ABSTRACT

Nanotechnology is the emerging field due to its extensive range of applications in different areas of science and technology. Different methods are used for the synthesis of nanoparticles (NPs) due to their extensive applications. Biogenic synthesis of NPs is more eco-friendly and cost-effective approach as compared to the conventional chemical synthesis. The current study aims to synthesize biogenically silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs) using bacterial isolates. Four bacterial strains *Escherichia coli* (MT448673), *Pseudomonas aeruginosa* (MN900691), *Bacillus subtilis* (MN900684) and *Bacillus licheniformis* (MN900686) were used for the synthesis of AgNPs from silver nitrate ($AgNO_3$) solution and AuNPs from gold ($AuCl_4$) solution. Antibiotic susceptibility test was performed to check the antibiotic susceptibility pattern of selected bacterial strains. It was observed that *P. aeruginosa* was highly resistant against all tested antibiotics ampicillin; 50 μgmL^{-1}, rifampicin; 50 μgmL^{-1}, erythromycin; 20 μgmL^{-1} and lincomycin; 50 μgmL^{-1}. Among tested four isolates, rifampicin was found to be highly effective against *B. subtilis* and *B. licheniformis* (Gram positive) and was used as positive control in subsequent study. As first step, the biogenic synthesis of AgNPs and AuNPs was confirmed by color change from pale yellow to brown and from yellow to dark purple, respectively. Next, the characterization of biogenically synthesized AgNPs and AuNPs was performed using UV-visible spectroscopy, fourier transform-infrared (FTIR) spectroscopy, scanning electron microscopy (SEM) and X-ray diffraction (XRD). *E. coli, P. aeruginosa, B. subtilis* and *B. licheniformis* based AgNPs showed peaks at 432, 430, 426 and 414 nm, respectively through UV-visible spectroscopy analysis. UV-visible spectroscopy of *E. coli, P. aeruginosa, B. subtilis* and *B. licheniformis* based AuNPs showed peaks at 540, 533, 543 and 549 nm, respectively. FTIR spectroscopy was performed to check the influence of biological molecules responsible for the reduction of $AgNO_3$ and $AuCl_4$, stabilization and capping of AgNPs and AuNPs, as well as to determine the functional groups of the AgNPs and AuNPs. FTIR of *P. aeruginosa* AgNPs showed bands at 3261, 3061, 2947, 2358, 1338, 1394, 1363.43, 1388.5, 1641.13 and 1650.77 cm^{-1} corresponding to the

stretching vibrations of O-H, N-H, C-H , C-N, C-O, C-H and C = O groups, respectively. *B. licheniformis* based AgNPs showed bands at 3550, 3062, 2945, 2358, 1337, 1391, 1683, 1508,1558, 1456 and 1411 cm^{-1} corresponding to the stretching vibrations of O-H, N-H, C-H, C-N, C-O, C-H and C = O groups, respectively. FTIR of *B. subtilis* based AgNPs showed peaks at 3368, 2936, 1738, 1651, 1532, 1460, 1352, 1058 and 615 cm^{-1}, presenting the H-C-H asymmetric and symmetric stretch N-H band and C-O stretch, respectively. *E. coli* based FTIR showed absorption bands at 3417, 2922, 2856, 1740, 1630, 1440 and 1045 cm^{-1} indicating the presence of capping agents with the NPs. The bands at 3417, 2922 and 2856 cm^{-1} corresponded to O-H and C-H stretching. FTIR spectrum of *P. aeruginosa* AuNPs showed peaks at 3418, 2928, 1637, 1404,1116 and 755 cm^{-1}, which were assigned to O-H stretching vibration and C-H band. FTIR analysis of *B. licheniformis* AuN Psshowed peaks at 1039, 1080, 1242, 1417, 1539, 1636, 2366, 2924, 3364 and 3704 cm^{-1}, corresponding to O-H, amide I and amide II stretching respectively. *B. subtilis* based AuNPs showed absorption peaks at 1040, 1079, 1240, 1420, 1542, 1866, 2365, 2924 and 3419 cm^{-1}. The peaks showed intermolecular H-bondand stretching peaks of amide I and II groups of proteins. In *E. coli* AuNPs, the peak at 3436 cm^{-1} was due to intermolecular H-bond. The peaks 1700, 1600, 1766 and 1638 cm^{-1}, presented amide (I/II) region and C=O and C =C stretching vibrations, respectively.

Air-dried AgNPs and AuNPs were coated for the analysis of their size and morphology by examining them using SEM. *P. aeruginosa* AgNPs were mostly spherical in shape with size ranging from 32-46 nm. SEM analysis of *B. licheniformis* based NPs were also spherical with size ranging from 28-48 nm. *B. subtilis* based AgNPs analysis showed spherical AgNPs having aggregations with size ranging from 10-19 nm in diameter. *E. coli* based AgNPs showed NPs spherical shape with size ranging from 20-52 nm. *P. aeruginosa* based AuNPs showed spherical, uniform aggregates with size variations from 50- 65 nm. *B. licheniformis* based AuNPs results clearly showed the uniform spherical shape AuNPs with size around 40–70 nm. *B. subtilis* based AuNPs were mono and polydispersed form having size in between 23 and 68 nm. SEM analysis of *E. coli* based AuNPs showed the size variation from 30-60 nm. Surface morphology of AuNPs showed NPs adherence to the surface in a scaly pattern.

X-ray diffraction (XRD) was used to analyze the structure of crystalline nature of NPs. XRD pattern of the *P. aeruginosa* AgNPs showed diffraction peaks at about 34.4°, 46.8°, 62.0° and 79.1°. *B. licheniformis* based AgNPs showed unique diffraction peaks at about 37°, 44.8°, 64.9° and 77.1°. XRD analysis of *B. subtilis* AgNPs showed four peaks at 38.1°, 44.2°, 64.1° and 77.7° respectively. XRD pattern of *E. coli* based AgNPs showed diffraction peaks at 44.3°, 46.9°, 64.7° and 77°. *P. aeruginosa* based AuNPs showed peaks at 38.28°, 44.27°, 64.52° and 77.79°. XRD profile of AuNPs by *B. licheniformis* showed peaks at 38.18°, 44.28°, 64.9° and 77.76°. Three peaks were indexed *i.e.* (111), (200) and (220) from *B. subtilis* and *E. coli* based AuNPs which were assigned to the planes of face-centered cubic structure. The observed changes in positions of diffraction peaks were used to relate crystal structure changes with the change in nanoparticles size and shape.Following characterization, the biogenically synthesized NPs were used to check the antibacterial activity against pathogenic *E. coli*, *P. aeruginosa*, *B. subtilis* and *B. licheniformis* strains. Among all biogenic AgNPs, *P. aeruginosa* AgNPs showed significantly ($p \leq 0.05$) higher antibacterial potential with zone of inhibition (ZOI) ranging from 19.0 ± 0.57 to 22.5 ± 0.28 mm. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of biogenic AgNPs again showed that *P. aeruginosa* based AgNPs showed significantly ($p \leq 0.05$) less MIC (4.3 ± 0.6 μgmL^{-1}) and MBC (5.6 ± 0.3μgmL^{-1}) values against *B. subtilis* strain. Overall, lowest MIC and MBC of *P. aeruginosa* based AgNPs was observed against all tested human pathogenic bacteria, indicating that *P. aeruginose* based AgNPs has high potential to adhere to the cell wall and penetrate into the bacteria cell easily, which in turn improves their antimicrobial activity against bacteria. Likewise, biogenically synthesized AuNPs were also tested for antibacterial potential against four human pathogenic isolates using agar well diffusion assay. Again, *P. aeruginosa* AuNPs showed higher efficacy with significantly ($p \leq 0.05$) bigger ZOI (22.0 ± 1.0 to 31.0 ± 1.0 mm) against all tested isolates. MIC and MBC of biogenic AuNPs showed that *P. aeruginosa* based AuNPs showed less MIC (4.7 ± 0.3 μgmL^{-1}) and MBC (5.4 ± 0.3 μgmL^{-1}) values against *B. subtilis* strain, hence it is concluded that *P. aeruginosa* based AuNPs releases gold ions and electrostatic attraction of positive

charged gold and negative charged cell surface of microorganism increases the antimicrobial potential of AuNPs.The biofilm time kinetics of four bacterial isolates (*P. aeruginosa*, *E. coli*, *B. licheniformis* and *B. subtilis*) was analyzed by incubating bacterial cultures at 37°C in test tubes over a period of different time intervals *i.e.,* 2, 3, 5 and 7 days following crystal violet staining method. All the four strains had ability to form strong biofilms between 48 to 72 hours of incubation. Two strains (*B. subtilis* and *B. licheniformis*) formed significant ($p < 0.05$) biofilm after 3 days of incubation period. The other two strains (*E. coli* and *P. aeruginosa*) showed strong biofilm formation after 2 days of incubation. Next, the antibiofilm activity of biogenically synthesized AgNPs and AuNPs (10 - 100 μgmL^{-1}) was analyzed against biofilm forming human pathogenic bacteria like *P. aeruginosa*, *B. licheniformis*, *E. coli* and *B. subtilis*. Findings of the work revealed that 60-90% inhibition was observed at 60 μgmL^{-1} of AgNPs and AuNPs, while maximum inhibition (*i.e.*100%) was found at highest concentration (90 μgmL^{-1}). It was evident that highly significant ($p < 0.05$) decrease in biofilm formation was observed with increasing concentration of AgNPs and AuNPs.Followed by antibiofilm activity, biogenically synthesized AgNPs and AuNPs were used for *in vivo* toxicity evaluation. The toxicity assessment of AgNPs and AuNPs was performed using albino male mice. Log-probit regression analysis was used to measure the dosage response to determine the median lethal dose (LD_{50}). The mice were administered 200, 400, 600, 800 and 1000 $mgkg^{-1}$ (per body weight) biogenic AgNPs and AuNPs, orally. The LD_{50} value was determined to be 670 and 770 $mgkg^{-1}$ for AgNPs and AuNPs, respectively. It was also observed that higher value of LD_{50} makes the NPs less toxic to the environment as well as to the living tissues.Histopathological studies of liver and kidney after exposure to biogenically synthesized AgNPs and AuNPs showed more toxicity at higher concentration (dose), *i.e.,* 150 $mgkg^{-1}$ compared to the groups administered with 50 and 100 $mgkg^{-1}$ concentrations. Male albino mice administered with 50 and 100 $mgkg^{-1}$ AgNPs and AuNPs showed normal hepatocellular architecture. At lower concentration, normal renal structure with normal architecture of glomeruli (G) and renal tubules was observed. However, at higher concentration (150 $mgkg^{-1}$) kidneys showed smooth

surface and dark red color with proliferation of podocytes. AgNPs and AuNPs treatments did not produce significant changes regarding mortality, body weight even at 150 $mgkg^{-1}$.

The toxic effects of biogenic AgNPs and AuNPs were analyzed in liver and kidney of male albino mice by measuring serum concentration of liver and renal enzymes. The levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatinine of the experimental male albino mice were measured and compared with control mice. AST level of untreated control groups varied from 155 to 159 U/L. There were no significant differences ($p < 0.05$) in serum AST levels between the control and the treatment groups at 50 $mgkg^{-1}$ concentration after 30 days treatment period. However, there was significant increase ($p > 0.05$) in AST levels at 100 and 150 $mgkg^{-1}$ concentrations. On the other hand, no significant difference ($p < 0.05$) in serum ALT and creatinine levels of untreated and treated groups at 50, 100 and 150 $mgkg^{-1}$ concentration was observed after 30 days of treatment. This study showed the effective antimicrobial potential of biogenically synthesized AgNPs and AuNPs and suggested these as alternative source of antimicrobial agents against the human pathogenic bacteria. AgNPs and AuNPs also showed strong bactericidal effect even at lower concentration against the tested isolates, which suggested that these NPs could be the magic bullet for combating the disease causing bacteria in the near future. Nanotechnology has the potential to revolutionize human health and medical industry with novel tools for the treatment of diseases at a faster and noninvasive way. Therefore, Long-term *in vivo* and *in vitro* biosafety and experimental observations are crucial in transferring this innovative therapeutics into clinical practice and in guiding their development.