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IJIEMR Transactions, online available on 18th Feb 2022. Link :http://www.ijiemr.org/downloads.php?vol=Volume-12&issue=Issue 02

DOI: 10.48047/IJIEMR/V12/ISSUE 02/65

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Volume 12, ISSUE 02, Pages: 416-422

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Exploring the Antimicrobial Properties of Silver Nanoparticles against Various Bacterial Strains

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Abstract
The advancement in the field of nanobiotechnology and nanomedicine in recent years has opened up new avenues for exploring the antibacterial and antifungal properties of metal nanoparticles, including Silver nanoparticles (Silver NPs). Silver NPs have gained popularity due to their broad range of biological applications, such as antibacterial, antifungal, antiviral, and anti-inflammatory properties, making them suitable for biomedical applications, particularly in wound care. This study presents the synthesis and characterization of Silver NPs using both green and chemical methods. The antibacterial efficacy of the synthesized Silver NPs was evaluated against Gram-negative bacteria (Klebsiella pneumonia, Escherichia coli) and Gram-positive bacteria (Staphylococcus aureus) using the disk diffusion method.

Keywords: Silver nanoparticles; antibacterial studies; Gram-positive; Gram-negative; disk diffusion method;

Introduction

The field of nanotechnology holds great potential for advancing medicine and biomedical applications, including diagnosis [1], treatment [2], medical device coating [3], drug delivery [4], medical textiles [5], and wound dressings [6]. Silver nanoparticles (Silver NPs) are particularly attractive due to their unique physical, chemical, and biological properties at the nanoscale, including increased surface area-to-volume ratio. These properties have made Silver NPs a commonly used nanomaterial in healthcare for centuries, in the form of metallic silver, silver nitrate, and silver sulfadiazine for the treatment of burns, wounds, and bacterial infections [7 -12].

The antibacterial, antifungal, antiviral, and anti-inflammatory properties of Silver NPs make them a highly sought-after material in biomedical applications [13, 14]. Silver has been normally used for curing infections, mending of wound and treating diseases. Silver nano particles are less harmful, safe inorganic in nature, they are being used in medical applications and is known to kill around 650 micro-organisms that cause infections. Silver NPs have a broad antibacterial effect on a range of Gram-negative and Gram-positive bacteria, including antibiotic-resistant strains, such as Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli [15-18]. Many pharmaceutical companies are exploring the antimicrobial properties of Silver NPs for the development of antibiotics.

In addition to their antibacterial properties, Silver NPs have antiviral activity and may interfere with fusion of the viral membrane, inhibiting viral penetration into host cells and thus act against HIV-1[19], hepatitis B virus [20], respiratory syncytial virus [21], herpes simplex virus type 1[22], and monkeypox virus[23]. They have also been shown to exhibit anti-inflammatory properties, promoting the healing of chronic leg

ulcers by reducing bacteria and inflammation in the wound bed, and thus decreasing inflammatory response [24]. Silver NPs' ability to reduce cytokine release and matrix metalloproteinases [24, 25], decrease lymphocyte and mast cell infiltration [26], and induce apoptosis in inflammatory cells [24, 27], may explain their anti-inflammatory mechanisms.

Furthermore, Silver NPs are effective antifungal agents against a wide range of common fungi, including Aspergillus, Saccharomyces, Candida albicans, Candida glabrata, Candida parapsilosis, Candida krusei, and Trichophyton mentagrophytes, by disrupting cellular membranes and inhibiting their growth [28-31].

Synthesis methods

The production of silver nanoparticles (Silver NPs) with varying sizes, shapes, morphologies, and stability can be achieved by utilizing different synthesis methods. Biosynthesis using plant extract is a promising and eco-friendly technique for producing well-defined and controlled silver nanoparticles. This method is cost-effective and can be used as an agar medium. The aim of the present study is to produce silver nanoparticles at room temperature with minimal energy consumption and increased stability. The green method uses the extract from Catharanthus roseus leaves, while the chemical method uses tannic acid as a reducing and stabilizing agent under alkaline conditions at room temperature. The antimicrobial effectiveness of the silver nanoparticles produced by these two methods has been compared to that of the antibiotic Ciprofloxacin.

Experimental Details

Materials

The following chemicals are used for sample preparation and purification without further purification: silver nitrate ($AgNO_3$, 99.999% purity from Merck India), tannic acid (C_{76} $H_{52}O_{46}$ from Fluka), potassium carbonate (K_2CO_3 from Merck India), and ethanol (from Merck India). The chemicals used are of analytical grade and the solutions are prepared using deionized water obtained from a Deionizer Millipore Simplicity UV system. The glassware used is cleaned with distilled water and dried in an oven.

Microbial Cultures

For the purpose of the antimicrobial activity study, a set of three clinical isolates, including Gram-positive and Gram-negative organisms, is obtained from the Department of Microbiology at Osmania Medical College in Hyderabad. The strains used in the study are Klebsiella pneumonia, Escherichia coli, and Staphylococcus aureus. The purity of these strains is tested using standard microbiological methods. The bacterial stock cultures are maintained on Mueller Hinton agar (MHA) slants and stored at 4°C.

Sample Preparation by Green Synthesis

Twenty grams of finely chopped Catharanthus roseus leaves are boiled in 100 ml of water for 20 minutes and filtered using Whatman filter paper to obtain the extract. The extract (10 ml) is mixed with 90 ml of 2 mM silver nitrate ($AgNO_3$) solution. The reduction of Ag^+ to Ag^0 is confirmed by observing the change in colour of the solution from colourless to dark brown. The mixture of Catharanthus roseus broth and Silver NPs is centrifuged at 4000 rpm for 15 minutes, and the precipitate is thoroughly washed with sterile distilled water to remove any impurities. The purified sample is then dried at 60°C and the sample has been sent for further characterizations.

Sample Preparation by Chemical Synthesis

The synthesis of Silver NPs is achieved through the chemical method using tannic acid, a plant-derived polyphenolic compound, as both the reducing and stabilizing agent in an alkaline environment at room temperature [32]. This approach is efficient, eco-friendly, and cost-effective. Fresh working solutions are made using de-ionized water, and weight measurements are taken with a Citizen CX 285 N electronic balance with a high degree of accuracy of 1×10^{-5} g, with readability 0.01/0.1 mg and repeatability ± 0.03/0.1 mg. 4 ml Silver nitrate solution (1.32×10^{-3} M) is added to tannic acid while stirring, following a specific ratio proposed by Franco Cataldo [33]. This particular

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composition is chosen as it has been found to have least size by TEM and DLs studies and with remarkable properties with desirable size and properties. The slight alkaline nature (pH = 8.0) of tannic acid is confirmed using an Elico pH meter before adding $AgNO_3$. The formation of Silver NPs is indicated by a rapid change in colour to a pale yellow hue. Stirring is continued until the colour remained unchanged, resulting in monodispersed and non-agglomerated particles with a uniform size distribution. The solution gradually darkened as more silver is added. The precipitated nanoparticles are washed several times using ethanol as the solvent and dried at 60^0C. The purified sample is then sent for further characterization.

Characterization techniques

XRD Analysis

The crystalline nature of the prepared Silver NPs from both methods is confirmed through X-ray diffraction (XRD) analysis. The XRD measurement is performed using a Panalytical Xpert-PRO 3050/60 X-ray diffractometer, operating at 30 kV and 100 mA. The XRD spectrum is recorded using CuKα radiation with a wavelength of 1.5406 Å, covering a 2θ range of 20°–80°. The XRD patterns of both samples are displayed in Figure 1.1.

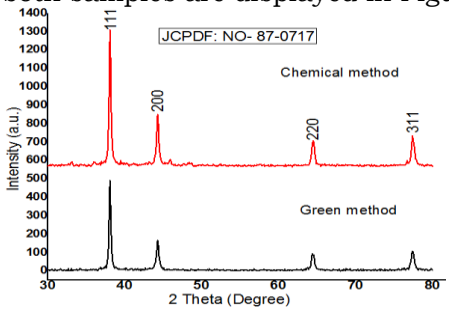

Fig.1.1. The XRD pattern of Silver NPs (a) by Chemical method and (b) by green method

SEM Analysis

The surface morphology of the Silver NPs is examined using a Scanning Electron Microscope (SEM), as shown in Figure 1.2. This global technique provided a detailed examination of the silver powder morphology and chemical composition analysis. The SEM images clearly show that the silver particles prepared through the green method are composed of micrometer-sized conglomerates made up of much smaller particles, compared to those prepared through the chemical method. However, the structure of the observed nanoparticles could not be determined due to difficulties in obtaining higher magnification.

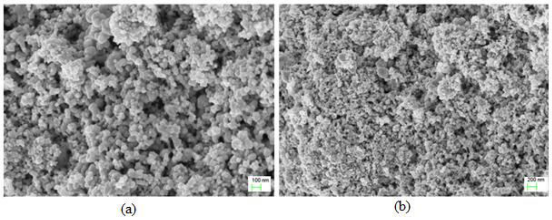

Fig.1.2. The SEM pattern of Silver NPs by (a) by Chemical method (b) by Green method

Antimicrobial activity assay of silver nanoparticles by Disk diffusion method

The antimicrobial activity of synthesized silver nanoparticles (NPs) against Klebsiella pneumonia, Escherichia coli, Staphylococcus aureus, and the antibiotic Ciprofloxacin was studied using the disk diffusion method [34-37]. The standard antibiotic disks are purchased from Sigma Aldrich in Delhi. The bacterial strains are reactivated from stock cultures by transferring them into Mueller Hinton Broth (MHB) and incubating them at 37°C for 18 hours. An inoculum containing 10^6 colonies forming units per milliliter (1 x 10^6 CFU/ml) is added aseptically to Mueller Hinton Agar (MHA) medium and poured into sterile petri dishes. Different test compounds at concentrations of 50-200 µg are added to wells (8 mm in diameter) punched on the agar surface. The plates are incubated overnight at 37°C, and the diameter of the inhibition zones (DIZ) around each well is measured in millimeters and is shown in Figure 1.3. The minimum inhibitory concentration (MIC) is measured in triplicates on three different days. Ciprofloxacin at a concentration of 50 µg is used as a reference to determine the sensitivity of

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the microorganisms under test. Distilled water is used as a negative control. The diameters of the inhibition zones around each well with the green synthesized silver NPs and chemically synthesized silver NPs are recorded and are shown in Table 1.1 and Figure 1.4.

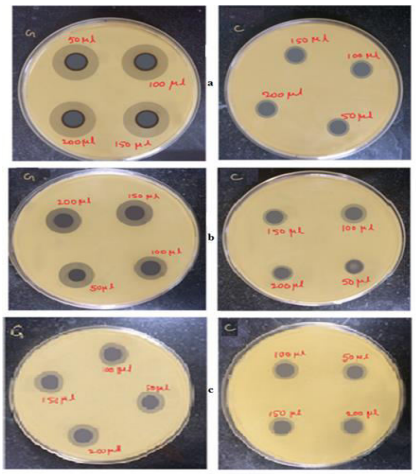
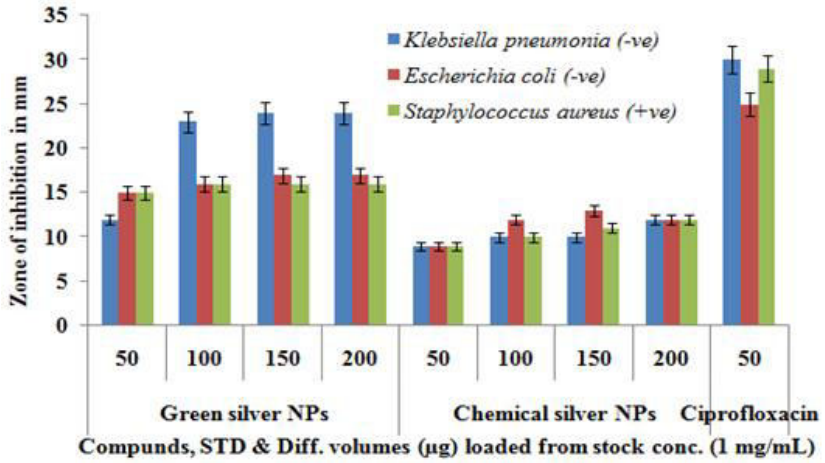


Fig.1.3. Antibacterial activity of silver NPs, G) by Green synthesis C) by Chemical synthesis against (a) *Klebsiella pneumonia (b) Escherichia coli, & (c). Staphylococcus aureus*

Table 1.1. Zone of inhibition (Diameter in mm at 1 mg/mL) of different organisms

S. No.	Organisms	Zone of inhibition (Diameter in mm at 1 mg/mL)								
		Green silver NPs				Chemical silver NPs				Ciprofloxacin
		50 µg	100 µg	150 µg	200 µg	50 µg	100 µg	150 µg	200 µg	50 µg
1	Klebsiella pneumonia (-ve)	12	23	24	24	09	10	10	12	30
2	Escherichia coli (-ve)	15	16	17	17	09	12	13	12	25
3	Staphylococcus aureus (+ve)	15	16	16	16	09	10	11	12	29



Results & discussion

The X-ray Diffraction (XRD) pattern of silver nanoparticles (NPs) synthesized using a chemical method revealed four prominent Bragg reflections, which could be indexed based on the face-centered cubic (FCC) structure of silver NPs. The comparison of the XRD spectrum with the standard spectrum confirmed that the silver NPs formed in the experiment are in

the form of nanocrystals, as indicated by peaks at 2θ angles of 38.02, 43.58, 64.32, and 77.22 degrees, corresponding to (111), (200), (220), and (311) planes, respectively. These peaks could be indexed according to the FCC crystal structure of silver. The interplanar spacing (d-spacing) values for (111), (200), (220), and (311) planes are calculated to be 2.336, 1.955, 1.436, and 1.224 Å, respectively, and the hkl values are obtained from JCPDS No. 87-0717. The average crystalline size
is calculated using the Debye-Scherrer formula. $$D = \frac{K\lambda}{\beta \cos\theta}$$

The average crystalline size of the silver nanoparticles is calculated using the Debye-Scherrer equation, which takes into account the average crystalline size (D), the geometric factor (k=0.9), the wavelength of the X-ray radiation source (λ), and the angular full-width at half maximum (FWHM) of the XRD peak (β) at the diffraction angle (θ) [39]. The calculated average crystalline size of the silver NPs synthesized using the green method was 27.5 nm.

The XRD pattern of silver NPs prepared by the green method confirmed that the Ag0 NPs formed from the reduction of Ag+ ions are also nanocrystalline in nature, with an average crystalline size of 24.5 nm calculated from the XRD data using the Debye-Scherrer equation. There are no additional peaks detected in the XRD pattern of the green method-prepared silver NPs, due to the low concentration of bio-compounds compared to the XRD pattern of silver NPs prepared using the chemical method.

The antibacterial activity of silver nanoparticles synthesized using both the green and chemical methods is tested against Gram-negative bacteria (Klebsiella pneumonia and Escherichia coli) and Gram-positive bacteria (Staphylococcus aureus) using the disk diffusion method. The results showed that the silver NPs displayed good antimicrobial activity against the test organisms. The highest antibacterial activity is observed in Klebsiella pneumonia and Escherichia coli when the NPs are synthesized using the green method, followed by Staphylococcus aureus. In the case of NPs synthesized using the chemical method, the highest antibacterial activity is observed against Escherichia coli and Staphylococcus aureus, followed by Klebsiella pneumonia. A reference antibiotic, ciprofloxacin at a concentration of 50 µg, is used to determine the sensitivity of the test organisms and the results are reported in Table 1.1.

Despite the widespread study of the antimicrobial effect of silver nanoparticles, the exact mechanism remains unclear. One proposed explanation is that the small size of synthesized silver NPs, which have a large surface area, allows for better contact and interaction with the bacterial cell wall, compared to larger NPs [40]. It is believed that silver ions released from Ag NPs can bind to and penetrate the cell wall, causing structural changes in the membrane, increasing cell permeability, and interacting with vital enzymes [41] containing sulfur and thiol groups, such as NADH dehydrogenases. This interaction can disrupt the respiratory chain and phosphorus containing compounds such as proteins and DNA may inhibit some functions in cells, such as preventing cell division and DNA replication and lead to the loss of viability and ultimately cell death [42]. The formation of free radicals by silver NPs also induces oxidative stress, which is another potential mechanism of cell death [43]. The greater surface area in smaller silver NPs enables a lower concentration of the particles to inhibit bacterial growth. Studies have shown that the combination of silver NPs with antibiotics such as amoxicillin can result in a greater antibacterial effect on E. coli than the use of either substance alone [44]. Silver NPs may also modulate signal transduction by changing the phosphotyrosine profile of bacterial peptides, offering a potential mechanism for antibacterial activity.

Conclusion

The production of Silver NPs through green and chemical methods is simple, cost-effective, and environmentally friendly, avoiding the risks associated with the use of hazardous reducing or capping agents. These methods have the potential to be a viable alternative for

producing Silver NPs for biomedical applications. The disruption of bacterial cell walls by the adsorption of nanosilver particles on the surface indicates that the Silver NPs solution has high antimicrobial activity due to the impregnation of Silver NPs inside the bacteria. This study highlights the potential for using Silver NPs as a treatment for infectious diseases caused by bacteria. The combination of Silver NPs and antibiotics has also shown improved efficacy against bacterial pathogens, making them promising candidates for the treatment of infectious diseases.

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