

Aerobic synthesis of biocompatible copper nanoparticles: Promising antibacterial agent and catalyst for nitroaromatic reduction and C-N cross coupling reaction

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Supporting information

Materials

Copper chloride, ammonium hydroxide, hydrazine, aniline, bromobenzene and 3-chloroaniline were purchased from Merck, India. Pectin (from citrus fruit) was purchased from Alfa Aesar, India. Tyramine, dopamine, pyrrole and indole were obtained from Sigma, US. Luria broth (LB), Nutrient broth (NB), glycine, ofloxacin and kanamycin were purchased from Himedia chemicals India Pvt. Ltd (Mumbai, India). Millipore water with a resistivity more than 18.0 MΩ was used for preparation of all aqueous solutions. All the glasswares were cleaned with freshly prepared aqua regia (3:1, HCl: HNO₃) and rinsed thoroughly with water. Then they were dry sterilized using hot air oven at 160 °C for 3 hrs, prior to use.

Preparation of copper nanoparticles using biopolymer pectin

Aqueous pectin solution was prepared by dissolving 50 mg pectin in 50 ml of water in warm water bath (~ 60 °C) for about 30 min for complete solubilization and then

brought down to room temperature. To the biopolymeric solution, aqueous CuCl_2 (50 mM final concentration) were added slowly under vigorous stirring. The pH of the solution was adjusted to basic using 200 μL of concentrated ammonia solution. The reaction was allowed to proceed under gentle stirring at room temperature for 5 min. The color changes from greenish blue to blue due to the formation of copper-ammonia complex. After 5 min of gentle mixing, 200 μL of hydrazine hydrate was added as a mild reducing agent. After stirring the solution for 5 min, the reaction was allowed to proceed at room temperature for 5 hrs. The formation of copper nanoparticles was easily noticeable due to the change in the color of the solution to reddish brown – the characteristic color of the copper nanoparticles (CuNPs).

Characterization of nanoparticles

UV-Vis absorption spectroscopy was performed on Thermo Scientific Evolution 201 spectrophotometer operated at 1 nm resolution. The size and morphology were examined using high-resolution transmission electron microscopy (HR-TEM) (JEOL-JEM 1011, Japan) operating at an accelerated voltage of 200 kV. The samples were prepared by placing a drop of NP solution on a graphite grid and drying it in vacuum. X-ray diffraction measurements of CuNPs were done on a XRD-Bruker D8 Advance X-ray diffractometer using monochromatic $\text{Cu K}\alpha$ radiation.

Catalytic reduction of nitroaromatic compounds

Typically, 0.1 mM of nitroaromatic compounds and 50 mM of NaBH₄ were added into a quartz cuvette containing 3 mL water. Then 200 μL of an aqueous solution containing CuNPs (1 mM) was injected into the cuvette to start the reaction. The intensity of the absorption peak maximum in UV-Vis spectroscopy was used to monitor the process of the conversion of nitro compound to amino compound. The time dependent change in the absorption maximum was used for kinetic analysis. Since the concentration of NaBH₄ was much higher than that of nitro compounds, the reduction kinetics was described by first-order rate law. Therefore, the reaction kinetics was described as $\ln(C_t/C_0) = -kt$, where k is the apparent first-order rate constant, t is the reaction time. C_t and C_0 are the concentrations of substrate at time t and 0, respectively. The rate constant, k was obtained directly from the slope of the linear part of the kinetic trace.

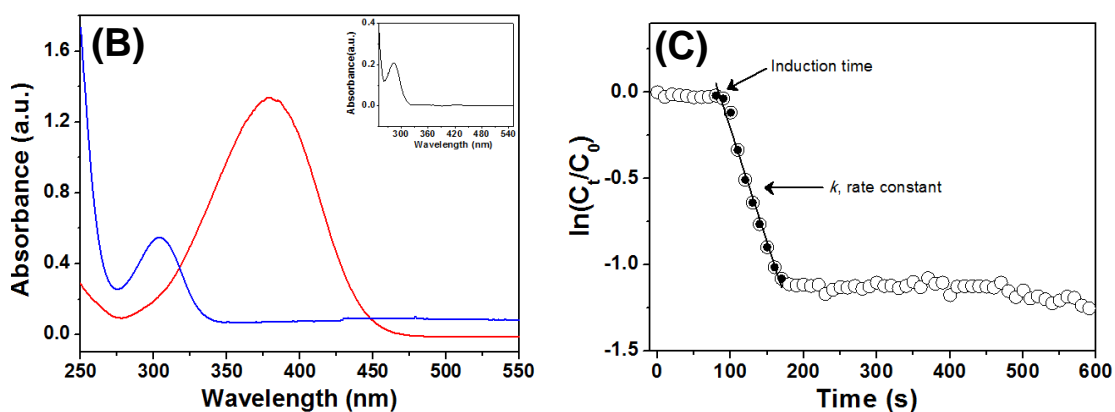
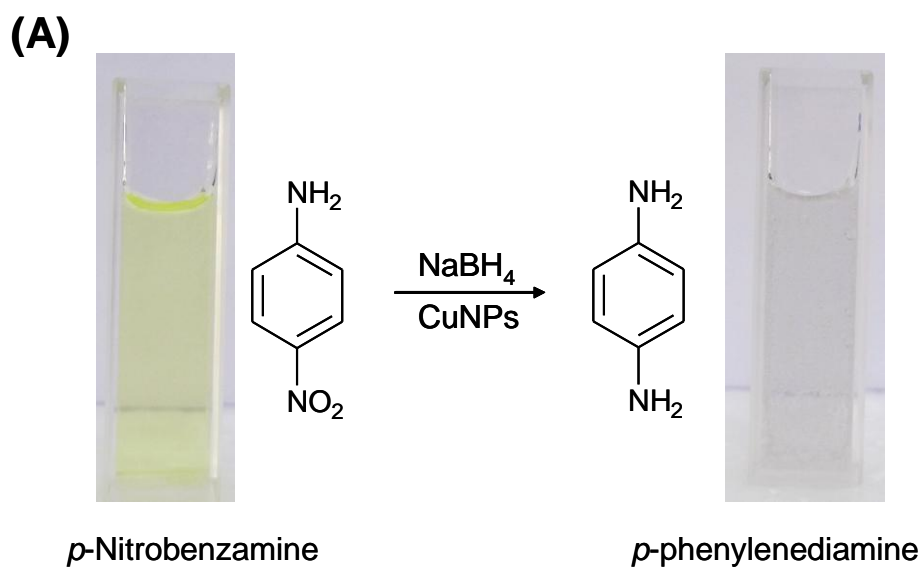


Figure S1: (A) Catalytic reduction of *p*-Nitrobenzamine to *p*-Phenylenediamine, (B) UV-Vis spectra of *p*-Nitrobenzamine (red) and the reduced product *p*-Phenylenediamine (blue). *Inset* corresponds to the standard *p*-Phenylenediamine. The disappearance of peak at 380 nm and appearance of new peak at 300 nm indicates the reduction is complete, (C) Plot of $\ln(C_t/C_0)$ versus reaction time for the reduction of *p*-Nitrobenzamine. C_t/C_0 is calculated based on the absorbance at 380 nm.

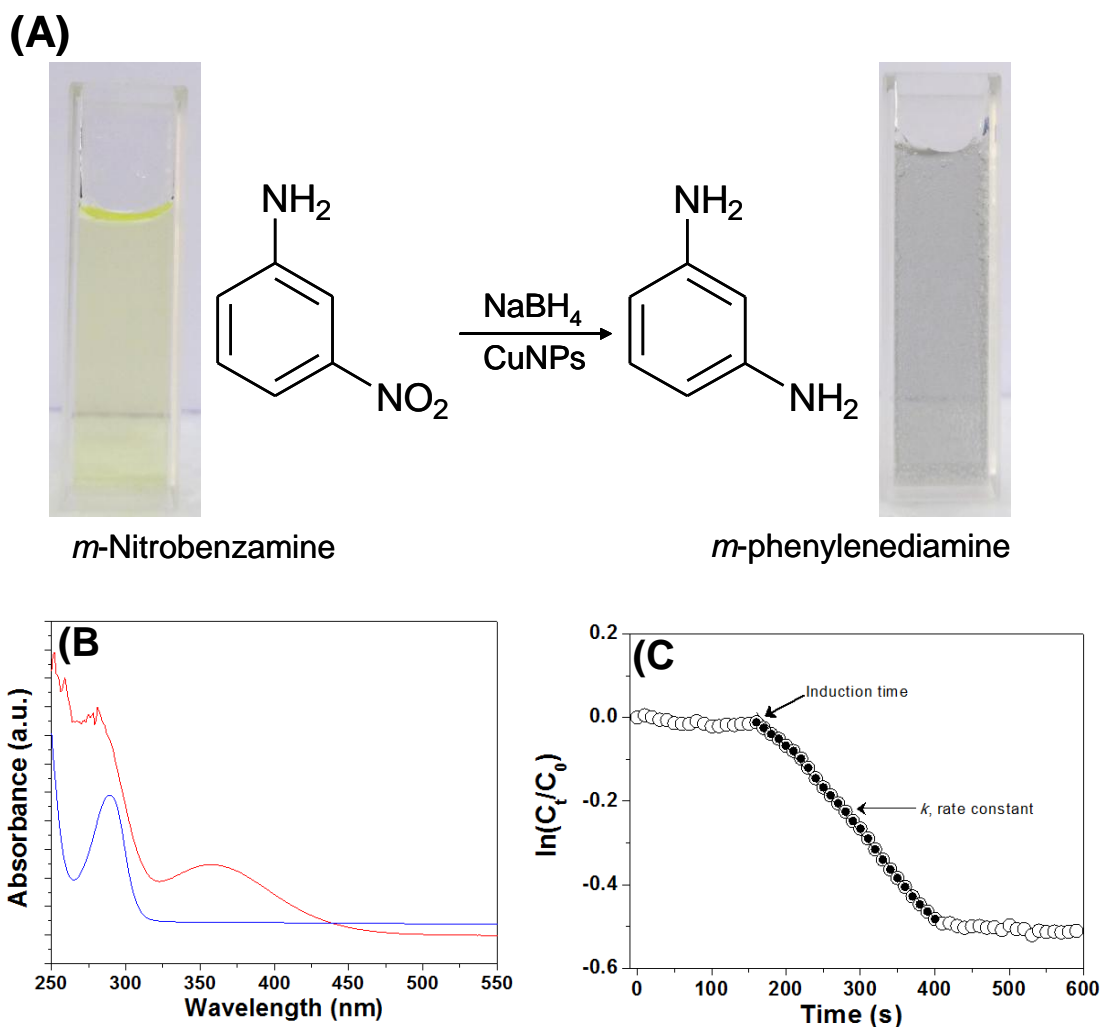


Figure S2: (A) Catalytic reduction of *m*-Nitrobenzamine to *m*-Phenylenediamine, (B) UV-Vis spectra of *m*-Nitrobenzamine (red) and the reduced product *m*-Phenylenediamine (blue). The disappearance of peak at 360 nm and appearance of new peak at 290 nm indicates the reduction is complete, (C) Plot of $\ln(C_t/C_0)$ versus reaction time for the reduction of *p*-Nitrobenzamine. C_t/C_0 is calculated based on the absorbance at 360 nm.

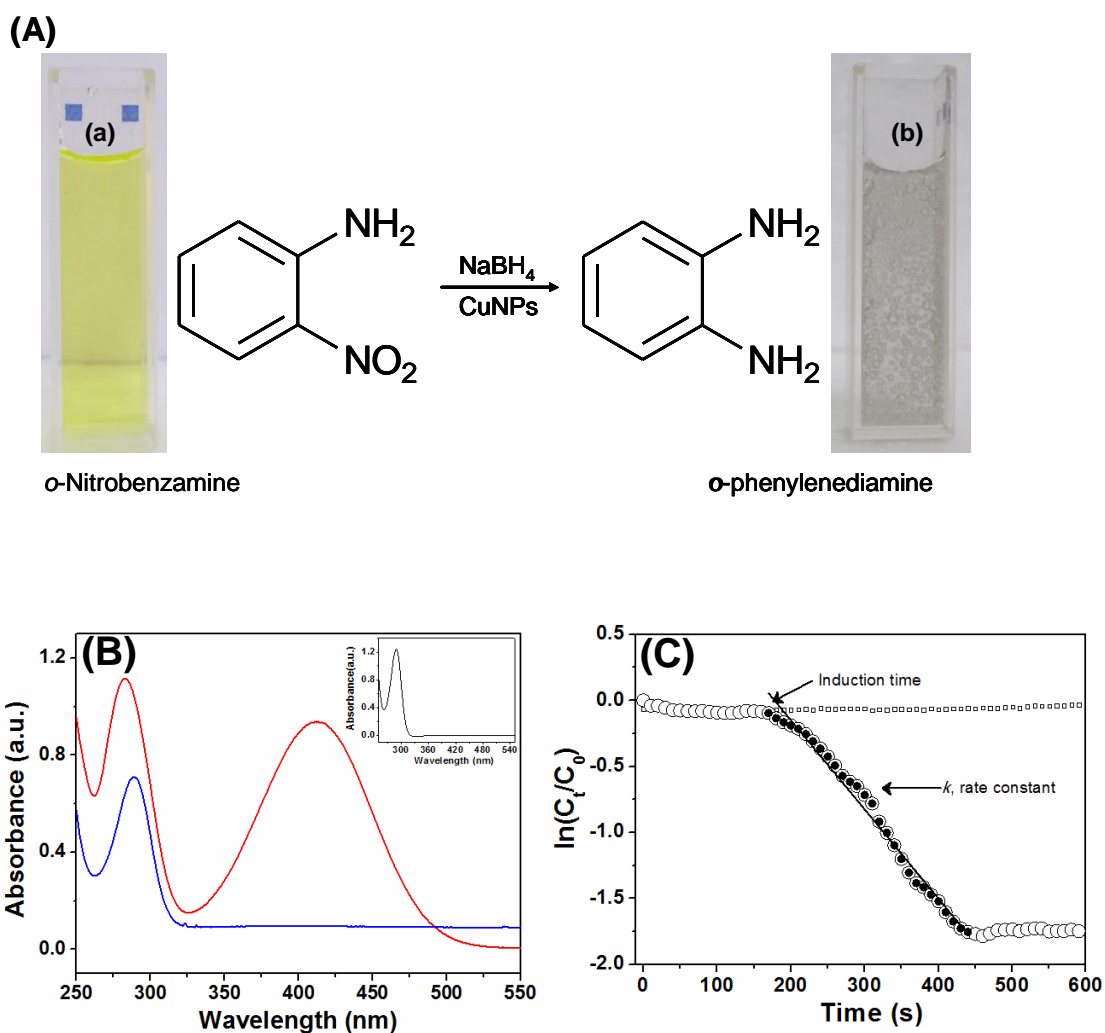


Figure S3: (A) Catalytic reduction of *o*-Nitrobenzamine to *o*-Phenylenediamine, (B) UV-Vis spectra of *o*-Nitrobenzamine (red) and the reduced product *o*-Phenylenediamine (blue). *Inset* corresponds to the standard *o*-Phenylenediamine. The disappearance of peak at 410 nm and appearance of new peak at 290 nm indicates the reduction is complete, (C) Plot of $\ln(C_t/C_0)$ versus reaction time for the reduction of *o*-Nitrobenzamine. C_t/C_0 is calculated based on the absorbance at 412 nm.

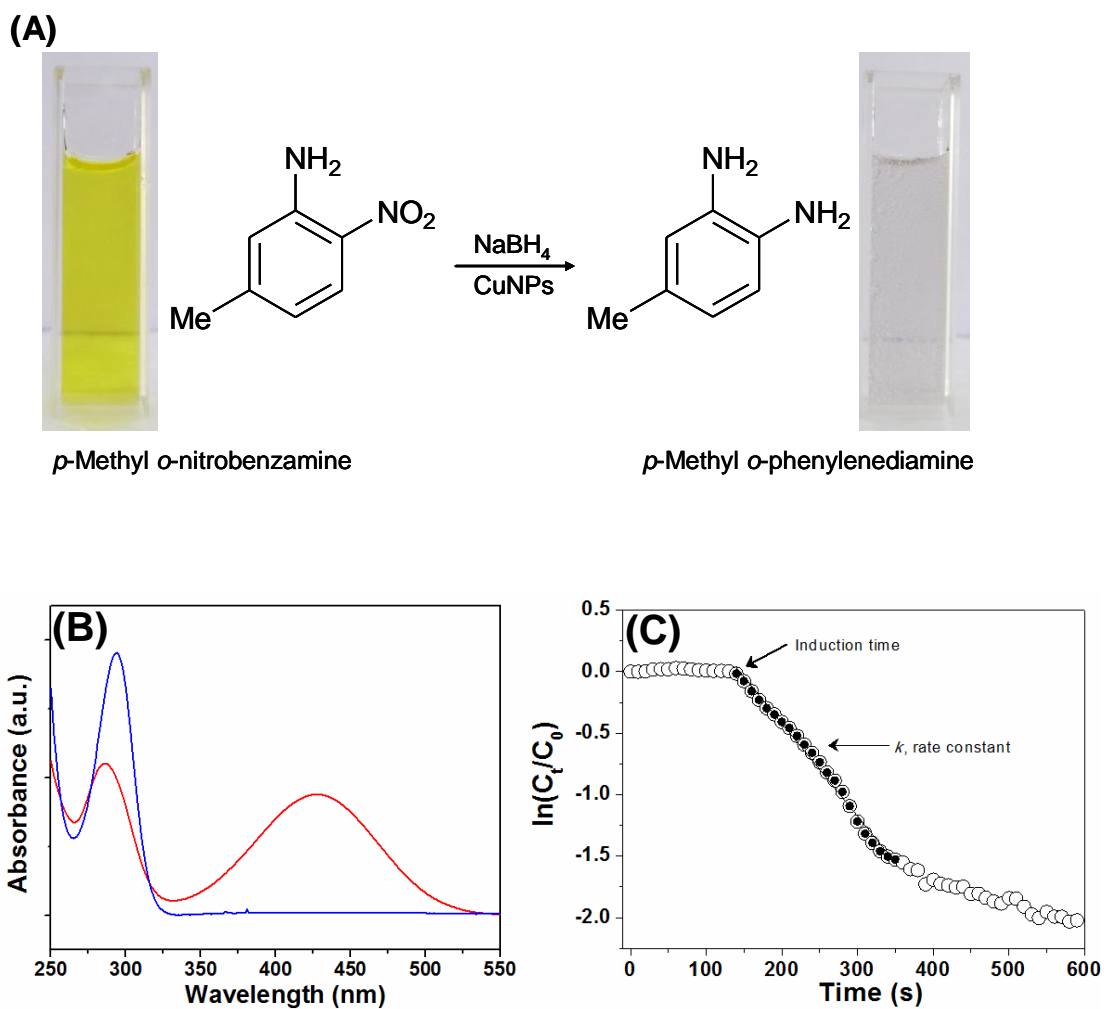
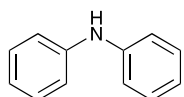


Figure S4: (A) Catalytic reduction of *p*-Methyl *o*-nitrobenzamine to *p*-Methyl *o*-phenylenediamine, (B) UV-Vis spectra of *p*-Methyl *o*-nitrobenzamine (red) and the reduced product *p*-Methyl *o*-phenylenediamine (blue). The disappearance of peak at 430 nm and appearance of new peak at 290 nm indicates the reduction is complete, (C) Plot of $\ln(C_t/C_0)$ versus reaction time for the reduction of *p*-Methyl *o*-nitrobenzamine. C_t/C_0 is calculated based on the absorbance at 430 nm.

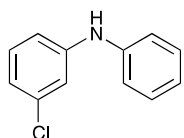
Copper nanoparticles catalyzed C-N cross coupling

Aryl bromide (1 mol %), amine (1 mol %), sodium hydroxide (1 mol %) and aqueous pectin stabilized CuNPs (2 mol %) were suspended in 5mL dimethylsulfoxide. The resulting mixture was heated at 110 °C under stirring until the reaction was complete as judged by TLC. The reaction mixture was diluted with ether, washed with brine and dried over magnesium sulfate. Evaporation of the solvent in vacuo gave a crude product which was further purified by silica gel flash chromatography. The purified products were confirmed by ¹H-NMR recorded with TMS as internal standard at 25°C on Bruker spectrometer 300 MHz. FTIR spectroscopy measurements were carried out on a Perkin Elmer spectrum-one instrument in the diffuse reflectance mode at a resolution of 1 cm⁻¹ in KBr pellet.



Diphenylamine (1a): white solid; Mp 50–52°C (lit. mp 51–53°C);

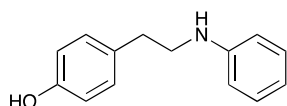
$R_f = 0.7$ (1:9 ethylacetate: hexane); IR (KBr) 3405, 3380, 3041, 1580, 1518, 1490, 1452, 1303 cm⁻¹; ¹H-NMR (300 MHz in CDCl₃) = 7.29 – 7.22 (m, 4H), 7.08 – 7.05 (m, 4H), 6.95 – 6.89 (m, 2H), 5.68 (br s, 1H, NH).



3-chloro-N-phenylaniline (2a): yellow solid Mp 61–63°C $R_f = 0.46$

(3:7 ethylacetate: hexane); IR (KBr) 3404, 3380, 3040, 1582, 1513, 1493, 1457, 1307 cm⁻¹; ¹H-NMR (300 MHz in CDCl₃) = 7.08 - 7.03 (m, 3H), 6.73 – 6.66 (m, 3H), 6.56 – 6.52 (m 3H), 3.72 (br, s, 1H).

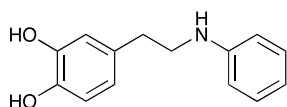
4-(2-(phenylamino)ethyl)phenol (3a): white solid Mp 50–



51°C; $R_f = 0.6$ (3:7 ethylacetate: hexane); IR (KBr) 3404, 3380, 3040, 1582, 1513, 1493, 1457, 1307 cm^{-1} ; $^1\text{H-NMR}$

(300 MHz in CDCl_3) = 6.96 (d, $J = 9$ Hz, 4H), 6.67 – 6.64 (m, 5H), 2.70 – 2.66 (m, 2H), 2.53 – 2.48 (m, 2H).

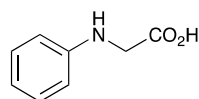
4-(2-(phenylamino)ethyl)benzene-1,2-diol (4a): Brown



solid Mp 65–67°C; $R_f = 0.6$ (3:7 ethylacetate: hexane); IR (KBr) 3401, 3370, 3035, 1581, 1533, 1483, 1450, 1302 cm^{-1} ;

$^1\text{H-NMR}$ (300 MHz in $(\text{CD}_3)_2\text{SO}$) = 8.88 (brs, 1H), 8.06 (brs, 1H), 7.59 – 7.55 (m, 3H), 7.39 – 7.32 (m, 2H), 6.70 – 6.63 (m, 2H), 6.49 – 6.46 (m, 1H), 2.91 (dd, $J = 15.6, 6.9$ Hz, 2H), 2.70 (dd, $J = 15.9, 8.7$ Hz, 2H).

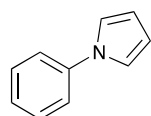
2-(phenylamino)acetic acid (5a): viscous oil; $R_f = 0.43$ (3:7



ethylacetate: hexane); IR (KBr) 3409, 3385, 3042, 1698, 1535, 1492, 1451, 1305 cm^{-1} ; $^1\text{H-NMR}$ (300 MHz in $(\text{CD}_3)_2\text{SO}$) = 7.59 –

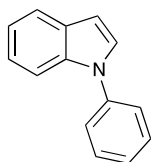
7.56 (m, 2H), 7.41 – 7.32 (m, 3H), 4.00 (brs, 2H).

1-phenyl-1H-pyrrole (6a): yellow solid Mp 52–54°C; $R_f = 0.16$ (1:9



ethylacetate: hexane); IR (KBr) 2886, 1582, 1513, 1493, 1457, 1307 cm^{-1} ; $^1\text{H-NMR}$ (300 MHz in CDCl_3) = 7.49 – 7.46 (m, 2H), 7.30 – 7.18

(m, 3H), 6.78 (q, $J = 2$ Hz, 2H), 6.25 (q, $J = 2$ Hz, 2H).



1-phenyl-1*H*-indole (7a): yellow solid Mp 60–62°C $R_f = 0.46$ (3:7 ethylacetate: hexane); IR (KBr) 3043, 1563, 1510, 1480, 1444, 1323 cm^{-1} ; $^1\text{H-NMR}$ (300 MHz in CDCl_3) = 7.67 – 7.64 (m, 2H), 7.42 – 7.39 (m, 2H), 7.26 – 7.10 (m, 5H), 6.57 – 6.56 (m, 2H).

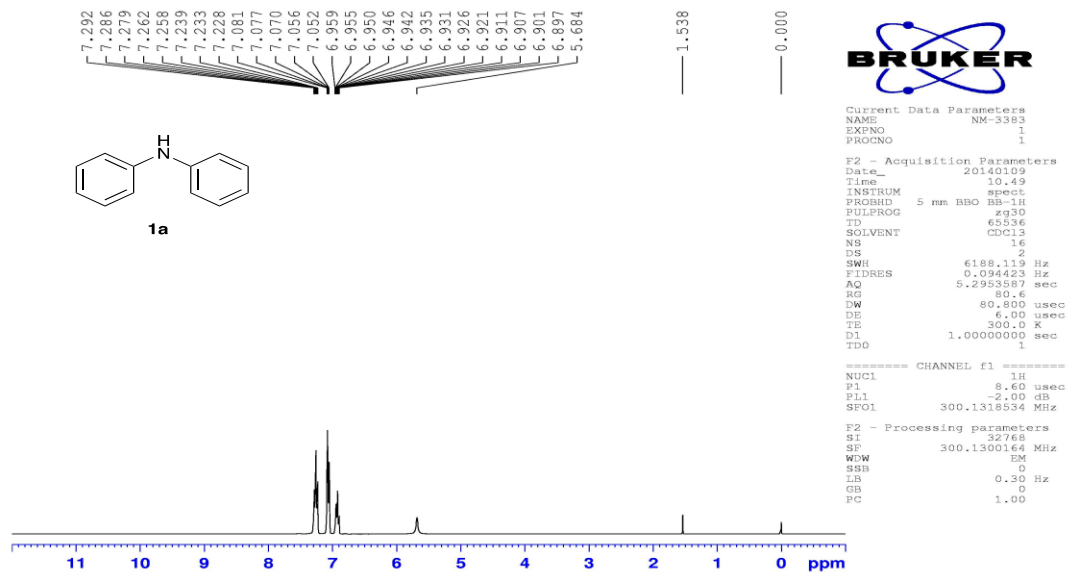


Figure S5: ^1H -NMR spectra of diphenylamine (1a) in CDCl_3

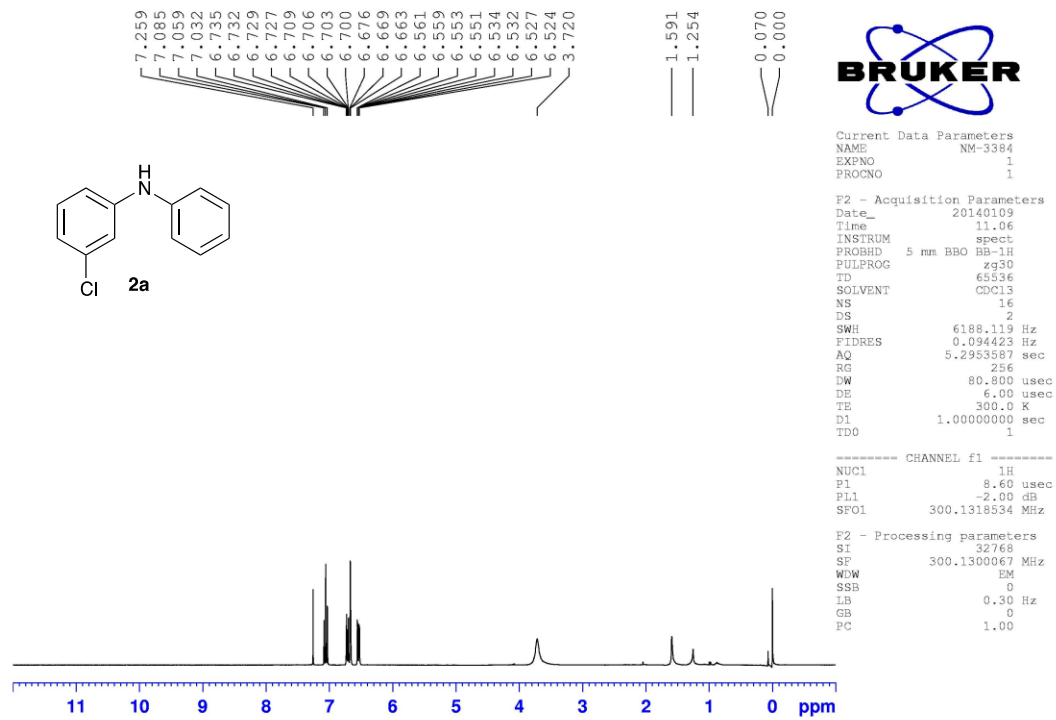


Figure S6: ¹H-NMR spectra of 3-chloro-N-phenylaniline (2a) in CDCl₃

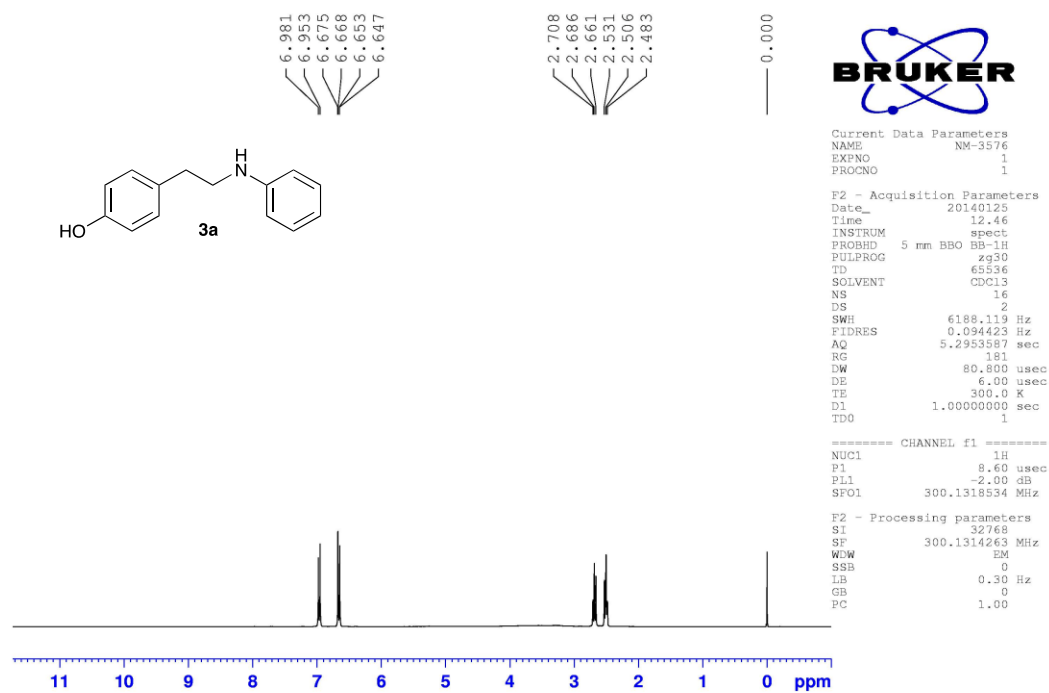


Figure S7: ¹H-NMR spectra of 4-(2-(phenylamino)ethyl)phenol (3a) in CDCl₃

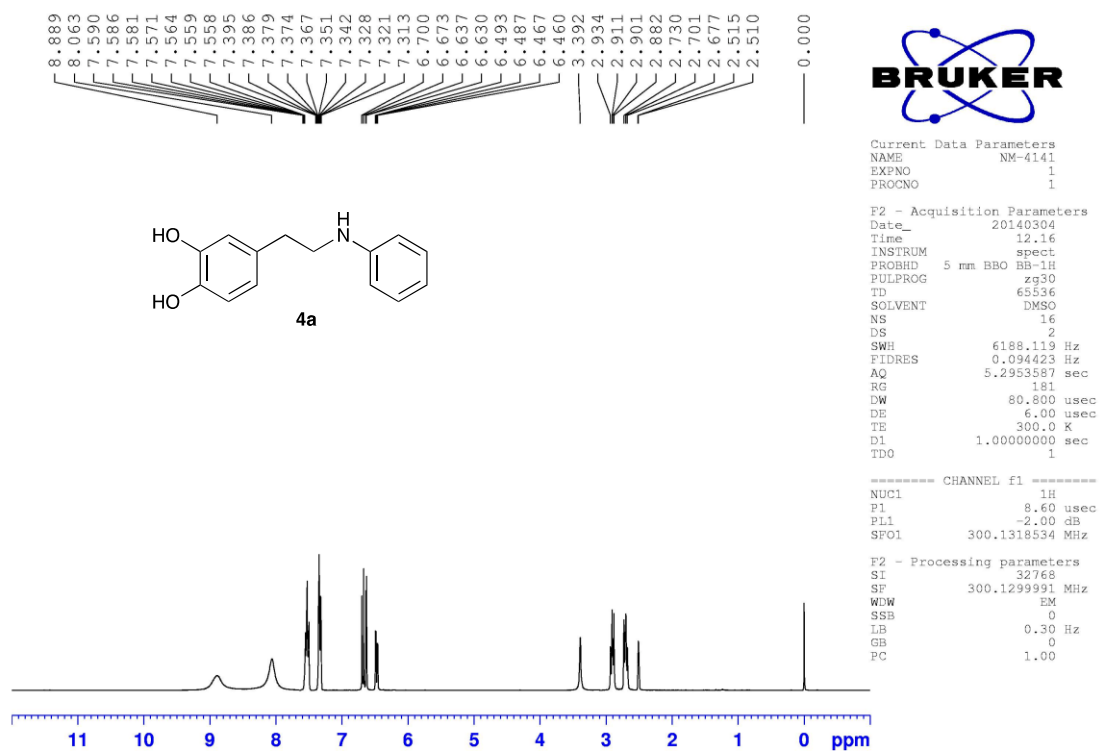


Figure S8: ^1H -NMR spectra of 4-(2-(phenylamino)ethyl)benzene-1,2-diol (4a) in $(\text{CD}_3)_2\text{SO}$.

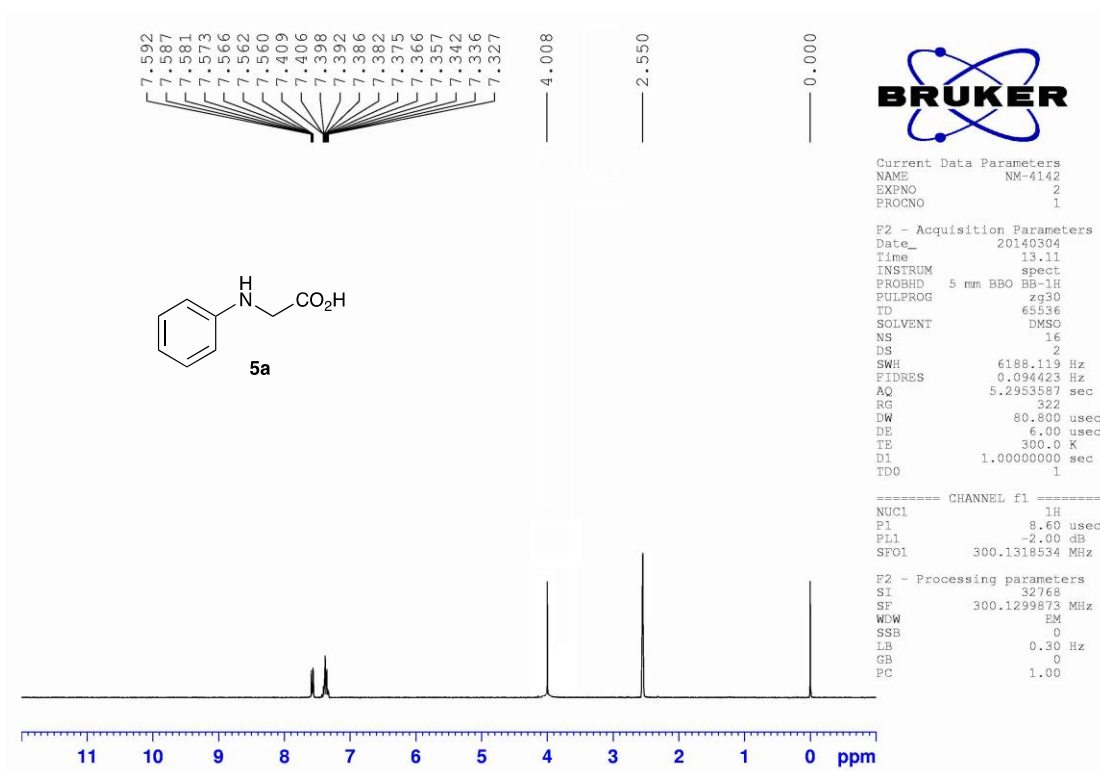


Figure S9: ^1H -NMR spectra of 2-(phenylamino)acetic acid (5a) in $(\text{CD}_3)_2\text{SO}$.

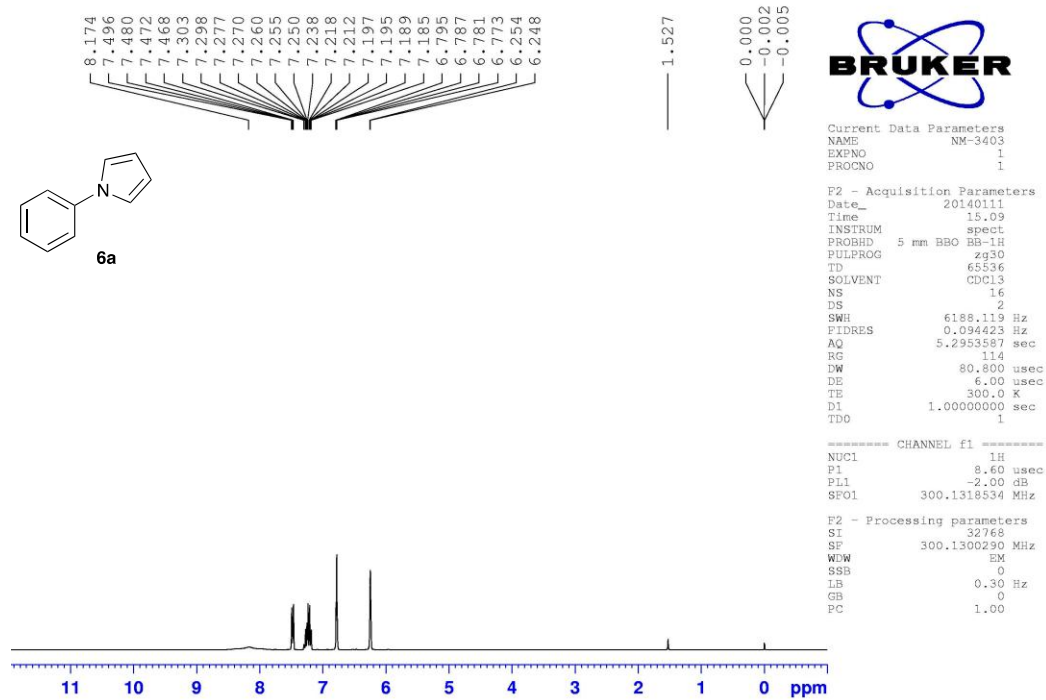


Figure S10: ¹H-NMR spectra of 1-phenyl-1*H*-pyrrole (6a) in CDCl₃.

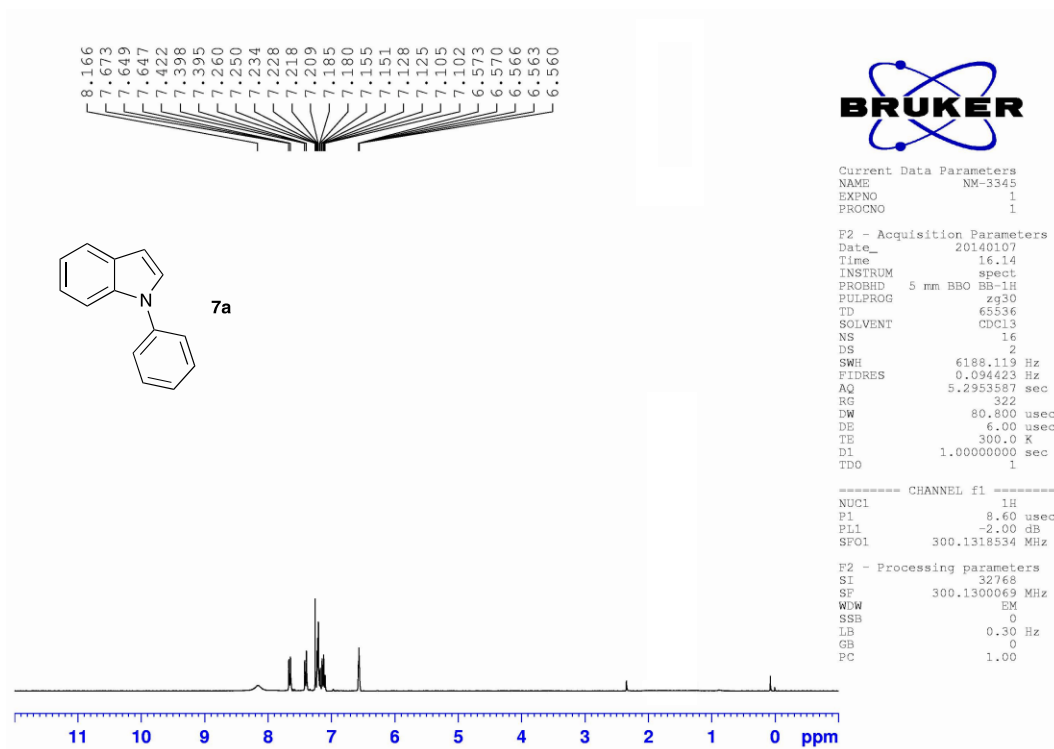


Figure S11: ^1H -NMR spectra of 1-phenyl-1*H*-indole (**7a**) in CDCl_3 .

Antibacterial activity

The antibacterial activity of the pectin stabilized CuNPs were evaluated by zone of inhibition using disc diffusion method. *Escherichia coli* (MTCC 1655), *Bacillus thuringiensis* (MTCC 869), *Klebsiella pneumoniae* (MTCC 109), *Proteus mirabilis* (MTCC 425), *Pseudomonas aeruginosa* (MTCC 1688), *Salmonella typhimurium* (MTCC 98), *Shigella flexneri* (MTCC 1457) and *Staphylococcus aureus* (MTCC 3160) were used in this study. The paper disc (Whatman No.1) was cut downed into small disc (4mm diameter) and sterilized in an autoclave. Approximately 20 ml of molten Nutrient agar was poured in sterilized Petri plates and plates were left overnight at room temperature. Meanwhile, the sterilized discs were impregnated with the aqueous solution of CuNPs (20 µg) and positive control drug. Then the disc was dried in sterile condition. The pure cultures of bacteria were subcultured on nutrient agar medium. Inoculum (10^5 cfu/mL) of each strain was swabbed uniformly over the individual plates using sterile cotton swabs. The CuNPs impregnated discs were placed on the plates and incubated at 37°C for 24 h. After incubation, the different levels of zone of inhibition of bacteria were measured. Ofloxacin and kanamycin was used as control antibacterial drugs. The diameter of the inhibition zones was measured in mm and the mean values were presented.

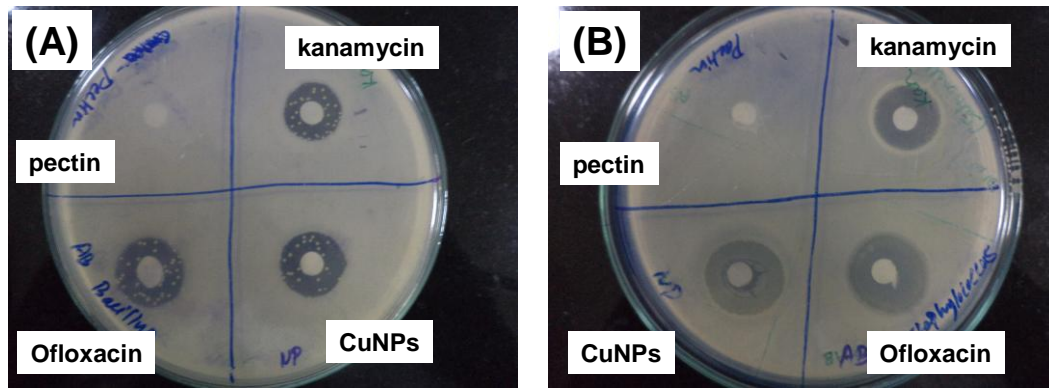


Figure S12: Antibacterial activity of CuNPs against Gram positive bacteria (A) *Bacillus Thuringiensis* and (B) *Staphylococcus aureus*. The visible zone around the disk is the indication of no bacterial growth. Ofloxacin and kanamycin, are standard antibiotics used as positive control.

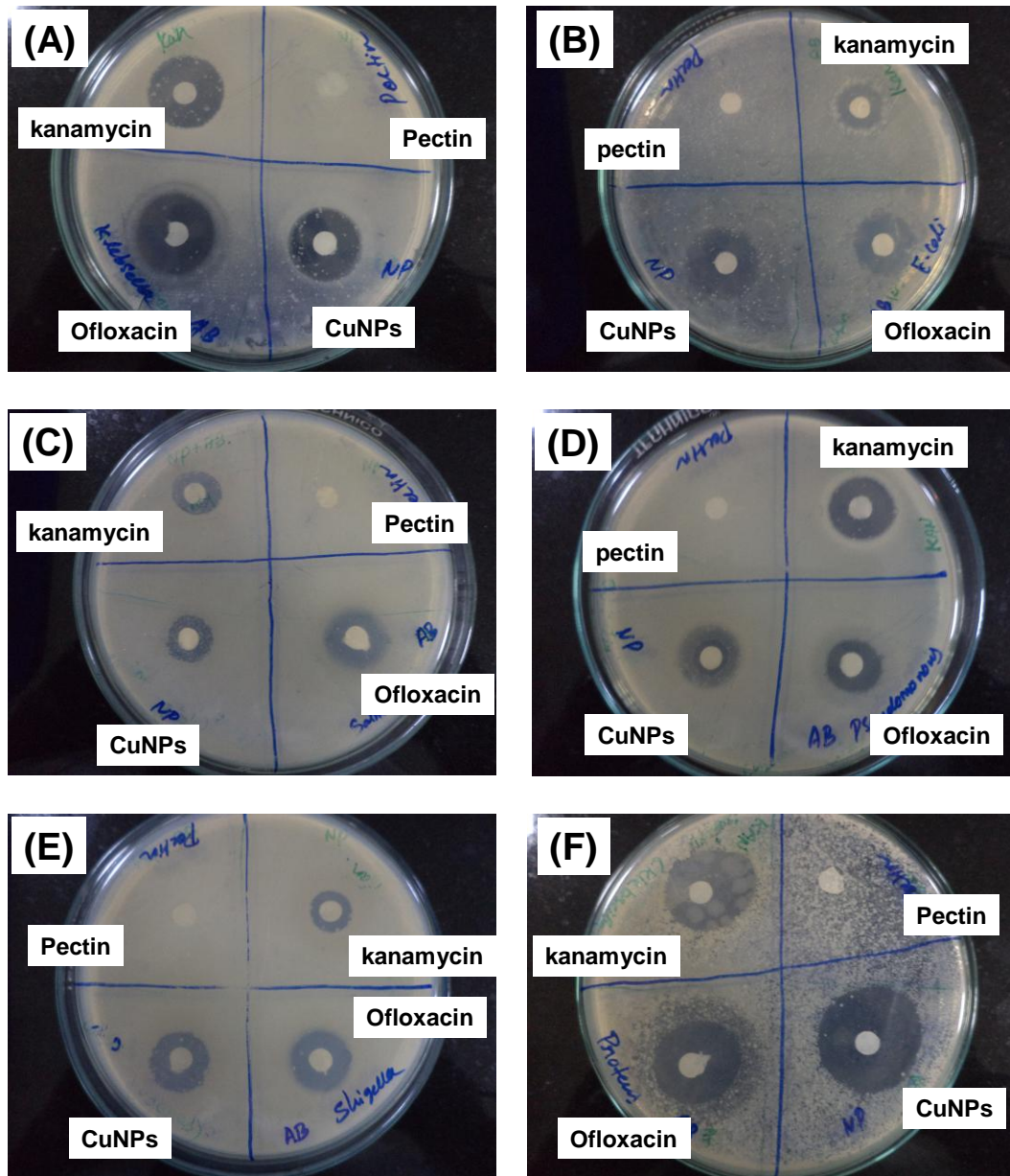


Figure S13: Antibacterial activity of CuNPs against Gram negative bacteria (A) *Klebsiella pneumonia* (B) *Escherichia coli* (C) *Salmonella typhi* (D) *Pseudomonas aeruginosa* (E) *Shigella flexneri* and (F) *Proteus mirabilis*. The visible zone around the disk is the indication of no bacterial growth. Ofloxacin and kanamycin are standard antibiotics used as positive control.