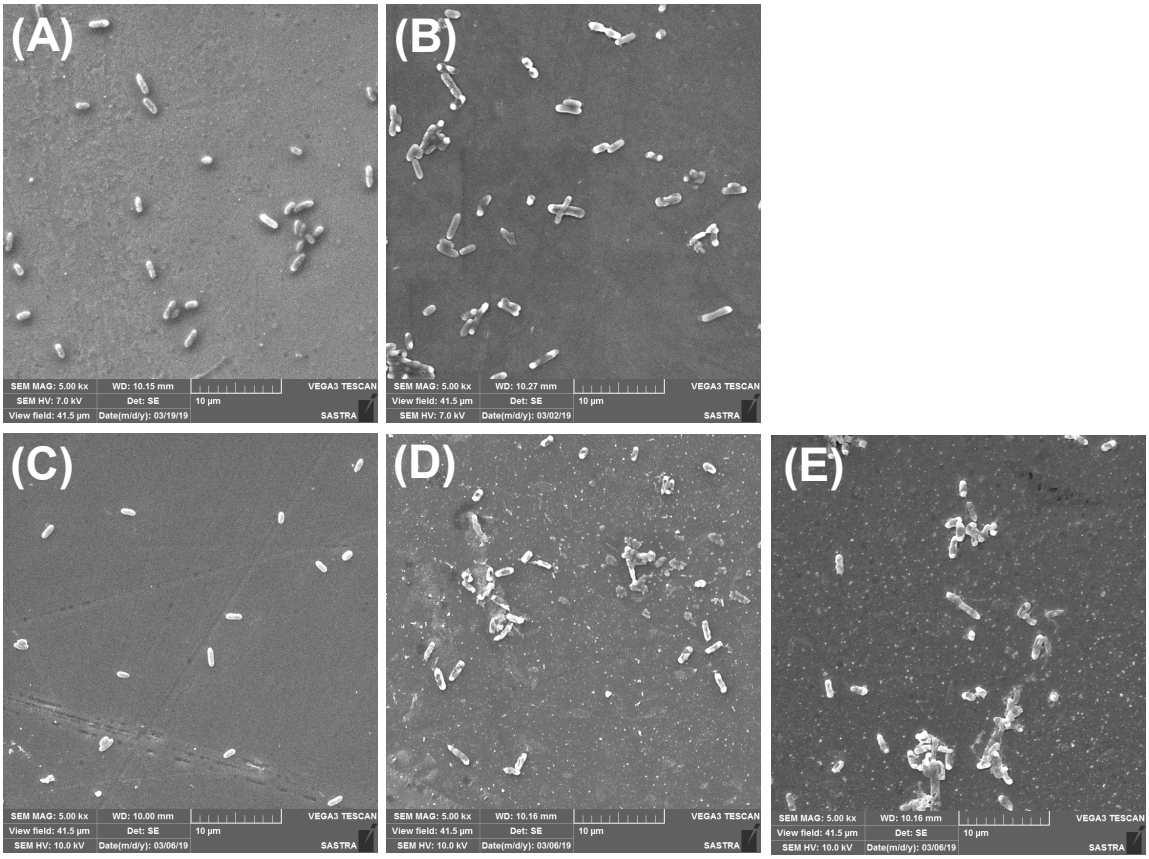


Figure S9: SEM study. (A) Untreated; (B) 12.5 µM pCuS; (C) 12.5 µM pCuS + 50 µM BSA; (D) 12.5 µM pCuS + 50 µM jacalin; and (E) 12.5 µM pCuS + 50 µM BSA + 50 µM jacalin. It is noted that untreated cells showed intact membrane and smooth surface. Also, cells treated with 12.5 µM pCuS in the presence of BSA showed intact membrane and smooth surface, indicating that the serum protein interfere with the antibacterial activity of the NPs. Cells treated with NPs (B), (D), and (E) showed damaged membrane and the cellular content also leaked. Noteworthy, 12.5 µM pCuS interact with jacalin showed membrane damaged cells (E) even in the presence of BSA.

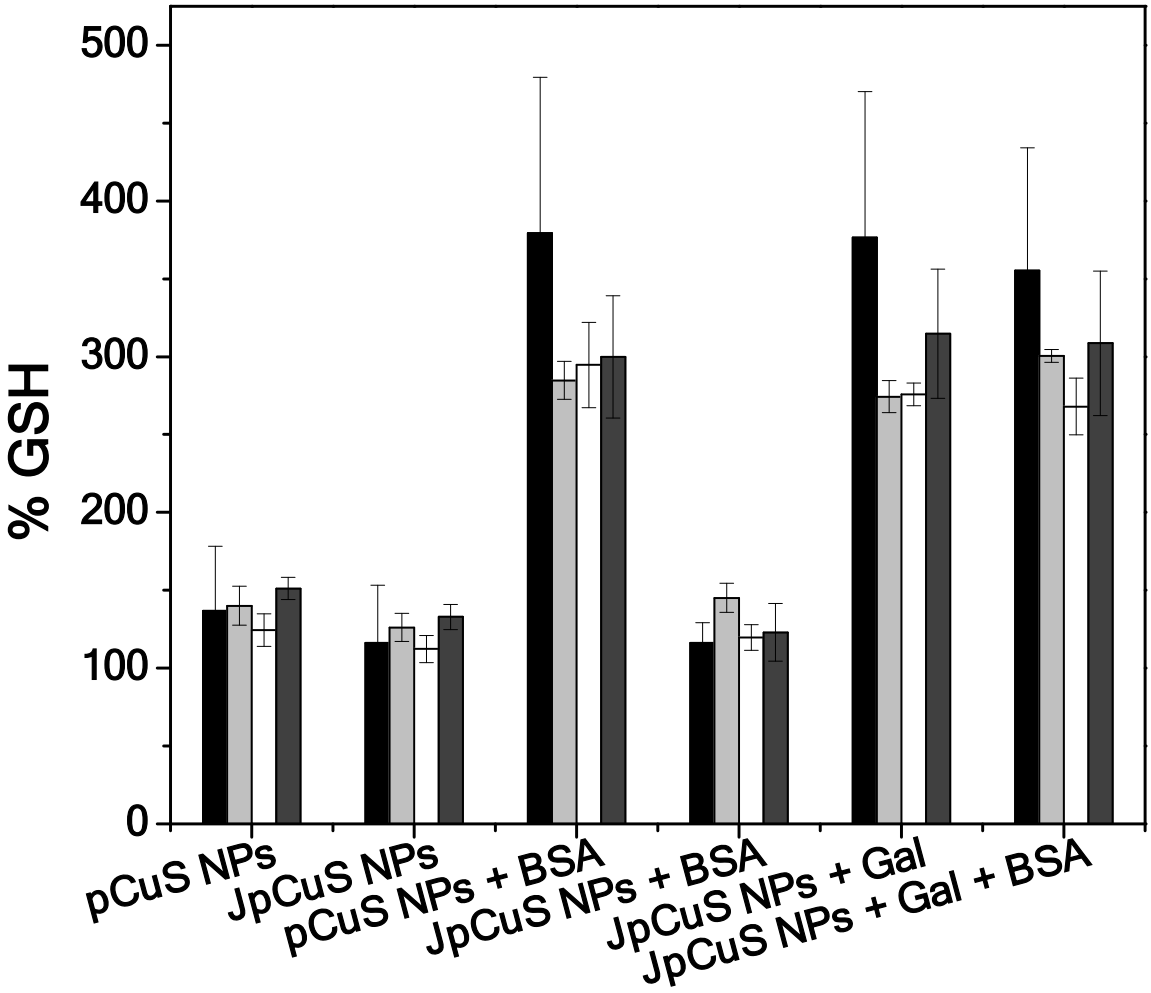


Figure S10: Effect of pCuS on GSH level. All test strains were treated with different condition of 12.5 µM pCuS for 2 h and the quantity of GSH was measured as described in material and methods. The results are expressed as the means ± SD of three separate experiments. It is noted that the amount of GSH depleted largely in the cells treated with pCuS and JpCuS as compared to the cells treated with BSA-pCuS, indicating that the JpCuS deplete the antioxidant GSH level.