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Biosynthesis of Silver Nanoparticles Using Vinca Rosea Leaf Extract and its Biological Applications against Human Pathogens

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ABSTRACT

We report green synthesis of silver nanoparticles (Ag-NPs) for biological applications against human pathogens. Vinca rosea leaf extract was used to swiftly generate silver nanoparticles which were characterized by different techniques. The structural and optical properties of the synthesized nanoparticles were measured using UV-visible spectrophotometer, X-ray diffractometer, scanning electron microscope (SEM) coupled with energy-dispersive X-ray spectroscopy (EDS), and transmission electron microscopy (TEM). The surface morphology of the nanoparticles was found to be of spherical shape with crystalline phase. Further, the antibacterial activity, anti-cancer, anti-diabetic, anti-microbial, anti-oxidant properties, anti-helminthic, anti-ulcer, hypotensive, anti-diarrheal, wound healing, and hypolipidemic activity of Vinca rosea reduced silver nanoparticles reveal that they are highly effective agents for gram-positive and gram-negative bacteria. The green silver nanoparticles have shown significant antibacterial potential against different bacterial strains.

Keywords: *Antibacterial Activity, Biosynthesis, E. coli, S. Aureus, Vinca rosea*

1. Introduction

The nanotechnology area has been a fascinating field, as lots of research work is going to be done to explore the various possible applications of nanoparticles [1, 2]. Various approaches that have been explored to synthesize nanoparticles are broadly classified into bottom-up and top-down processes. An essential component of nanotechnology research is the creation of an environmentally friendly method for creating silver nanoparticles [3, 4]. There have been reports [5-8] on the synthesis of metal nanoparticles utilizing various techniques. A novel method of synthesis involves producing metal nanoparticles from bacteria, algae, plant leaves, stems, and flowers [9-12]. The green synthesis of nanoparticles using leaf extract has been attracting the scientific community because of its environmentally benign and cost-effective nature. This method is environment-friendly, non-toxic, reliable, and low-cost, whereas the synthesized nanoparticles have various applications [13-17]. Noble metal nanoparticles like Au, Ag, and Pt as well as their composites have been synthesized for various applications like sensors, solar cells, catalysis, and biomedical [7, 18–21]. Due to their unique physiochemical properties like high surface-to-volume ratio, surface plasmon resonance in the visible region, and reactivity towards the bacterial cell wall, they can be used in enormous applications. Syntheses of different metal nanoparticles using green methods for biological applications have been reported so far [9, 22–32]. Silver nanoparticles have shown good antibacterial activities against both gram-positive and gram-negative bacteria like S. aureus and E. coli. The antibacterial activity of silver species has been well-known since ancient times because silver is not toxic to human cells.

In this article, we reveal a novel approach to synthesizing silver nanoparticles using Vinca rosea leaf extract. Leaf extract has the potential to have anti-cancer, anti-diabetic, anti-microbial, anti-oxidant properties, anti-helminthic, anti-ulcer, hypotensive, anti-diarrheal, wound healing, and hypolipidemic activities. Hence, our approach takes a new direction in the medical field by using Vinca rosea-reduced silver nanoparticles to treat deadly diseases effectively and economically as well as prevent hazardous chemicals.

2. Materials and Methods

2.1 Materials

Vinca rosea leaves collected from the botanical garden, silver nitrate ($AgNO_3$) of analytical grade purchased from Hi-media, doubly distilled water, and Whatman’s filter paper No. 4. All chemicals were used without further purification.

2.2 Preparation of leaf extract

Vinca rosea plant leaf extract was prepared after washing twice with doubly distilled water, drying and chopping 30 g of Vinca rosea leaves into 30 ml of water and kept at 70^{o}C for 45 minutes until the color of the sample was changed. The formation of the extract was confirmed by a change in the dark green color of the solution-binary mixture. Whatman's filter paper No. 4 was employed to filter the obtained extract twice to remove the broth and get a pure extract. The process of Vinca rosea leaf extraction is shown in Fig. 1.

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Fig. 1: Preparation of Vinca rosea leaves extract

2.3 Synthesis of silver nanoparticles

A typical reaction procedure for the formation of Ag nanoparticles was performed in aqueous medium. 0.01M of $AgNO_3$ was taken and dissolved in 5 ml of deionized water, kept for some time to form the binary mixture, and then heated to 70°C with constant stirring for 20 min.

The Vinca rosea leaf extract was added drop by drop until it showed a color change from colorless to dark yellow.

Fig. 2: Green synthesis of silver nanoparticles using Vinca rosea

A change in color confirms the formation of Ag nanoparticles, as shown in Fig. 2. Ag-NPs were centrifuged and washed several times to get an impurity-free Ag nanoparticle colloidal solution, then dried to get a dry powder the sample. The prepared Ag nanoparticles were used for further characterization to study the surface morphology and particle size of the prepared material.

2.4 In-vitro bactericidal activity

In-vitro efficacy of Ag-NPs effect on gram-positive Staphylococcus aureus and gram-negative Escherichia coli was verified utilizing the disc agar-diffusion method. The testing bacteria (1×10^6 CFU/mL in nutrient broth) were spread on nutrient agar (NA) plates. A clean sheet of sterilized paper disc (6 mm) was placed on the NA plates to observe the bacterial susceptibility to NPs. 30 and 60 µg concentrations of NPs suspension (based on the Minimum Inhibitory Concentration assay) were placed on the paper disc. As controls, 100 g of streptomycin and distilled water were used. The inhibitory zone was measured after 24 hours of 37°C incubation on the plates. The findings of the experiment which were completed in triplicate are displayed as the average size of the inhibitory zone.

2.5 Characterization techniques

UV-Vis spectra are employed to investigate the surface plasmon resonance (using Ocean OpticsHR4000 spectrophotometer). To access the crystalline nature of the prepared AgNPs, the data was collected utilizing X-ray diffractometer device (Rigaku) with radiation of Cu Kα = 1.54178 Å wavelength and angle 2θ ranging from 20° to 80°. The size and shape of the nanoparticles were observed using SEM (JEOL JSM-6360, Mira-3, Tescan, Brno, Czech Republic). The grain particle sizes of the synthesized nanoparticles were confirmed by TEM analysis.

3. Results and Discussion

Silver nanoparticle fabrication using Vinca rosea leaf extract was carried out. The produced nanoparticles have been identified based on their optical characteristics, structural analysis, morphology, and crystalline nature. Furthermore, the antibacterial activity was evaluated against gram-negative bacteria (E. coli) as well as gram-positive bacteria (S. aureus). The Phytochemicals of Vinca rosea (Fig. 3) are alkaloids like vinblastine, vincristine, vindesine, and vinorelbine, which are responsible for reducing the silver salt into silver nanoparticles.

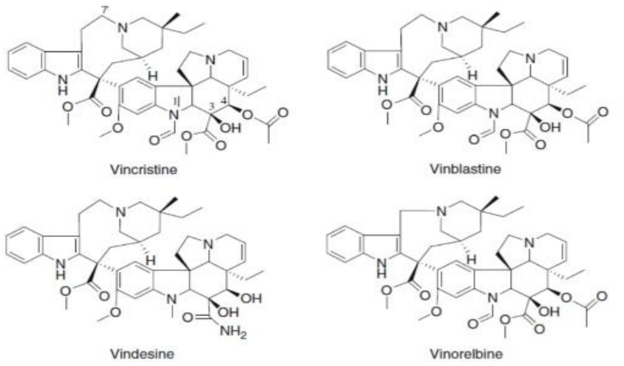

Fig. 3: Phytochemicals in Vinca rosea

3.1 Optical analysis of Ag nanoparticles

The absorption spectrum of synthesized Ag nanoparticles was recorded using UV-visible spectrophotometer. The absorption peak at 420 shows preliminary confirmation of the formation of nanoparticles (Fig. 4). It has been reported that maximum absorption occurs around 420 nm [10, 33–35], which is the characteristic of Ag nanoparticles.

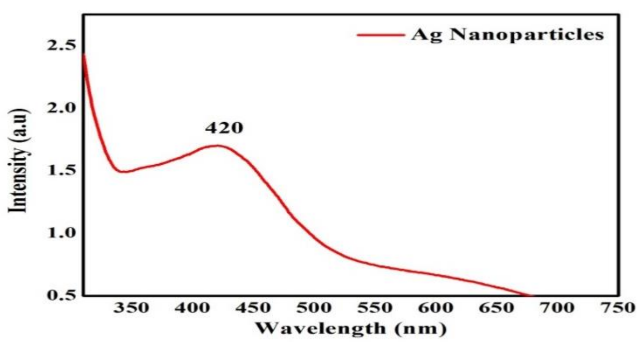

Fig. 4: UV- Visible spectrum of Ag nanoparticles.

3.2 Surface morphology and elemental analysis

The scanning electron microscope (SEM) images displayed in Fig. 5 (a, b) exhibit spherical-shaped synthesized nanoparticles with an average diameter of 30 nm. The SEM images confirm well-dispersed Ag nanoparticles within the nanometer range [10, 33–38]. Elemental examination of the synthesized Ag nanoparticles was performed using Energy Dispersive X-ray (EDX) analysis coupled with SEM. The EDX analysis shows the formation of Ag nanoparticles (Fig. 5c). The spectra show the presence of C, O, and Ag in the synthesized nanoparticles. The phytochemicals present in the extract help reduce particles to nanosize [38, 39]. These biochemicals have capping and reducer effects.

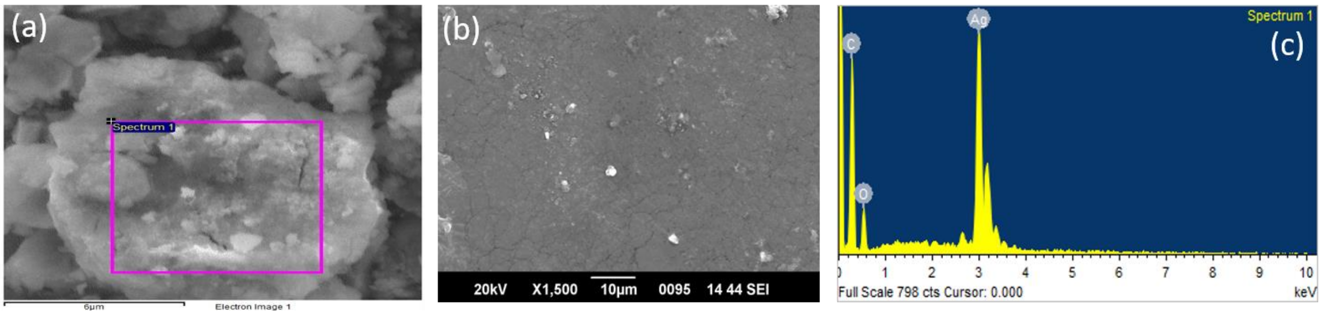

Fig. 5 (a, b): Scanning electron microscope images and (c) EDX image of synthesized Ag nanoparticles

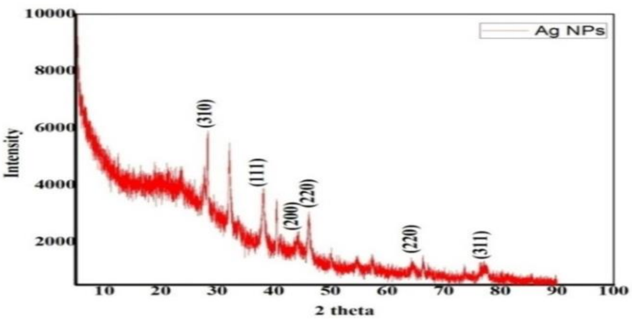

Fig. 6: X-ray diffraction patterns of Vinca rosea-produced silver nanoparticles (Ag-NPs)

3.3 XRD analysis of Ag nanoparticles

Fig. 6 shows the XRD images of AgNP's synthesized using Vinca rosea. The XRD pattern of synthesized nanoparticles reveals the FCC (Face-centered Cubic) structure of the prepared nanoparticles. In addition, all the AgNP's have a similar diffraction pattern and XRD peaks at 2θ of 28.39°, 38.08°, 44.13°, 46.15°, 64.26°, and 77.08° and could be attributed to 310, 111, 200, 220, 220 and 311 crystallographic planes of the FCC cubic silver crystals, respectively (JCPDS file no. 84-0713 and 04-0783) [12, 38, 40].

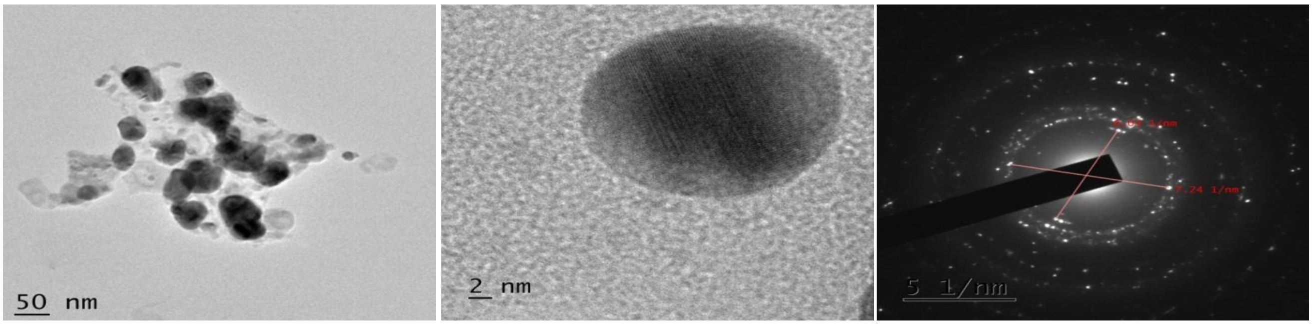

Fig. 7: (a) TEM image of Ag nanoparticles (b) A HR-TEM image of a single Ag nanoparticle (c) SAED pattern

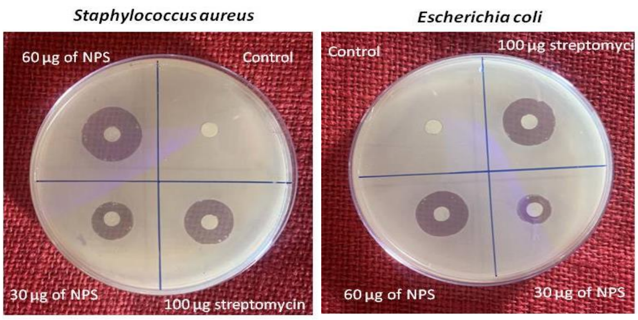

Fig. 8: Antibacterial activity of silver nanoparticles using gram-positive and gram-negative bacteria

Thus, it is evident from the XRD spectrum that the produced in this work were crystalline in nature. Due to the imperfections, it is believed that silver was the only crystalline phase present in the prepared samples. In both XRD and EDX analyses, it was confirmed that the prepared samples were silver nanoparticles.

3.4 HR-TEM analysis of Ag nanoparticles

The prepared silver nanoparticles were treated with nitrate $AgNO_3$ solution using Vinca rosea leaf extract. Fig. 7(a, b) shows the HR-TEM analysis of synthesized nanoparticles with the green synthesis method. UV-Vis absorption spectra show that the broad SPR band contains a peak at 420 nm. The existence of a peak shows that there is a wide dispersion of Ag hydrosol present. Thus, the UV-visible spectral investigation [41–43] is consistent with the existence of large distributions of particles in the HR-TEM image. The selected area electron diffraction (SAED) pattern shown in Fig. 7(c) confirms the well-crystalline structure of Ag nanoparticles [44, 45]. Additionally, the HR-TEM micrographs verified that the nanoparticles were fabricated in the 30-35 nm size range. Similar SAED patterns of silver nanoparticles have also been observed in other works [44–46].

3.5 In-vitro bactericidal activity

Different NP concentrations' *in vitro* bactericidal efficacy against S. aureus and E. coli was evaluated by the disc diffusion method. At 60 µg mL^{-1} NPs, S. aureus showed an inhibition zone of 17.8±0.8 mm, whereas 30 µg mL^{-1} NPs showed an inhibition zone of 12.5±1 mm [3, 6, 47–49]. Similarly, E. coli showed 16.2±1.1 mm and 11.4±0.8 mm at 60 and 30 µg mL^{-1} NPs, which are statistically significant compared to the control.

Moreover, positive control streptomycin shows an inhibition zone of 17.2±0.8 mm and 16.8±1.2 for S. aureus and E. coli, respectively (Fig. 8). This shows that the synthesized Ag nanoparticles play an active role with excellent activity for the gram-negative bacteria with MIC 16.8±1.2.

4. Conclusion

We have successfully synthesized silver nanoparticles via a Vinca rosea leaf extract-mediated process. The synthesized nanoparticles were capped by the phytochemicals present in the leaf extract. The UV-Vis spectrum shows the formation of Ag nanoparticles, with the surface plasmon resonance peak at 420 nm. The synthesized nanoparticles have a crystalline structure as confirmed by the XRD pattern. The analysis indicates the formation of Ag nanoparticles with an average size of 30 nm. The synthesized Ag nanoparticles have excellent free radical and antibacterial activities. From our findings, the synthesized nanoparticles show growth-inhibitory activities against both gram-positive and gram-negative pathogenic bacteria. Thus, the synthesized nanoparticles can be used in medicine as therapeutic agents due to their antimicrobial activities against the bacteria evaluated in vitro.

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