

# Polymer Capped Silver Nanoparticles from *Ziziphus nummularia* Leaves Extract: Potent Antibacterial and Antioxidant Activity

Faruk Arodiya, Chirag Makvana and Kokila Parmar

Department of Chemistry, Hemchandracharya North Gujarat University, Patan, Gujarat, India.

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Generally, synthesis and encapsulation process improve therapeutic value of nano encapsulated drugs. The silver nanoparticles (AgNPs) biosynthesized from *Ziziphus nummularia* leaves and encapsulated with polyvinyl pyrrolidone (PVP) polymer as antibacterial agents, due to its high bioavailability, better encapsulation and less toxic properties. The nanoparticles (AgNPs) biosynthesized from *Ziziphus nummularia* leaves and capped with polyvinyl pyrrolidone (PVP) polymer, The acquired AgNPs and polymeric functionalized AgNPs were fully characterised by the UV- Visible spectroscopy, Transmission electron microscopy (TEM), X-Ray diffraction pattern (XRD) and Fourier transform infrared spectroscopy (FTIR). The crystalline Ag NPs and Polymer Functionalized AgNPs have a face-centered cubic structure with an average size of 9.20 nm, according to X-ray Diffraction spectroscopy. Fourier Transform Infrared spectroscopy revealed that biomolecules such as proteins are incapable of reducing metal ions and the formation of an encapsulating layer in terms of metal ions. High-Resolution transmission electron microscopy revealed that Polymer functionalized AgNPs ranged in size of 10 nm. AgNPs and Polymer functionalized AgNPs showed effective antimicrobial and antioxidant activity. The biosynthesized monodisperse silver nanoparticles and encapsulated silver nanoparticles demonstrated better antimicrobial and antioxidant activity which can be used in various biomedical applications.

**Keywords:** Antibacterial and antioxidant; Characterization; Green synthesis; Polyvinyl pyrrolidone (PVP); *Ziziphus nummularia*.

Silver nanoparticles are among one of the most extensively studied nanomaterials. Which metal ions have been used for the treatment of various diseases and biomedical applications<sup>1</sup>. Silver and Gold were used in the form of “Bhasma (Swarna and Rajat) for asthma, anemia, chronic fever, cough, sleeplessness, muscle weakness, and weak digestion<sup>2,3</sup>. Concern for environmental issues led to the development of eco-friendly

methods in chemistry and chemical technology<sup>4</sup>. Silver nanoparticles are highly commercialised materials<sup>5</sup>. As a result, a biological technique for the manufacture of silver nanoparticles that is simple, cheap, and ecofriendly is needed<sup>6</sup>. Recent advances in chemistry methodologies for the manufacture of silver nanoparticles have demonstrated their potential in all biomedical applications. Silver nanoparticle production from

\*Corresponding author E-mail: drkap\_chem@yahoo.com



biosources has always been an attractive task for researchers due to its numerous applications. For the manufacture of silver nanoparticles, some scientists have used plant sources (extract of leaves, roots, flowers, seeds, stems, and fruits) and microbiological sources (such as bacteria, fungus, and their culture media<sup>7</sup>). The reduction and stability of nanoparticles have been linked to a number of medicinally important biomolecules, including alkaloids, proteins, phenols, saponins, tannins, enzymes, and terpenoids<sup>8</sup>. Diseases such as cancer, bacterial infections, cardiovascular disease, and neurological disease can be detected and treated using metal nanoparticles<sup>9</sup>. There are numerous uses for Phyto nanoparticles, including antibacterial, anticancer, image contrast agents, fluorescence probes, and drug delivery systems due to their superior biocompatibility as well as their medical potential<sup>10</sup>. Various analytes related to agriculture, diagnostics and environmental sector used sensors which are developed by the phytosynthesized silver nanoparticles<sup>11</sup>. An extract of *Ziziphus nummularia* has been found to contain the DPPH radical. This is important. Using plants to synthesise AgNPs is based on the fact that the process is faster, less hazardous to the environment, more efficient, and cost-effective than traditional approaches. The formation of silver nanoparticles and also functionalized with polymers to further enhance their biocompatibility for the desired application. Many plants have been recently used in the synthesis of AgNPs, such as *Crateva Religiosa*<sup>12</sup>, *Bauhinia Variiegata*<sup>13</sup>, *Cleistanthus collinus*<sup>14</sup>, *Morinda citrifolia*<sup>15</sup>, *Iris germanica*<sup>16</sup>, *Ceropegia thwaitesii*<sup>17</sup>, *Sauropus androgynous*<sup>18</sup>, *Rhizophora stylosa*<sup>19</sup>, *Ganoderma lucidum*<sup>20</sup> etc. There are so many medicinal plants used to synthesize metal nanoparticles<sup>21-24</sup>. *Ziziphus nummularia* possesses various pharmacological activities like antioxidant, analgesic and anti-inflammatory, antinociceptive, antipyretic activity. The leaves are used to treat coughs, colds, and typhoid, as well as to heal cuts and skin illness<sup>25</sup>. Significantly, DPPH radical scavenging activity in *Ziziphus nummularia* extracts has been demonstrated<sup>26</sup>. In consideration of these findings, we studied the green production of polymer functionalized AgNPs from *Ziziphus nummularia* leaves aqueous extract and examined their antibacterial and antioxidant properties.

## MATERIAL AND METHOD

### Material

Salt of AgNO<sub>3</sub> was received from Sigma Aldrich, fresh leaf of *Ziziphus nummularia* was collected from farm of North Gujarat region. Bacterial culture was purchased from MTCC Chandigarh. DPPH was purchased from ACS, sigma Aldrich product of polyvinyl pyrrolidone (PVP mw 40,000) in its purity was procured commercially and used without further purification.

### Plant extract preparation

Fresh *Ziziphus nummularia* leaves were thrice cleaned in double-distilled water to remove any impurities. The leaves were rinsed, dried, and then finely cut into fine pieces. 10 gram of plant material (fine pieces) was cooked in 100 ml of double-distilled water for 10 minutes at 40-50°C and then cooled. The obtained extract was filtered by Whatman filter paper no.1 and kept in a freezer at 4°C for later use in the synthesis.

### Green synthesis of silver nanoparticles

90 ml of 1mM AgNO<sub>3</sub> solution was added to 10 ml leaf extract and this reaction mixture was placed on a hot plate at 60°C with constant stirring with a magnetic stirrer for 2 hours. Silver nanoparticle generation was preliminarily confirmed by a change in colour from yellowish to dark brown at the beginning of the reaction. The reduction of Ag<sup>+1</sup> to Ag<sup>0</sup> in the reaction mixture was due to the biomolecules present in the plant which acts as a reducing agent. UV visible analysis was used to demonstrate the production of silver nanoparticles. For isolation of AgNPs from the reaction mixture centrifugation process is used. The reaction mixture was centrifuged at 10,000 rpm for 20 minutes. In the bottom of the centrifuge tube, nanoparticles were found, which had been twice purified with double distilled water, then collected and dried at 70-75°C in an oven for 2 hrs. Dry crystalline powder of AgNPs was kept in an airtight bottle for biological activity and characterization (FT-IR, XRD, and HR-TEM).

### Preparation of PVP formulated silver nanoparticles

0.2 gm PVP were dissolved in 100 ml of distilled water and stirred for 1 hr at 80°C. Subsequently, the solution was progressively added to the homogenous solution of AgNPs

that had been generated by leaf extraction. Final confirmation of formation of PVP functionalized silver nanoparticles was studied by UV visible Analysis. Using centrifugation, silver nanoparticles encapsulated with PVP were isolated. For 15 minutes, the reaction mixture was rotated at 6000 rpm. Nanoparticles were observed purified twice by double distilled water then collected and dried at 80-85 °C.

#### **Characterization of Green synthesized Silver nanoparticles**

A UV-visible spectrophotometer was used to measure the absorption spectra of produced AgNPs in the range of 200 nm to 800 nm (Shimadzu UV-1800 UV-visible spectrophotometer). The shape and size of the generated AgNPs were determined using high-resolution transmission electron microscopy (HR-TEM). Nanoparticles were investigated using Fourier transform Infrared spectroscopy (FTIR) to determine their surface chemistry. The Rigaku D/max 40 kV diffractometer equipped with the graphite chromator was used to conduct an XRD investigation to purify crystalline structure with an average particle size.

#### **Antibacterial activity of silver nanoparticles**

Antibacterial activity of synthesized AgNPs and polymer functionalized AgNPs was carried out by Harsh Mistry *et al.* (2020) with some modifications<sup>27</sup>. All of the test bacterial strains were cultured in nutrient broth overnight at 37°C and adjusted to 0.5 according to McFarland standards. 100 µL of gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) strains were dispersed on each nutrient agar plate under sterile conditions. Using a cork borer, a 10 mm diameter well was punched in the agar plate, and the synthesized AgNPs, polymer functionalized AgNPs, and AgNO<sub>3</sub> were injected in each well. Plates were incubated for 24 hours at 37°C, and the inhibitory zone's diameter was measured using a zone scale to determine bacterial activity (HiMedia).

#### **Antioxidant activity by 2,2-diphenyl -1-picrylhydrazyl (DPPH) method**

Antioxidant capacity of synthesized AgNPs and polymer capped AgNPs was performed according to Harsh *et al.*<sup>27</sup> with slight modification. Ascorbic acid was taken as standard due to its high antioxidant properties. The radical scavenging activity of AgNPs, polymer functionalized AgNPs,

and vitamin C was determined using the DPPH. 1 mL of various concentration (10, 50, 80, 100 µg/ml) AgNPs and PVP functionalized AgNPs and standard ascorbic acid solution were mixed separately with 1ml of DPPH solution and incubated for 30 min. The absorbance was measured by UV- Visible Spectrophotometer at 517nm. The free radicals scavenging activity was calculated as a percentage of inhibition. The following formula is used to calculate.

$$\% \text{ of scavenging} = \frac{[(P_{\text{controlled}} - P_{\text{sample}}) / P_{\text{controlled}}] \times 100}{}$$

Where  $P_{\text{controlled}}$  is the absorbance of the control and  $P_{\text{sample}}$  is the absorption of AgNPs/polymer capped AgNPs/vitamin C.

## **RESULT AND DISCUSSION**

#### **UV-visible spectroscopic analysis**

A UV-visible spectrophotometer was used to confirm the creation of AgNPs. The reduction of Ag<sup>+1</sup> to Ag<sup>0</sup>, as indicated by a colour change from light brownish to dark brownish, was due to the excitation surface plasmon resonance (SPR) of AgNPs<sup>28</sup>. The observed results are substantially similar with those of a recent research<sup>29</sup>. The successful formation of AgNPs and polymer functionalized AgNPs absorbance peak at 431 and 443 nm show in figure 2<sup>30</sup>. This result is consistent with earlier research<sup>31-33</sup>. According to studies, the SPR of the majority of metallic compounds is shape and size dependent<sup>34-36</sup>.

#### **Fourier transform infrared spectroscopy (FTIR) analysis**

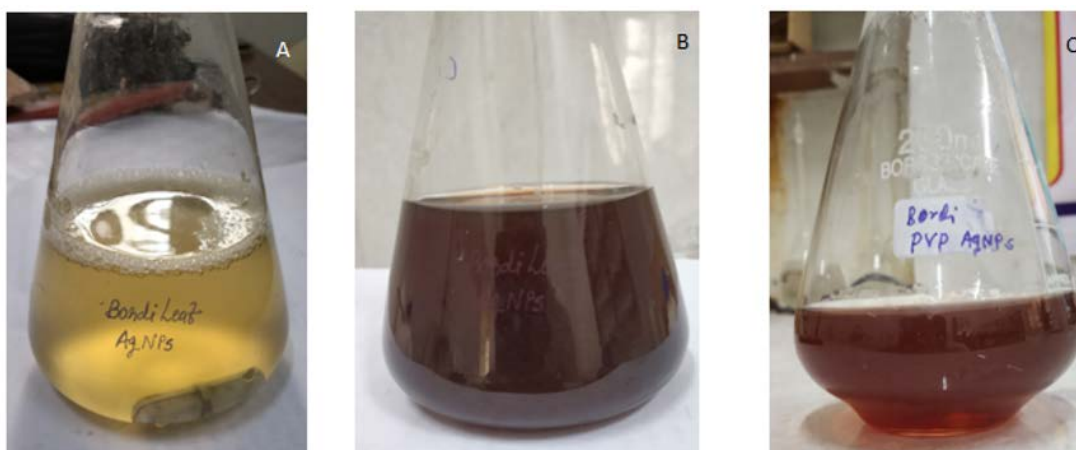
The FTIR spectrum of synthesized AgNPs is shown in figure 3. This result in absorption peaks positioned between the area about 4000 cm<sup>-1</sup> and 500 cm<sup>-1</sup>. FTIR spectrum of plant extract and AgNPs displays peak at 3300, 3050, 2890, 1597, 1350, 1050 cm<sup>-1</sup> for silver nanoparticles. The obtained results are congenial with the previous report<sup>[29]</sup> The AgNPs show several peak at 3200, 613, 532 cm<sup>-1</sup> region. The peak at 1421 cm<sup>-1</sup> is common to both the extract and AgNPs and is characteristic of the C-H bending vibration. Vibration stretching at 3300 cm<sup>-1</sup> peak correspond to O-H stretching of water and phenolic compounds. The peak at 1350 and 1050 cm<sup>-1</sup> is

evidence of the C-H stretching for respective amines. The existence of a peak at  $420\text{ cm}^{-1}$  shows metal oxide bonding. The peak at  $1640\text{ cm}^{-1}$  corresponds to metal carbonyl stretching polymer mediated samples have prominent peaks where the stretching vibration associated with O-H and C-H/CH<sub>2</sub> groups are located at  $3350\text{ cm}^{-1}$  and  $2930\text{ cm}^{-1}$  is associated with the aliphatic hydrocarbons group in polysaccharide, proteins or polyphenols of water molecule bounds in Ag surface respectively<sup>37-40</sup>. The observed vibration bands below  $600\text{ cm}^{-1}$  for AgO surface<sup>41,42</sup>. The obtained results are consistent with previous publications confirming *Ziziphus*

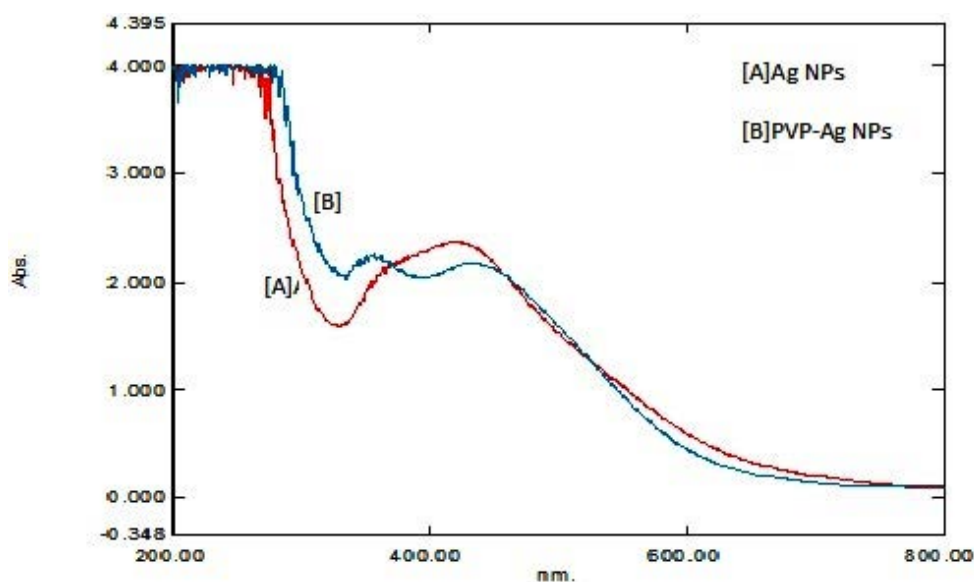
*nummularia*'s efficacy as a reducing agent in the synthesis of AgNPs and polymer functionalized AgNPs<sup>43,44</sup>.

#### X-RAY diffraction analysis

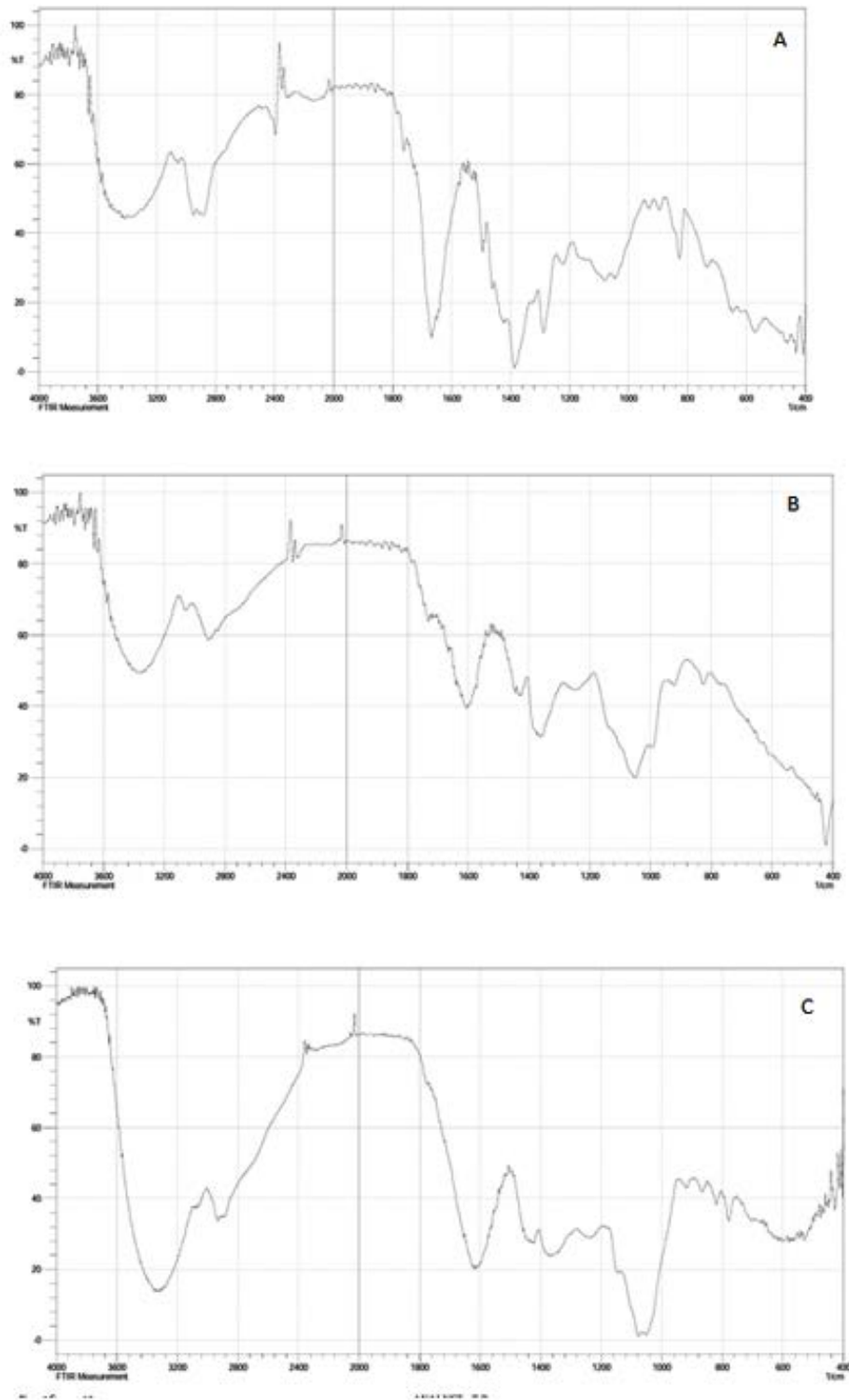
The XRD pattern of polymer-functionalized AgNPs (as shown in Fig.4) revealed a well-crystallized sample with the principal diffraction peaks located at 2 theta values of  $27.12^\circ$ ,  $32.39^\circ$ ,  $46.33^\circ$  and  $77.4^\circ$  which corresponds to the plane(100), (111), (200), (311) respectively. The phase change modification caused by PVP might be attributed to the bio conjugate formed between the polymer component and the produced



**Fig. 1.** Colour change from yellowish to dark brown [A] Initial Time, [B] After 2 hr and [C] After adding PVP Ag NPs



**Fig. 2.** UV-Visible absorption spectra of [A] Ag NPs [B] PVP Ag NPs



**Fig. 3.** FT IR Spectrum of [A] *Ziziphus nummularia* leaf extract [B] Ag NPs [C] PVP functionalized Ag NPs

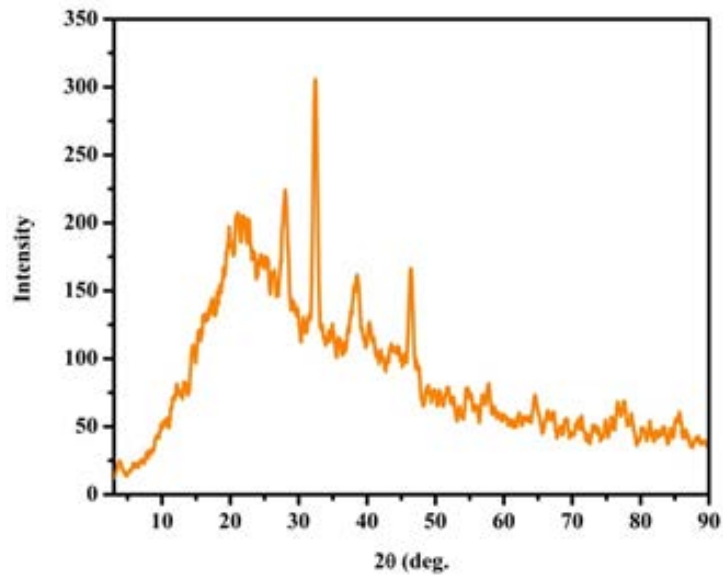


Fig. 4. XRD pattern of PVP functionalized Ag NPs

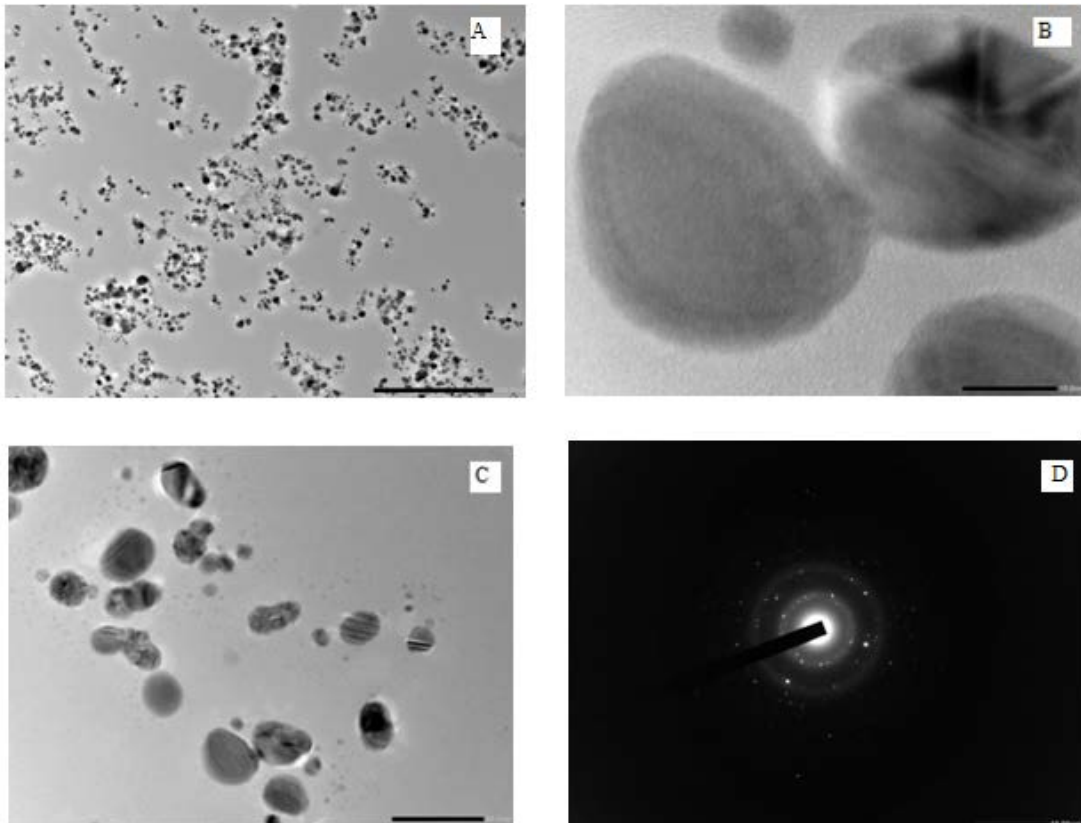


Fig. 5. HR-TEM image [A],[B],[C] and SAED image [D] of PVP functionalized Ag NPs

polymer-capped AgNPs. The mean particle size of PVP AgNPs was determined using the Debye-Scherrer formula which is given as  $D = 0.9k/b \cos$ , where D is the crystalline size (nm), k is the X-ray wavelength (0.1541 nm), b represents the angular line full width at half maximum (FWHM) of the peak (in radians), and his is the Bragg's angle (in radians)<sup>45</sup>. Calculations indicate that the PVP AgNPs have an average particle size of 9.20 nm, which is in good agreement with the HR-TEM average particle size of 10 nm.

#### HR TEM analysis

The H-7500 model was used for high-resolution transmission electron microscopy (HR-TEM). HR-TEM was used to investigate size and shape morphology, as illustrated in figure 5. The polymer functionalized AgNPs with a spherical morphology and uniform size, with an average particle size of 10 nm. The crystallinity of the biosynthesized polymer functionalized AgNPs was demonstrated using a selected area electron

diffraction (SAED) pattern with bright circular spots.

#### Antibacterial activity of AgNPs and Polymer functionalized AgNPs

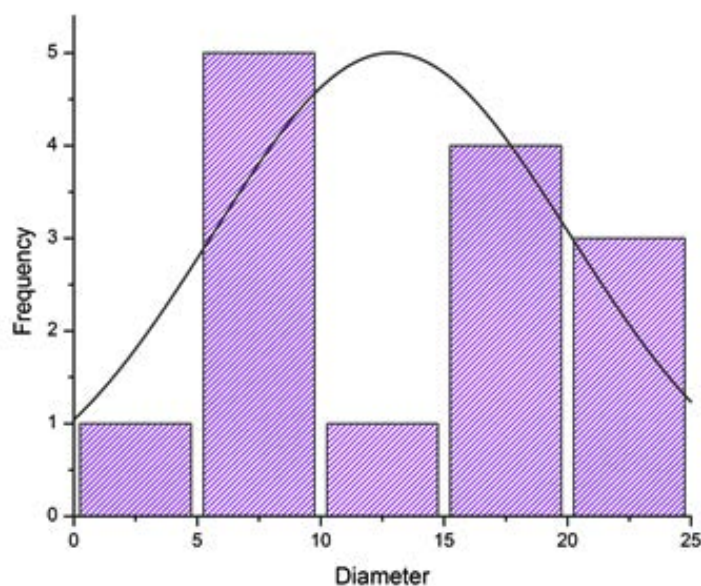
The antibacterial activity of AgNPs was examined by measuring the Inhibition zone of plant extract, silver nanoparticles and polymer functionalized nanoparticles was summarized in table 1. The PVP AgNPs inhibit the growth of *Escherichia coli*, *Staphylococcus aureus*, shown in figure 7 It also shows good activities then the all the organisms in comparison with standard drug<sup>27</sup>. Due to their activity at lower concentrations, PVP AgNPs have a higher chance of competing with conventional antibacterial medicines.

#### Antioxidant activity of AgNPs and Polymer capped AgNPs

DPPH is a stable molecule that may be reduced by absorbing hydrogen or electrons, and it is often used to evaluate antioxidant activity. AgNPs displayed strong antioxidant activity,

**Table 1.** Antibacterial activity of Ag NPs and PVP Ag NPs

Sr. No.	Organism	Zone of Inhibition (In mm)			
		AgNO <sub>3</sub> (10 mM)	AgNPS	PVP AgNPS	Ampicillin (1 mg/ml)
1	<i>Escherichia coli</i>	15	19	20	15
2	<i>Staphylococcus Aureus</i>	15	18	19	17



**Fig. 6.** The size distribution curve of PVP functionalized AgNPs as determined by TEM and SAED images

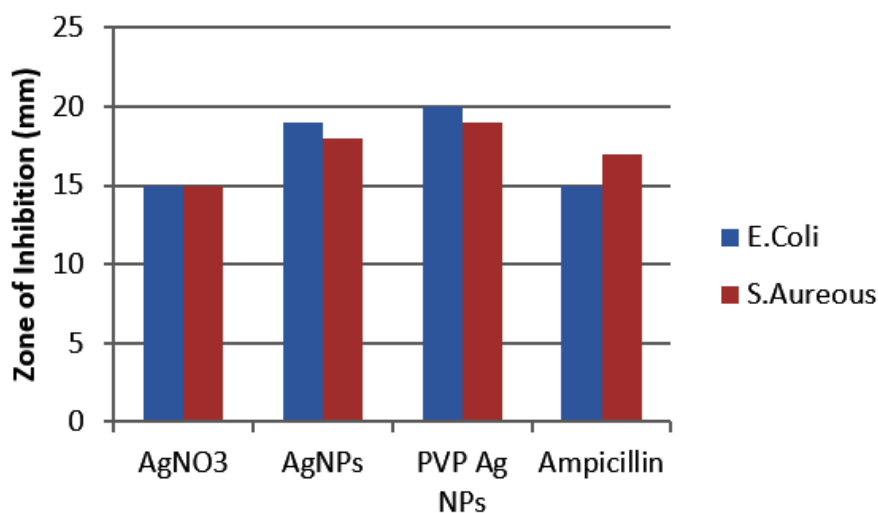


Fig. 7. Antibacterial zones of inhibition of AgNPs and PVP AgNPs in comparison with Standard Ampicillin

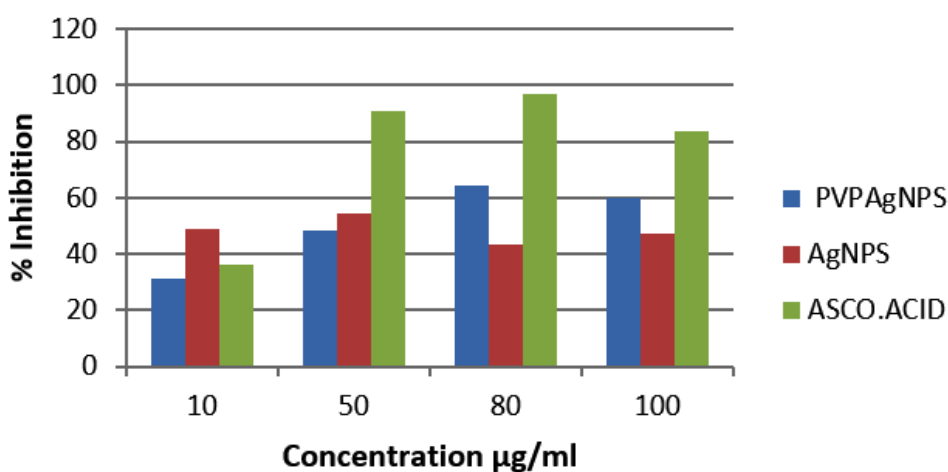


Fig. 8. Antioxidant activity (%) of synthesized silver nanoparticle and polymer functionalized Ag NPs in comparison with standard ascorbic acid

with their ability to scavenge free radicals increasing with concentration. The figure displays AgNPs' antioxidant activity, which is around 48.83 percent. The proportion of PVP-AgNPs was around 51.15 percent. Polymer-capped AgNPs had better antioxidant activity than AgNPs, according to the findings. AgNPs have antioxidant properties due to plant component absorption on silver nanoparticles<sup>46</sup>.

## CONCLUSION

Using an extract of *Ziziphus nummularia* leaves and AgNO<sub>3</sub> salt solution silver nanoparticles were successfully synthesized. To improve biocompatibility, the AgNPs were further functionalized with PVP without the addition of any hazardous or poisonous chemicals. At the preliminary level, the colour change of



solution was used to confirm the synthesis of AgNPs and polymer capped AgNPs. Various characterization procedures were employed to confirm the comparison of AgNPs and polymer capped AgNPs. UV visible confirmed the formation of AgNPs trolls by changing the visible colour to dark brown after 2 hours of peak at 431 nm. The FTIR spectrum identified the several functional groups present in the *Ziziphus nummularia* extract that are involved in the synthesis of AgNPs and polymer-formed AgNPs. XRD examination confirmed the crystallinity and an average particle size of 9.20 nm of AgNPs and polymer capped AgNPs. HR-TEM imaging microscopy revealed that the particles were spherical in shape and ranged in size from 2 to 25 nm. The antimicrobial activity of AgNPs and polymer-capped AgNPs was evaluated against gram-positive and gram-negative bacteria with a significant zone of inhibition. Also, the good antioxidant activity of synthesized silver nanoparticles and polymer capped silver nanoparticles. This study proposed a biological technique for synthesis polymer-capped nanoparticles with antibacterial and antioxidant activity that is both eco-friendly and cost-effective.

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#### Conflict of interest

The authors confirm that the content of this manuscript has no conflict of interest.

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