Antibacterial activity of Green Synthesis of Silver Nanoparticles from Withania Somnifera (Ashwagandha) Root Extract

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Abstract

Biosynthesis (green synthesis) of metal nanoparticles such as Silver Nanoparticles (AgNPs) has been one of the safest, most cost-effective and environmentally sustainable method in recent years. In this research, AgNPs were synthesized using Withania Somnifera (Ashwagandha roots). For the characterization of synthesized AgNPs different techniques were used, such as X-ray diffraction (XRD), Ultraviolet Visible (UV-VIS) spectroscopy, Fourier transform Infra-Red (FTIR) spectroscopy, scanning electron microscopy (SEM), Energy Dispersive X-ray (EDX) analysis, and atomic force microscope (AFM(. X-ray diffraction analysis showed that the particles were crystalline, while in nature. UV-Visible absorption spectra of the reaction medium containing silver nanoparticles showed maximum in the visible region at 430 nm. FTIR analysis confirmed the reduction of Ag^+ ions to Ag^0 ions in synthesized silver nanoparticles. The SEM analysis the size 5 nm shape and spherical in structure. The antibacterial activity of silver nanoparticles was performed on the growth of both gram-positive and gram-negative bacteria. A bactericidal was observed through the highest zone of inhibition against the bacterial strains, with higher activity for Meropenem. The root extract of Ashwagandha quickly reduces Ag^+ to Ag^0 and enhances the synthesis of silver nanoparticles with highly significant antibacterial activity.

Keywords: *Green Synthesis, Silver Nanoparticles, Withania Somnifera, Characterisation, Antibacterial activity.*

Introduction

Nanotechnology was considered one of the most emerging fields of science and it deals with the synthesis of nanoparticles and nanomaterials, which have dimensions of 1 to 100 nanometers (1). The most important and distinct property of nanoparticles is that they have a larger surface to volume ratio (2). The properties of nanoparticles, such as high diffusion, durability and versatile chemical and biological activities have gained importance in technological applications (3). A metal nanoparticle synthesis study has increased in number due to possible applications in nanotechnology (4). The nanoparticles can be synthesized by physical-chemical and biological method, but the green synthesis of silver nanoparticles has several advantages over physical and chemical method as it is cheaper, can be achieved with a single process and eco-friendly (5-10). Silver Nanoparticles (AgNPs) have attracted growing interest due to their unusual physical, chemical and biological properties, including high electrical and thermal conductivity, Surface-Enhanced Raman Scattering, chemical stability, catalytic activity, and nonlinear optical behavior. AgNPs have wide applications in pharmaceuticals, cosmetics, medical devices, footwear, and textile industries. It has been also AgNPs were helpful in the purification of drinking water/effluent water by efficient removal of the water-born pathogen (11). The synthesis of AgNPs is speedy, cost-saving, eco-friendly and single step synthesis procedure (12).

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The roots of Withania Somnifera (L.) Dunal (Family-Solanaceae)-a vital Rasayana herb is traditionally known as 'Ashwagandha' or winter cherry. In Ayurveda, it is called 'Indian ginseng'. Ashwagandha is widely used in most Indian herbal drugs and nutraceuticals for the treatment of various diseases including nervous, infectious diseases, diabetes, cancer, ulcer, immunological disorders, stress, arthritis, etc[13].

Withania Somnifera (Ashwagandha) as Medicinal plants has therapeutic potential due to the presence of natural antioxidants functioning as reducing agents, free radical scavengers and quenchers of singlet oxygen. Majority of different medicinal plant and their antioxidant activity are due to bioactive compounds viz. flavones, isoflavones, flavonoids, anthocyanins, coumarins, lignans, catechins and isocatechins [14].

The aim of this work, Ashwagandha root extract was used for the production of AgNPs by green mothed. The different properties of biosynthesized AgNPs have been investigated by XRD, SEM, UV-visible, and FTIR to evaluate their shape, distribution of particle size distribution, and functional groups. The biosynthesized formulation of AgNPs was obtained to investigate the antibctrial against Gram-positive and Gram-negative bacterial strains in the inhibition zone compared to the standard antibacterial drug Meropenem.

Materials and Method

The plant extract used in the study was obtained from Ashwagandha root extract. In the synthesis method, Silver Nitrate ($AgNO^3$, purity 99.8%) was used.

Preparations of plant extract: The extract was prepared by the freshly Ashwagandha root extract. The root powder was weighed about (5g) and mixing with distilled water (100 ml) on a magnetic stirrer with a hot plate then the solution was boiling at 50 ºC for 15 minutes. The plant extract was filtered through filter paper after cooling, and the filtered extract was stored for further experiments at a temperature of 4 °C.

Green synthesis of silver nanoparticles: The Silver nitrate solution was freshly prepared about (0.09 g) with distilled water (100 ml) under dark conditions. Prepared root extracts were used to reduce Ag^+ to Ag^0 by combining it with silver nitrate solution ($AgNO_3$) at a ratio of 1 to 1 mol. These plant extracts and $AgNO_3$ mixtures have been kept under 27 ° C with continuous stirring. Reduction of silver ions in solution was monitored by a visible change in the color. This indicates the initial confirmation that Ashwagandha was formed as silver nanoparticles (As-Ag Nanoparticles). Figure 1 shows the steps to prepare silver nanoparticles using Ashwagandha root extract.

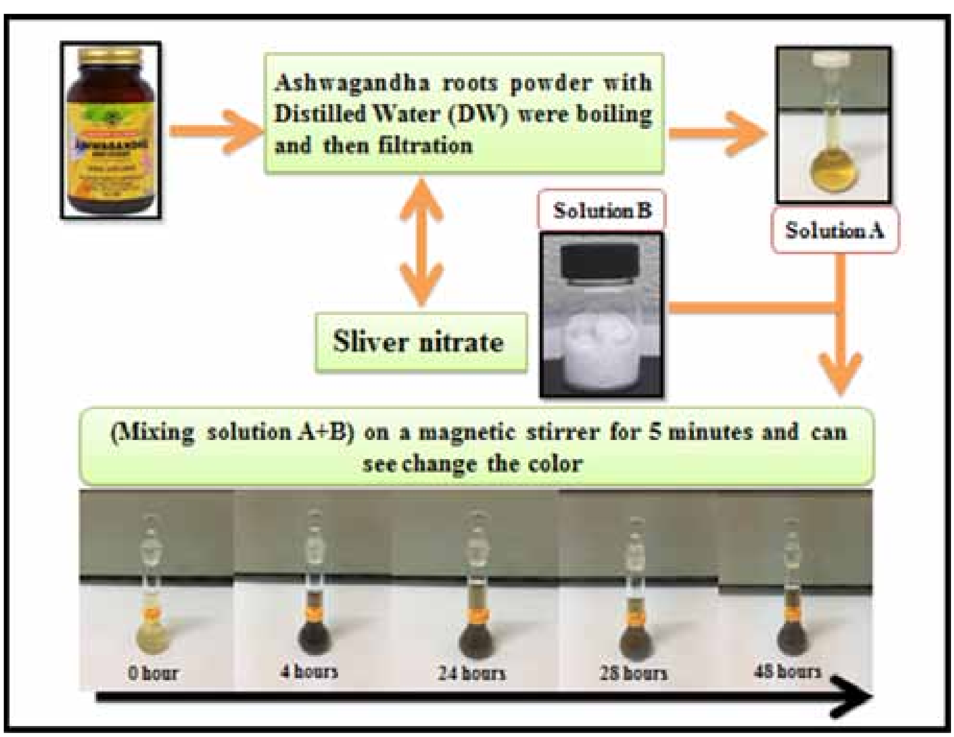

Figure 1: The steps to prepare Silver Nanoparticles using Ashwagandha root extract.

The Antibacterial activity for AgNPs by well diffusion method: Antibacterial of green synthesis for AgNPs was evaluated using a well-diffusion method against bacterial strains of gram-negative *(Escherichia coli)* and gram-positive *(Staphylococcus aureus)*. Microorganisms were grown at 37°C in the medium of Uti chromagar (UCA) and Mannitol salt agar (MSA). Holes of 6 mm in diameter were filled with 60 µl impregnated with equal concentrations of Ashwagandha plant extract, $AgNO_3$ solution, synthesized AgNPs and Meropenem, and kept at 37°C. The diameter of the growth inhibition zones was measured in millimeters (mm) after the 24-hour incubation time**.**

Characterization of Silver Nanoparticles: The crystal structures of AgNPs were analyzed using SHIMADZU LabX X-ray diffractometer with CuKá1 radiation at λ =1.5406 Å. While the absorbance spectra of the samples were recorded in the wavelength (190-1100 nm) using a UV-VIS (SHIMADZU1800) spectrophotometer. FTIR analysis was performed in the 400 cm^{-1} - 4000cm^{-1} range with (SHIMADZU 8400S) spectrophotometer device to determine which functional groups were involved in the reduction of plant extracts. Scanning electron microscopy (SEM) and Energy-dispersive X-ray analysis (EDX) was used to verify the presence of AgNPs in the elemental composition. Atomic force microscopy (AFM) used to study the surface morphology of the samples was the AFM model AA 3000 scanning probe microscope from Angstrom Advanced Inc., USA).

Results and Discussion

X-ray diffraction analysis (XRD): The XRD technique was used to confirm the crystalline nature and particle size of silver nanoparticles. The XRD pattern of green synthesized silver nanoparticles (AgNPs) that extracted from mixing $AgNo_3$ with Ashwagandha was analyzed as in figure (2). The sharp diffraction peaks indicate the good crystallinity of the prepared AgNPs. The intense and narrow peaks (111), (200), (220), (311), (222), (400), (331), (420) and (422) were assigned at 2θ = 38.11°, 44.27°, 64.42°, 77.47°, 81.53°, 97.88°, 110.49°, 114.92° and 134.88°,respectively. The sample indicated a face-centered cubic structure (FCC) of silver structure with the lattice constants (a = b = c = 4.086 Å). Figure (2) shows that silver nanoparticles (111) diffraction peak is the strongest ones which indicates that formed silver particles have a preferential crystallographic (111) orientation. This is due to the organic compounds which are present in the extract and responsible for silver ions reduction and stabilization of resultant nanoparticles. This observation confirms the silver nanoparticles synthesized from Ashwagandha roots were crystalline in nature. The average crystallite size (D) for synthesized silver particles was calculated according to the Debye Scherrer equation[15] and was equal to 21 nm.

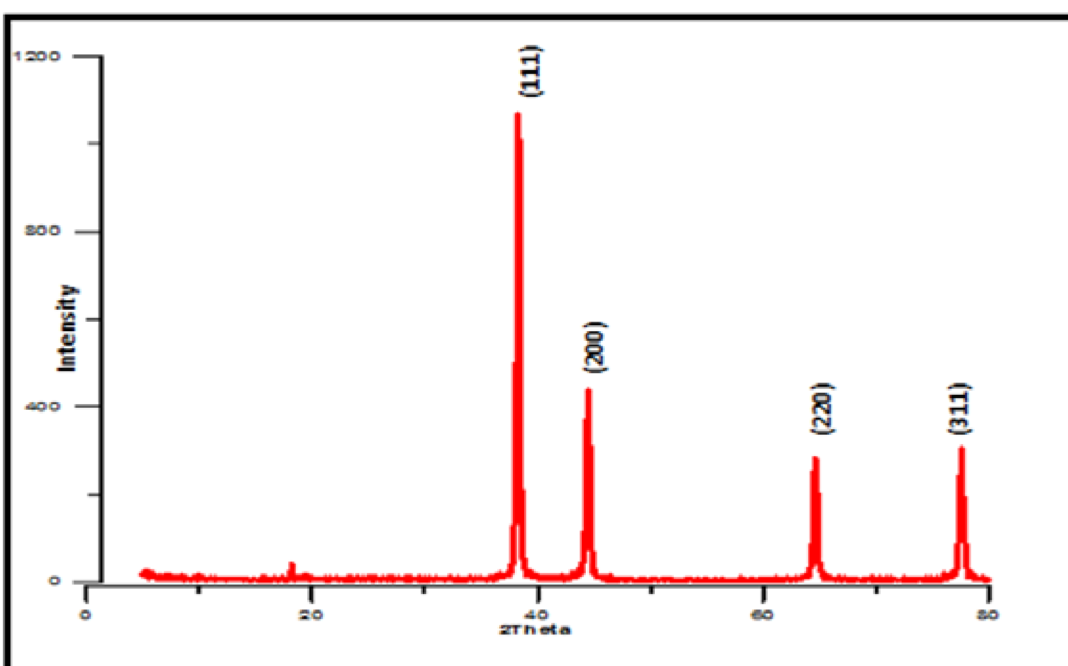

Figure (2): XRD pattern of synthesized AgNPs from Ahwagandha roots.

UV-Visible Spectrophotometer Analysis: The formation of silver nanoparticles was easily monitored with color change and it is due to excitation of surface plasmon vibration of metal nanoparticles. When colorless $AgNO_3$ solution mixed with Ashwagandha roots at 0 hour time watery pale yellow color was shown. The mixed solution color at time 4 hours, 24 hours,28 hours, 48 hours, and 72 hours, was gradually from light yellow to dark reddish-brown. The color change indicates the synthesis of AgNPs by UV–Visible spectrum analysis as shown in (Figure 3). In our result, maximum absorption was observed at 430 nm (Figure 3), the optical properties of silver nanoparticles change which depends upon the collective oscillation of free electron when particles aggregate and the conduction electrons near each particle surface become delocalized and are shared amongst neighboring particles. The resulted observe the spectrum shifts the surface plasmon resonance (SPR) to lower energies. i.e. The absorption peaks move to red shift of plasmon resonance (increases the wavelength at which plasmon resonance occurs) with wide and lower intensity spectrum towards blue shift because of the accumulation effect. The increase of color intensity and (SPR) band sharpness clearly indicates the reduction of Ag^+ into Ag^0.

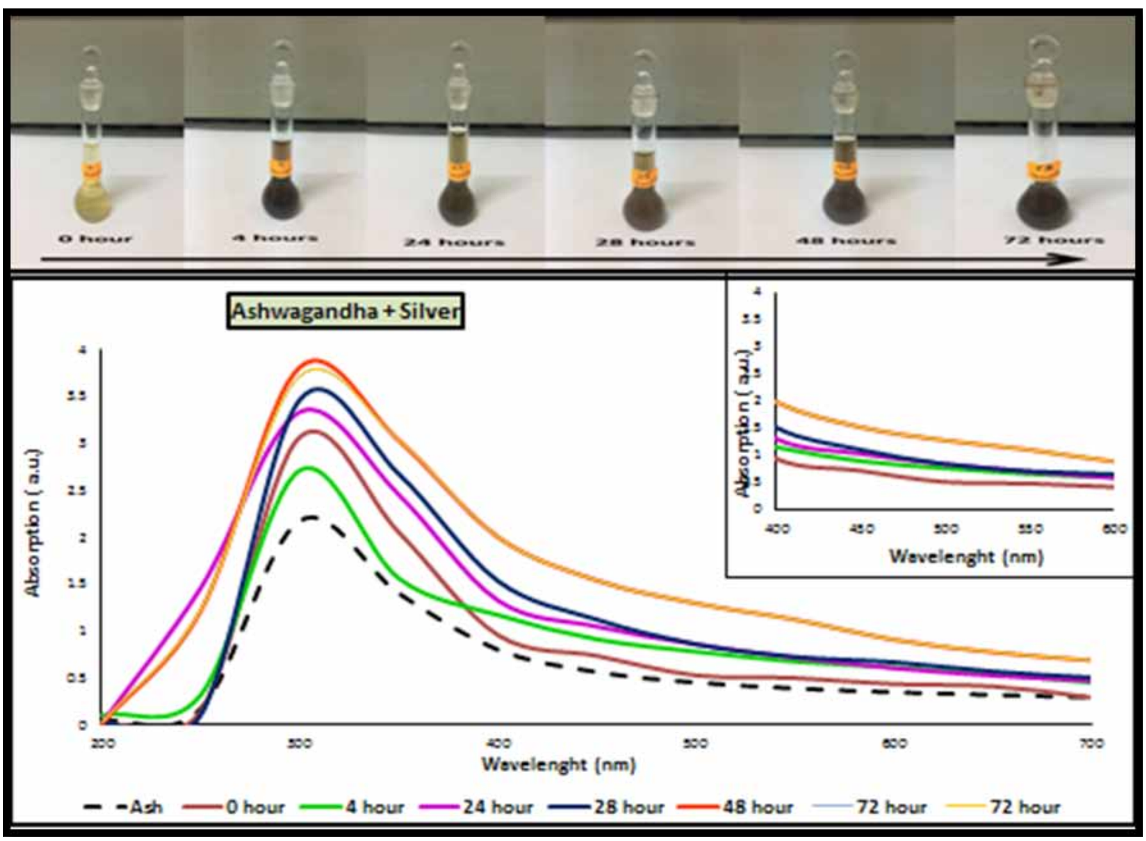

Figure (3): UV-visible spectra of Sliver Nanoparticales with Ashwaghandha at different time.

Fourier Transform Infrared Spectroscopy (FTIR): FTIR measurement was used to identify the possible biomolecules responsible for capping and reducing agents for the stabilization of Silver nanoparticles synthesized from Ashwagandha root extract. The FTIR spectrum of the silver nanoparticle is shown in Figure (4). The observed main peak at 495 cm^{-1} corresponds to metal confirms the formation of (Ag). The peak value at 3413 cm^{-1} may arise due to the O-H stretching vibrations, indicating the presence of hydroxyl groups on the root extract surface of the maize shifted due to the interaction between the Ashwagandha root extract and the silver metal. The medium peaks of 2968cm^{-1} and 2889 cm^{-1} indicate the C−H and C≡H of alkanes and alkynes, respectively. The peak at 1635 cm^{-1} is a signal of N−H bond vibrations from amide groups of the proteins**.**

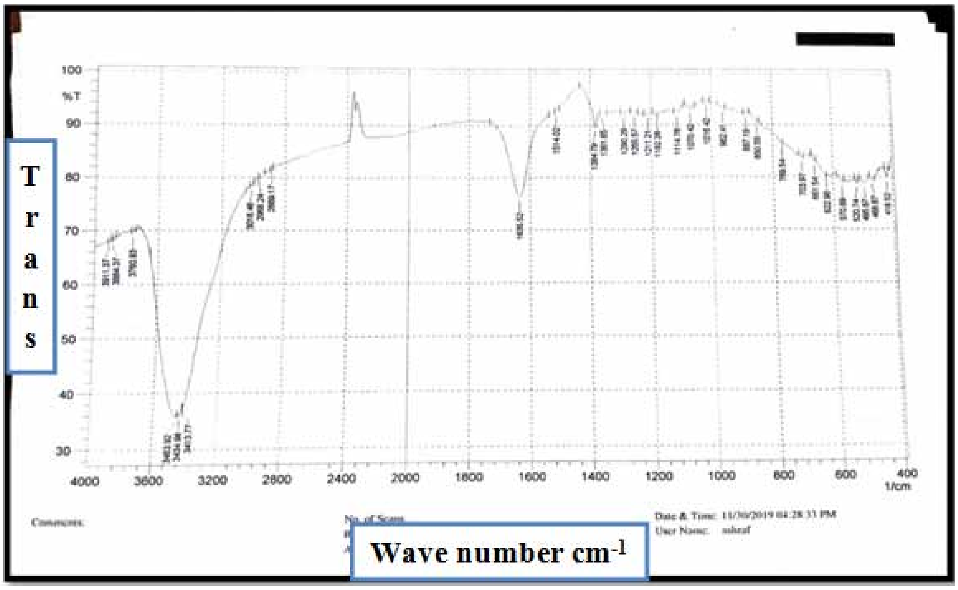

Figure (4): FTIR spectra of green synthesized AgNPs using Ashwagandha root extract.

Field Emission Scanning Electron Microscope (FESEM): AgNPs was analyzed by FESEM shows in the high density AgNPs image. It was shown that AgNPs synthesized by a green method using Ashwagandha root extract. The FESEM image of AgNPs indicates the interactions between the various phytochemical molecules bound to the AgNPs. Images of biosynthesized nanoparticles in SEM show surface morphology of AgNPs were clearly indicated that AgNPs were roughly spherical in shape and uniformly distributed and agglomeration was found in AgNPs at different magnification ranges at 500 nm - 1 µm as shown in Figure (5).

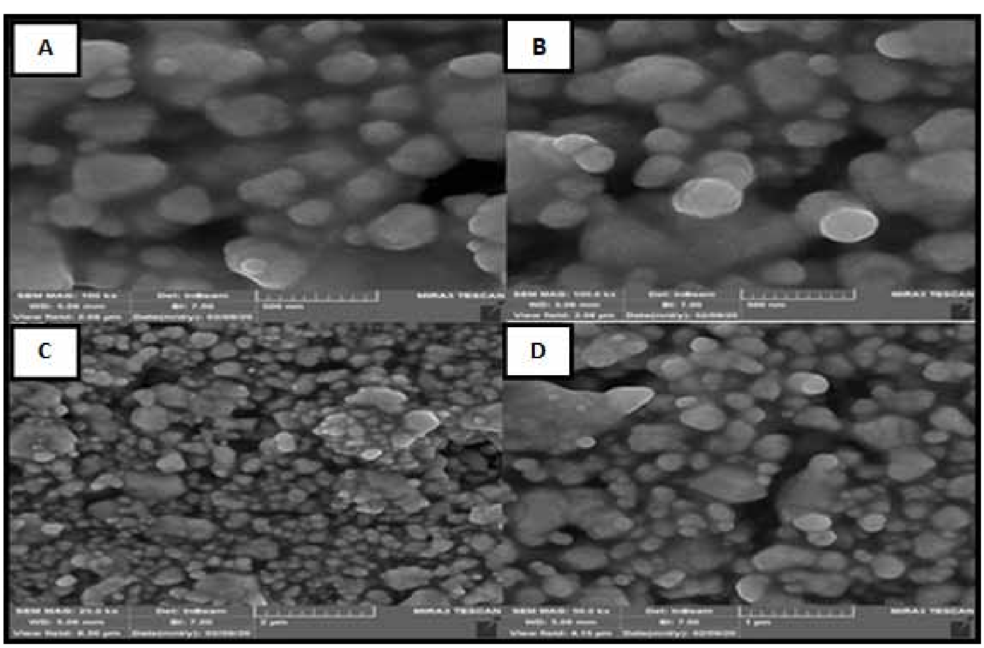

Figure (5): The FESEM images of AsAgNPs in different magnification ranges from 500 nm -1µm.

Antibacterial activity of Silver Nanoparticles (AgNPs): When the inhibition zone of silver nanoparticles against both bacterial groups was greater than 6 mm is considered active. Bacterial inhibition zone measurement by well diffusion method. However, the antibacterial activity of green synthesized AgNPs has shown positive results against both gram-negative. Initially, the antibacterial activities of AgNPs were examined along with plant extract of Ashwagandha, $AgNO_3$ solution, AgNPs and Meropenem against bacterial cultures through well diffusion method. The results showed that AgNPs were highly effective than all other solutions for gram-negative (E.coli) bacteria while for gram-positive (S.aurues) it was more effective than Ashwagandha, AgNO3, and Meropenem (Figure 6). In S. aureus bacterial cultures, AgNPs a greater maximum inhibition zone of 8 mm. In gram-negative bacteria, AgNPs highest maximum inhibition zone (17 mm for E. coli). Thus obtained results clearly indicate that prepared AgNPs were more beneficial against gram-negative bacterial growth than the gram-negative (E.coli) and gram-positive (S.aureus) bacterial pathogenic strains.

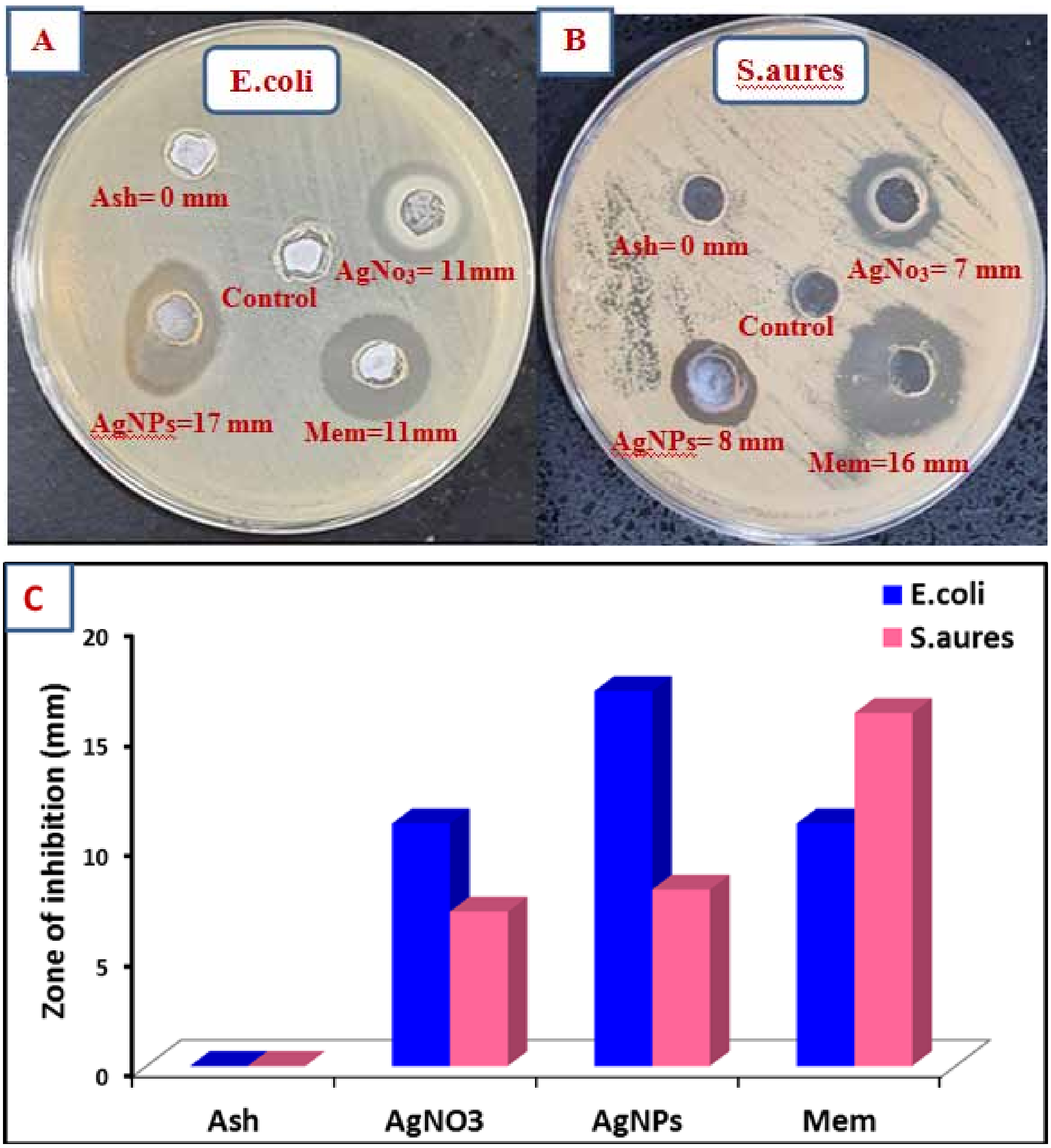

Figure (6): Antibacterial activity of AgNPs against pathogenic bacteria By well diffusion method (Ashwagandha, $AgNO_3$ solution, AgNPs, Meropenem).

Conclusion

The bio-reduction of Ag^+ ions by the root extracts of Ashwagandha has been demonstrated. Probably the biomolecules present in the extract of the roots is responsible for the reduction and stabilization of silver nanoparticles. The synthesized silver nanoparticles were characterized using XRD and confirmed the FCC phase. The obtained silver nanoparticles showed significant antibacterial. We found that the green synthesis of silver nanoparticles from Ashwagandha root extract might be used as antibiotics in the future due to non-toxic, eco-friendly, cost-effective, and highly effective against the bacteria.

Conflict of Interest: None

Funding: Self

Ethical Clearance: Not required

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