pellets. Transmission electron microscopy (TEM) images were taken using Philips CM 200 high resolution (2.4 Å) TEM operating at an accelerating voltage of 20-200 kV. The test sample was placed on the carbon-coated copper grid and dried before microscopy.

Antibacterial activity of AgNPs

Antibacterial activity of AgNps against clinical isolates of *Salmonella typhi*, *Vibrio cholerae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* was carried out by standard agar well diffusion method[9]. Bacterial strains were spread on the Petri dishes which contained autoclaved nutrient agar and then wells were made using a sterilized cork borer. The prepared wells were filled with an equal volume of AgNPs mixed in deionized water (50 µL) and standard drug (ciprofloxacin 125 µg/mL) separately. The plates were incubated at 37 °C. The zone of inhibition (ZI) was measured after 24 h. The same procedure was repeated for control (sterile water).

Results and Discussion

Synthesis of AgNPs and its UV–Vis characterization

The primary detection of the formation of AgNPs was done by visual observation. The change in colour of the reaction solution from pale yellow to a dark brown with the increase in time provides evidence of the formation of AgNPs (Fig. 2).

UV-visible spectroscopy is a widely used analytical technique to monitor the formation of AgNPs. Upon interaction with an electromagnetic field, the conducting electrons present in the outermost orbital of metal NPs collectively oscillate in resonance with certain wavelengths to exhibit a phenomenon called surface plasmon resonance (SPR). The excitation of SPR is responsible for the formation of colour and absorbance in a colloidal solution of AgNPs[2]. AgNPs are known to exhibit a UV–Visible absorption maximum in the range of 400–500 nm because of surface plasmon resonance[19].

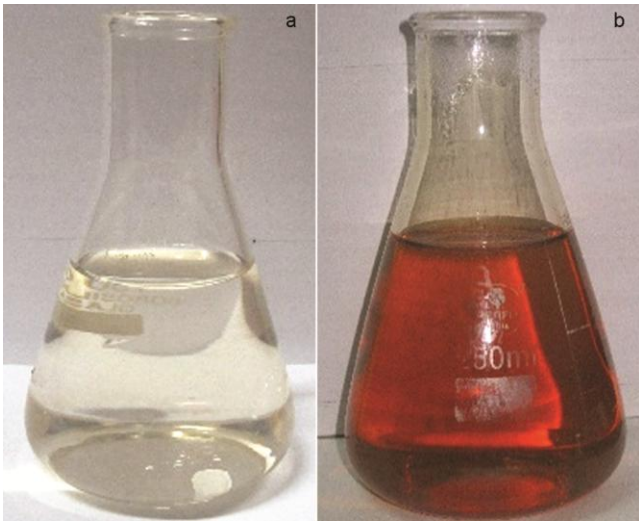

Fig. 2 — Aqueous seed extract of *E. superbum* with $AgNO_3$ solution, a) before incubation, b) after incubation.

The UV-Visible spectrum of the AgNPs synthesized using seed extract of *E. superbum* is shown in Fig. 3. The SPR of the nanoparticles produced a peak centred at 420 nm, indicating the reduction of silver nitrate into AgNPs.

TEM and selected-area diffraction (SAD) analysis

Transmission electron microscopy (TEM) technique was used to visualize the morphology of the Ag NPs[20,21]. TEM images of the samples show nanoparticles predominate with spherical, triangle, and hexagonal shapes (Fig. 4a). The image also shows loosely bound particles were created due to the effect of sonication treatment. Most of the nanoparticles were roughly circular with smooth edges. Fig. 4b shows a SAD pattern of the silver nanoparticles. The AgNPs are crystalline and are evident from the selected area diffraction pattern recorded from one of the nanoparticles in the aggregate. SAD spots that corresponded to the different crystallographic planes of the face-centered cubic (fcc) structure of elemental silver are seen in Fig. 4b.

XRD study of the AgNPs

The XRD peaks at 2θ°degrees of 38.12, 44.30, 64.45, 77.41, and 81.55° correspond respectively to (111), (200), (220), (311), and (222) plans, confirming

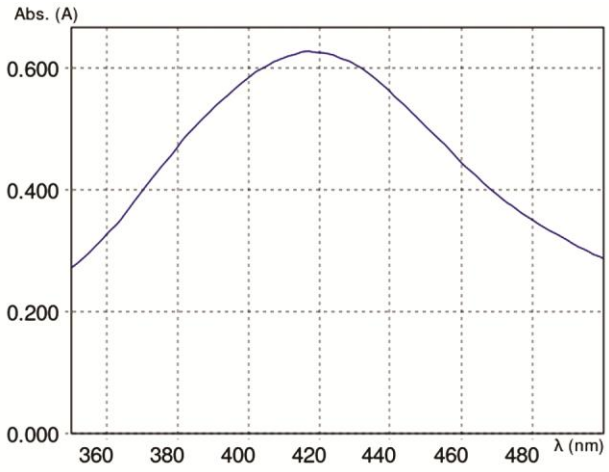

Fig. 3 — UV–Vis absorption spectrums of biosynthesized AgNPs.

Fig. 6 — FTIR spectrum of AgNPs.

Table 1 — Antibacterial activity of biosynthesized silver nanoparticles

Name of Organisms	Zone of inhibition in mm	
	AgNPs (Mean±SEM)	Ciprofloxacin (Mean±SEM)
Pseudomonas aeruginosa	19.67±0.88	22.00±1.00
Salmonella typhi	18.33±0.67	22.67±0.88
Escherichia coli	16.00±1.00	19.67±0.33
Klebsiella pneumonia	15.33±0.88	23.00±1.15
Vibrio cholerae	12.00±0.58	19.67±0.88

alkaloids, phenolic acids, and proteins in the reduction of metal ions into NPs and in supporting their subsequent stability has also been postulated[27].

Antibacterial activity

Antibacterial activity of AgNPs was carried out against 05 bacterial clinical isolates, such as *S. typhi, P. aeruginosa, E. coli, K. pneumoniae, and V. cholera*. The diameter of the inhibition zone (mm) around each well with AgNPs is given in Table 1. The control (deionized sterile water) exhibited zero zones of inhibition (ZI). The highest antibacterial activity was observed against *P. aeruginosa* (19.67±0.88 mm) and the lowest bactericidal action was recorded against *V. cholera* (12.00±0.58 mm). When the ZI was compared with the standard drug (ciprofloxacin), it was found that the antibacterial activity of AgNPs was close to the standard drug. A similar type of work was carried out using AgNPs[28], and CuNPs[29] synthesized from *Terminalia arjuna* bark extract. *Cestrum diurnum*[30], *Clitoria ternatea* and *Solanum nigrum*[31], honey[32] were also reported to synthesize AgNPs and evaluated for their antibacterial activity.

Several studies have proposed the mechanism of the bactericidal action of AgNps. Kvitek *et al.*[33] suggested that AgNPs may attach to the surface of the bacterial cell membrane via interacting with sulfur-containing proteins[34], troubling permeability and respiration functions of the cell resulting in cell death[35].

Conclusion

This study reports the synthesis and characterization of silver nanoparticles using an aqueous seed extract of *E. superbum*. The antibacterial susceptibility of the synthesized AgNPs against several pathogenic microbes is highlighted in the present study. These AgNPs showed promising antibacterial activity against *P. aeruginosa* and *S. typhi*. Although the rapid and green synthetic methods using plant extracts have shown great potential in AgNPs, understanding the mechanism by which phytochemicals of *E. superbum* are involved in the synthesis and the mode of antimicrobial inhibition is still not fully understood.

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Conflict of interest

The authors declare no conflict of interest.

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