**POLYMER CAPPED SILVER NANOPARTICLES FROM *ZIZIPHUS NUMMULARIA* LEAVES EXTRACT: POTENT ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY**

**Abstract:**

Generally, synthesis and encapsulation process improve therapeutic value of nano encapsulated drugs. The biodegradable monodisperse 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 responsible for metal ion reduction 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 mono disperse 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*.

**1. Introduction**

One of the most well researched nanomaterials is silver nanoparticles. where metal ions have been applied in biomedical applications and the therapy of numerous ailments [1]. For the treatment of asthma, anaemia, chronic fever, cough, insomnia, muscle weakness, and poor digestion, silver and gold were employed in the form of "Bhasma" (Swarna and Rajat) [2,3]. Concern for environmental issues led to the development of the eco-friendly approach in chemistry and chemical technology [4]. The commercialization of silver nanoparticles is extensive [5]. As a result, a biological technique is the best option for the production of silver nanoparticles since it is straightforward, affordable, and environmentally friendly [6]. Recent developments in silver nanoparticle synthesis techniques have demonstrated its promise for use in all biomedical applications. Researchers have always found it intriguing to create silver nanoparticles utilising biosources because of all the different uses they may be put to. For the manufacture of silver nanoparticles, some researchers have used a variety of biological sources, such as plant sources (extracts of leaves, roots, flowers, seeds, stems, and fruits) and microbial sources (such as bacteria, fungus, and their culture media)[7].Alkaloids, proteins, phenols, saponins, tannins, enzymes, and terpenoids are a few medicinally significant biomolecules from biological sources that are involved in the reduction and stabilisation of nanoparticles[8]. Metal nanoparticles can be used as both therapeutic and diagnostic tools for a wide range of diseases, including cancer, cardiovascular disease, and neurodegenerative illness [9]. Phyto nanoparticles are recommended for numerous applications, including antibacterial, anticancer, image contrast agents, fluorescence probes, and drug delivery systems [10] because of their outstanding biocompatibility and medicinal potential.

The creation of sensors for the identification of various analytes relevant to agriculture, diagnostics, and the environment sector is being done with the use of phytosynthesized silver nanoparticles [11].Importantly, it has been demonstrated that a Ziziphus nummularia extract contains DPPH radicals. The use of plants in AgNP synthesis relies on the fact that the procedure is quicker, simpler, environmentally friendly, affordable, and trustworthy, and produces more stable synthesised particles than other, traditional approaches. In order to further improve their biocompatibility for the intended use, silver nanoparticles were formed and then functionalized with polymers. Many plants have recently been used to make AgNPs, including Crateva Religiosa [12], Bauhinia Variegata [13], Cleistanthus collinus [14], Morinda citrifolia [15], Iris germanica [16], Ceropegia thwaitesii [17], Sauropus androgynous[18], Rhizophora stylosa[19], Ganoderma lucidum [20], etc. Metal nanoparticles can be made from a wide variety of medicinal plants [21–24]. Numerous pharmacological properties of ziziphus nummularia include antioxidant, analgesic and anti-inflammatory, antinociceptive, and antipyretic action. The leaves are used to heal wounds and treat cutaneous conditions as well as coughs, colds, and typhoid [25]. Importantly, DPPH radical scavenging activity in a ziziphus nummularia extract has been demonstrated [26]. In light of this findings, we looked at the environmentally friendly production of polymer functionalized AgNPs from Ziziphus nummularia leaves water extract and tested their antioxidant and antibacterial properties.

**2. Material and Method**

**2.1. Material**

Fresh leaves of Ziziphus nummularia were gathered from a farm in the North Gujarat region, and Sigma Aldrich delivered the salt of AgNO3. Bacterial culture was bought from MTCC Chandigarh, DPPH from ACS, and polyvinyl pyrrolidone (PVP mw 40,000) from Sigma Aldrich was bought in its purest form and used without further purification.

**2.2. Preparation of plant extract**

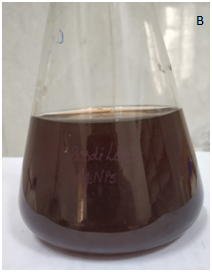
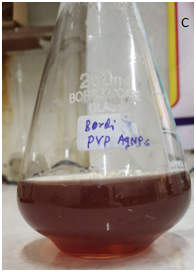
Ziziphus nummularia fresh leaves were cleaned twice with double-distilled water. After being washed, the leaves were cut into small pieces and allowed to dry at room temperature to eliminate any remaining water. 10 gm of plant material (fine pieces) were used, and 100 ml of double-distilled water was added. The mixture was heated at 40–500°C for 10 minutes, then cooled. In order to use the obtained extract for the synthesis, it was filtered using Whatman filter paper no. 1 and kept at 40°C in a freezer.

**2.3. Green synthesis of silver nanoparticles**

10 ml of leaf extract were combined with 90 ml of a 1 mM AgNO3 solution, and the mixture was then heated to 60 0C while being constantly stirred with a magnetic stirrer for two hours. We saw a colour change from yellowish to dark brown at the reaction's beginning, which provided a preliminary confirmation of the creation of silver nanoparticles. The presence of biomolecules in the plant, which function as a reducing agent, is what caused the reduction of Ag+ to Ag0 in the reaction mixture. UV visible analysis was used to determine the silver nanoparticles' final confirmation of their creation. AgNPs can be separated from the reaction mixture using centrifugation. Centrifuging the reaction mixture for 20 minutes at 10,000 rpm. The nanoparticles were collected, dried at 70-750C for two hours, and examined at the bottom of the centrifuge tube after being twice purified by double-distilled water. AgNPs were stored as a dry, crystalline powder in an airtight container for FT-IR, XRD, and HR-TEM assessment of biological activity.

**2.4. Preparation of PVP formulated silver nanoparticles**

For one hour at 800C, 0.2 gramme of PVP were dissolved in 100 ml of distilled water and stirred. The homogenous solution of AgNPs made from leaf extract was then gradually supplemented with the solution. UV visible analysis was used to confirm the production of PVP functionalized silver nanoparticles. Silver nanoparticles with PVP coatings were separated using centrifugation. 15 minutes were spent centrifuging the reaction mixture at 6000 rpm. Double-distilled water was used to purify the nanoparticles twice, after which they were collected and dried at 80 to 85 °C.

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

**2.5 Characterization of Green synthesis Silver nanoparticles**

A UV-visible spectrophotometer (Shimadzu UV-1800 UV-visible spectrophotometer) was used to evaluate the absorption spectra of synthesised AgNPs in the 200–800 nm range. High-resolution transmission electron microscopy (HR-TEM) was used to determine the size and form of synthesised AgNPs. Fourier transform infrared spectroscopy (FTIR) was used to analyse the surface chemistry of the nanoparticles, and X-ray diffraction (XRD) was used to purify the crystal structure with an average particle size using a Rigaku D/max 40 kV diffractometer outfitted with a graphite chromator.

**2.6 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[27]. All the test bacterial strains were grown in nutrient broth at 370C overnight and adjusted to 0.5 as per McFarland standards. Under sterile conditions, 100 μL of gram-positive (*Staphylococcus aureus*) and gram-negative strains (*Escherichia coli*) were spread on each nutrient agar plate. A diameter well of 10 nm was punched on the agar plate using a cork borer and the synthesized AgNPs, polymer functionalized AgNPs and AgNO3 were inoculated in each well. Plates were incubated at 370C for 24 hours and the bacterial activity was evaluated by measuring the diameter of the inhibition zone using zone scale (HiMedia).

**2.7Antioxidant 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. [27] with slight modification. The radical scavenging activity of AgNPs, polymer functionalized AgNPs, and vitamin C was determined using the DPPH. Various concentration (10,50,80,100 μg/ml) of 1 mL AgNPs were mixed with 1 ml of 1 mM freshly prepared DPPH solution followed by vortex. Then, the solution was kept for 30 minutes in dark at room temperature. The absorbance was recorded at 517 nm DPPH with all reagents except sample was used as a control and methanol was used as a blank. The free radical scavenging activity was represented as the percentage of inhibition which was calculated by using the following formula.

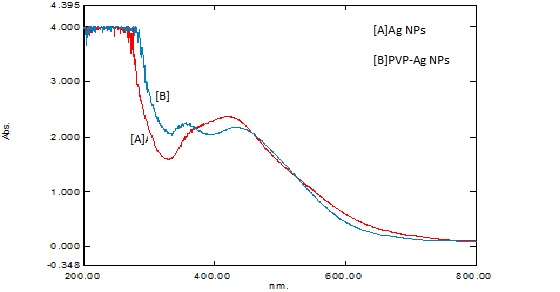
% of scavenging = [(Pc-Ps)/Pc] × 100

Where Pc is the absorbance of the control and Ps is the absorption of AgNPs/polymer capped AgNPs/vitamin C.

**3. Result and Discussion**

**3.1 UV-visible spectroscopic analysis.**

The aqueous reduction reaction mixture was subjected to a UV-visible spectrophotometer to confirm the formation of AgNPs reduction of Ag+1to Ag0 observed by a colour change from light brownish to dark brownish was due to excitation surface Plasmon resonance (SPR) [28] of AgNPs which finally confirmed the production of AgNPs. Observed results are highly in accordance with a recent report [29]. The successful formation of AgNPs and polymer functionalized AgNPs absorbance peak at 431 and 443 nm show in figure 2 [30]. This result is congenial with previous findings [31-33]. Studies show that the SPR of most metallic compounds is size and shape dependant [34-36].

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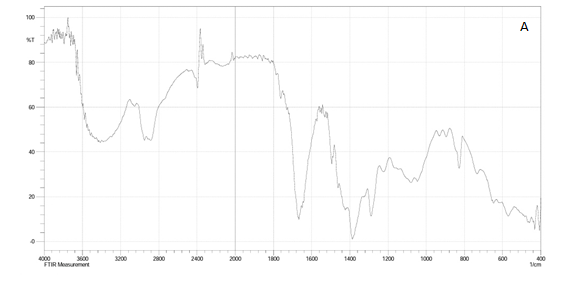
**Figure: 2 UV-Visible absorption spectra of [A] Ag NPs [B] PVP Ag NPs**

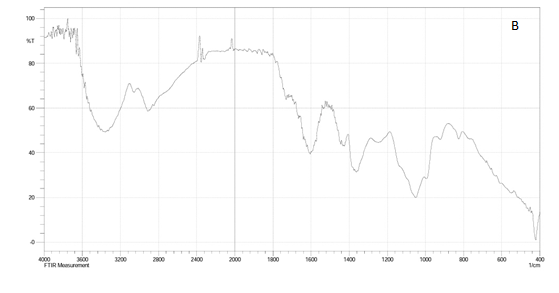
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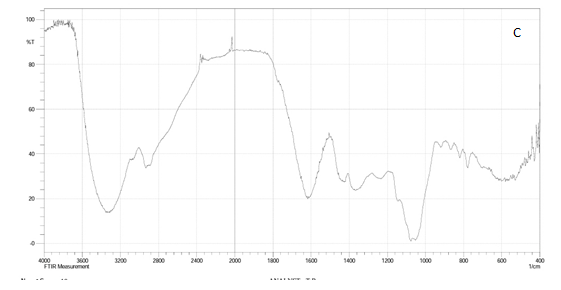
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**3.2 Fourier transform infrared spectroscopy (FTIR) analysis:**

The FTIR spectrum of synthesized AgNPs is shown in figure 3. Which manifests absorption peaks located between the region about 4000 cm-1 and 500 cm-1. FTIR spectrum of plant extract and AgNPs displays peak at 3300, 3050, 2890, 1597, 1350, 1050 cm-1 for silver nanoparticles. The obtain results are congenial with the previous report [29]. The AgNPs show several peak at 3200,613, 532cm-1 region. The peak at 1421 cm-1is common to both the extract and AgNPs and is characteristic of the C-H bending vibration. Vibration stretching at 3300 cm-1 peak correspond to O-H stretching of water and phenolic compounds. The peak at 1350 and 1050 cm-1 is evidence of the C-H stretching for respective amines. The existence of a peak at 420 cm-1 shows metal oxide bonding. The peak at 1640 cm-1 corresponds to metal carbonyl stretching polymer mediated samples have prominent peaks where the stretching vibration associated with O-H and C-H/ CH2 groups are located at 3350 cm-1 and 2930 cm-1 is associated with the aliphatic hydrocarbons group in polysaccharide, proteins or polyphenols of water molecule bounds in Ag surface respectively [37-40].The observed vibration bands below 600 cm-1 birth AgO surface [41,42]. The obtain results are congenial with previous reports demonstrating the applications of *ziziphus nummularia* as a reducing agent in the formation of AgNPs and polymer functionalized AgNPs [43,44].

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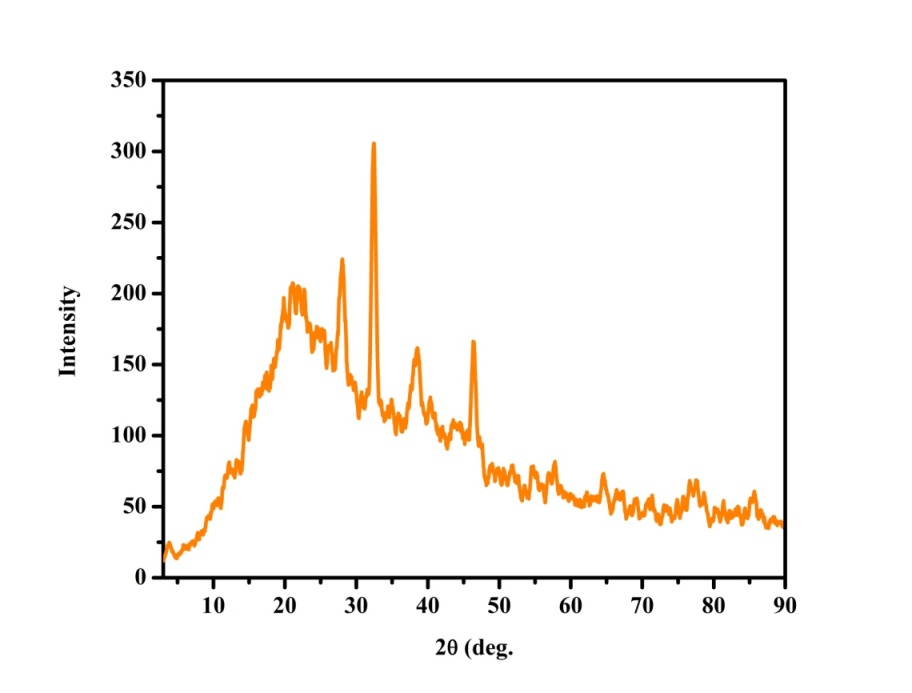
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**Figure : 3** **FT IR Spectrum of [A] *Ziziphus nummularia* leaf extract [B] Ag NPs [C] PVP functionalized Ag NPs**

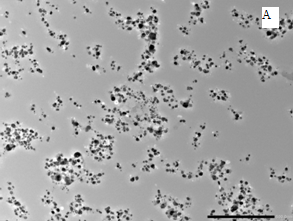
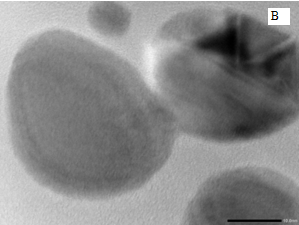
**3.3 X-RAY diffraction analysis:**

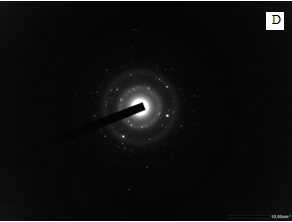
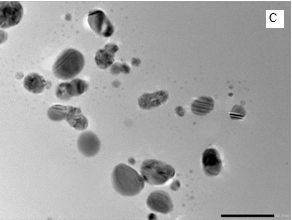
The XRD pattern of polymer functionalized AgNPs(as seen in Fig.4 ) showed a well-crystallized sample with the major diffraction peaks at 2 theta values of 27.12°, 32.39°,46.33° and77.4° which corresponds to the plane(100), (111), (200), (311) respectively. The alteration of the phase change by PVP may be adduced to the bioconjugate between the polymers component and the formulated polymer capped AgNPs. The mean particle size of PVP AgNPs was calculated using the Debye-Scherer formula given as D = 0.9k/b cos Ɵ, where D is the crystalline size (nm), k is the wavelength of X-ray (0.1541 nm), b represent the angular line full width at half maximum (FWHM) of the peak (in radians) and his the Braggs angle (in radians)[45]. By calculation, the PVP AgNPs were found with a 9.20 nm average particles size which is in fair agreement with the HR-TEM average particle size of ~10 nm.

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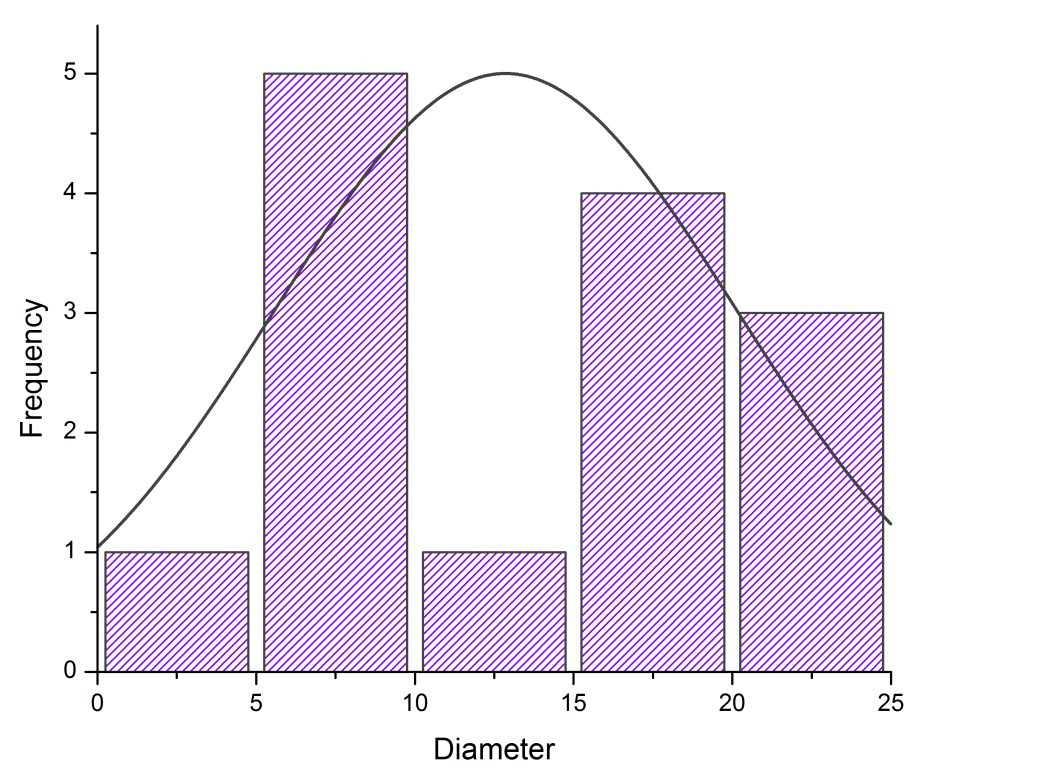
**Figure: 4 XRD pattern of PVP functionalized Ag NPs**

**3.4 HR TEM analysis**:

High-resolution transmission electron microscopy HR-TEM was performed using the H-7500 model. Size and shape morphology were studied by HR-TEM shown in figure 5. The polymer functionalized AgNPs has taken up a spherical morphology uniform size with the average particle size of 10 nm shown in figure 6.Using the Selected area electron diffraction(SAED) pattern with bright circular spots, the crystallinity of the biosynthesized polymer functionalized AgNPs was evidenced.



**Figure: 5 HR-TEM image [A],[B],[C] and SAED image [D] of PVP functionalized Ag NPs**

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**Figure: 6** **The size distribution curve from the TEM analysis and SAED pattern of PVP functionalized AgNPs**

**3.5 Antibacterial activity of AgNPs and Polymer functionalized AgNPs:**

The antibacterial potential of AgNPs was assessed 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[27]. PVP AgNPs stand a better chance as a potential substitute for the conventional antibacterial drugs sequel to its activities at a lower concentration.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **SR.NO.** | **Organism** | **Zone of Inhibition (In mm)** | | | |
| **AgNO3**  **(10 mM)** | **AgNPS** | **PVP AgNPS** | **Ampicillin**  **(1 mg/ml)** |
| 1 | Escherichia coli | 15 | 19 | 20 | 15 |
| 2 | Staphylococcus Aureus | 15 | 18 | 19 | 17 |

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

**Figure: 7**  **Antibacterial zones of inhibition of AgNPs and PVP AgNPs in comparison with**

**Standard Ampicillin.**

**3.6 Antioxidant activity of AgNPs and Polymer capped AgNPs**

DPPH is a steady compound that can be reduced by accepting hydrogen or electrons and has been widely applied to determine antioxidant activity. AgNPs showed effective antioxidant potential as their radical scavenging ability was increasing with the increment concentration. The figure 8 shows the antioxidant activity of the AgNPs what about 48.53%. PVP AgNPs was about 51.15%.Results confirmed that the polymer capped AgNPs have more antioxidant activity than AgNPs. The antioxidant property of the AgNPs is due to the absorption of plant constituents on the silver nanoparticles [46].

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

**4. Conclusion**

Silver nanoparticles were successfully synthesized using an extract of *ziziphus nummularia* leaves using AgNO3 salt solution. The formed AgNPs were further functionalized with PVP to enhance its biocompatibility without any hazardous or toxic material further. At the preliminary level, the formation of AgNPs and polymer AgNPs was confirmed by the colour change of solution various characterization techniques were used to confirm the comparison of Ag NPs and polymer capped AgNPs. The UV visible confirmed the formation of AgNPs trolls through visible colour change to dark brown after 2 hours peak at 431 nm. The FTIR spectra gave information of the different functional groups in the *Ziziphus nummularia* extract responsible for the biogenic formation of AgNPs and polymer-formed AgNPs. Crystalline nature and an average particle size of 9.20 nm AgNPs and polymer capped AgNPs were confirmed by XRD analysis.HR-TEM imaging microscopy which showed a spherical shape with a particle size of 2 to 25 nm. Good Antibacterial Activity of AgNPs and polymer capped AgNPs was performed against gram-positive and gram-negative bacteria which considerable zone of inhibition. Also, the good antioxidant activity of synthesized silver nanoparticles and polymer capped silver nanoparticles. This investigation described the eco-friendly and cost-effective biological method to synthesized polymer capped nanoparticles for antibacterial and antioxidant activity.

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