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Silver Nanoparticles & Its Antimicrobial Activity From*AzadirachtaIndica* (Neem) Leaf Extract.

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Abstract

Nanotechnology is an emerging field that focuses on manipulating matter at the atomic and molecular scale, typically within the size range of 1 to 100 nanometers in one dimension. This technology has gained attention due to the unique behaviour of materials when reduced to nanoscale dimensions. One significant application is the utilization of nanostructured materials, such as silver nanoparticles, for removing harmful substances from air and water. Silver nanoparticles are renowned for their ability to eliminate bacteria. They are now being incorporated into next-generation dressings to combat infections. These nanoparticles can also be integrated into materials and fabrics, rendering them sterile. Recent research has revealed that silver nanoparticles can be produced through a green synthesis method using leaf extracts from the *Azadirachta indica* (neem). The presence of flavonoids and terpenoids in the extract serves as both reducing and capping agents. This study involved treating an aqueous solution of silver nitrate (AgNO3) with neem leaf extract, resulting in the formation of crystalline silver nanoparticles. The confirmation of nanoparticle formation was achieved using UV-visible spectroscopy, which displayed a peak absorption at 440 nm. The silver nanoparticles displayed antimicrobial properties against both Gram-positive and Gram-negative bacteria. For instance, they formed clear zones around them, with a diameter of 4 mm for *Bacillus subtilis* and 3 mm for *Escherichia coli* when using pure silver nanoparticles. Additionally, the crude extract of silver nanoparticles produced a 2 mm clear zone for *Escherichia coli*. Antifungal activity of Silver nanoparticles against *Capnodium spp* showed 4 mm which indicated the inhibition of fungal growth.

Keywords:*Azadirachta indica* (Neem),Silver Nanoparticles, Antimicrobial Activity.

1. INTRODUCTION

Neem leaf extract was chosen as the bio reductant in this study for several reasons. Firstly, neem is readily available in India. Secondly, it eliminates the need for external stabilizing agents during particle synthesis. Lastly, neem leaf extract is known for its antibacterial properties, which could enhance the antimicrobial properties of the silver nanoparticles produced due to synergistic effects.

Nanotechnology has emerged as a highly captivating field in multidisciplinary science (Arvizo et al., 2012). Nanoparticles (NPs), owing to their small size, exhibit exceptional physical and chemical properties (Sau et al., 2010). These properties have found successful applications in various technological and biomedical domains (Doane and Burda, 2012). Extensive efforts have been directed towards creating novel materials based on nanoparticles for applications like biomedical devices, implants, and surgical instruments with enhanced functionality. Their use extends to areas such as biosensing, bioimaging, and healthcare (Pereira et al., 2015). Therefore, there is a strong desire to develop environmentally friendly methods for the synthesis of nanoparticles with tailor-made structural properties.

Traditionally, NPs have been synthesized using diverse physical and chemical methods based on their suitability for specific applications (Rao et al., 2012). Physical methods, including ball milling, electric arc discharge, flame pyrolysis, and laser ablation, often involve costly equipment and high temperature and pressure (Ladj et al., 2013). In contrast, chemical methods involve the reduction or decomposition of metal complexes in solutions using various chemical reductants, such as sodium borohydride or hydrazine, often at elevated temperatures (Betke and Kickelbick, 2014). However, these methods frequently employ toxic reagents, reductants, stabilizers, and organic solvents that can pose environmental and health hazards. Green chemistry has emerged as a solution, emphasizing the synthesis of chemical products, including NPs, with reduced environmental impact by eliminating hazardous materials from the preparation process (Nabikhan et al., 2010).

Various methods have been employed for the synthesis and stabilization of silver nanoparticles (AgNPs), including chemical (Hu et al., 2009), physical (Jung et al., 2006), and biological approaches (Velavan et al., 2012). Biological methods have gained significant attention due to their cost-effectiveness and eco-friendliness compared to chemical and physical methods. Many studies have reported the synthesis of AgNPs using biological materials such as bacteria (Deshmukh et al., 2012), algae (Xie et al., 2007), fungi (Ingle et al., 2008), and plants (Gardea-Torresdey et al., 2003). Among these biomaterials, plant extracts have garnered attention for their eco-friendly nature, affordability, and wide availability. Furthermore, nanoparticles synthesized using plant extracts tend to remain stable under various conditions (Tran and Le, 2013). Numerous studies have explored the synthesis of AgNPs using extracts from different plant parts.

Moreover, it is widely documented that AgNPs synthesized from plant extracts often exhibit strong antibacterial activity against various bacteria, including E. coli, S. aureus, and P. aeruginosa (Rai et al., 2009). The effectiveness of AgNPs in this regard is influenced by their size, shape, and the stabilizing properties of the nanoparticles (Morones et al., 2005). Capping agents are commonly used to stabilize nanoparticles, and capped AgNPs typically demonstrate superior antibacterial activity compared to uncapped ones (Amato et al., 2011). In this study, we investigated the antibacterial properties of AgNPs against a range of gram-positive and gram-negative bacteria, including some known human pathogens.

2. MATERIALS AND METHODOLOGY

2.1 Preparation of the leaf extract:

Fresh and healthy neem leaves were taken from Narsinh Mehta university, Junagadh campus Gardenand properly cleaned with tap water before being cut into small pieces and dried at roomtemperature to remove all dust and undesired visible particles. Approximately 10 g of finelyincised leaves were weighed separately and put to 250 mL beakers containing 100 mL distilled water, where they were heated for roughly 1 hour. The extracts were then filteredusing filter paper to eliminate particulate debris and get clear solutions, which were then stored in 250 mL Erlenmeyer flasks at 4°C for further experiment.

2.2 Silver nanoparticle synthesis:

In 250 mL Erlenmeyer flasks, aqueous solutions of silver nitrate ($AgNO_3$) (1 mM) wereproduced, and leaf extract was added for reduction to Ag+ ion. The colour change of the combination from weak light to yellowish brown to reddish brown was observed for a maximum of 30 minutes throughout this time (time and colour change were recorded togetherwith frequent sample and scanning by UV-visible spectrophotometry). The reactions werecarried out at room temperature in the dark (to minimise photo activation of $AgNO_3$). Thecolour change from colourless to colloidal brown confirmed the complete reduction of$AgNO_3$ to Ag+ ions. After irradiation, the dilute colloidal solution was cooled to ambienttemperature and left for 24 hours to allow for complete irradiation and saturation, asmeasured by UV-visible absorption.

2.3 Characterization of silver nanoparticles:

UV-Vis spectral analysis was done by using Shimadzu UV-visible spectrophotometer (UV-1800, Japan). UV-Visible absorption spectrophotometer with a resolution of 1 nm between 200 and 800 nm was used. One millilitre of the sample was pipetted into a test tube and subsequently analysed at room temperature. Dynamic light scattering (Spectroscatter 201) was used to determine the average size of synthesized silver nanoparticles.UV-visible spectrophotometric scanning was used to determine full bioreductionand saturation (Ahmedet al., 2016).

2.4 Determination of antimicrobial activity of silver nanoparticles by well diffusion method:

The silver nanoparticles were centrifuged 10000rpm and the silver dissolve the pellet indistilled water.

2.4.1 Antibacterial activity:

2.4.1.1 Preparation of inoculum:

The active young cultures for the study were prepared by sub-culturing a loopful of cellsto the nutrient broth and incubated for 24 hours at 37˚C.

2.4.1.2 Agar well diffusion method:

The Petri plates were prepared with 20 ml of Mueller Hinton agar media and the testcultures were swabbed on the surface of the solidified media and allowed to dry for 10minutes and pour into agar and make a well. Biosynthesized iron nanoparticles added in thewell. $AgNO_3$was used as a control. The plates were incubated for 24 hrs at 37˚Cfor bacterial growth. Zones of inhibition were recorded in millimetres.

2.4.2 Antifungal activity:

2.4.2.1 Preparation of fungal inoculum:

The fungal culture of*Capnodium spp* was grown on PDA plates at 37˚C, 4 days for fungi. Spore suspensions were prepared in sterile distilled water.

2.4.2.2 Agar well diffusion method:

The antifungal activity of the AgNPs was evaluated using the diffusion method. The Petri plates were prepared with 20 ml of potato dextrose agar media and the fungi colony were perpendicular on the surface of the solidified media and allowed to dry for 10 minutes and pour into agar and make a well. Biosynthesized iron nanoparticles added in well. $AgNO_3$was used as a control. Then Petri plate incubated at 37ºC for 48 hours. Finally, the inhibition zones were measured.

3. RESULT AND DISCUSSION

3.1 Silver nanoparticle synthesis and UV-Vis spectroscopy

The addition of *A. indica* leaf extract to beakers containing an aqueous solution of silver nitrate (1 mM) resulted in a color change from light to yellowish brown, progressing to reddish brown, and eventually becoming colloidal brown after 24 hours. This color transformation indicated the production of silver nanoparticles (AgNPs), as depicted in Figure 1. The phenomenon is attributed to the excitation of surface plasmon vibrations in the silver nanoparticles, a process elucidated by Veerasamy et al. in 2011.

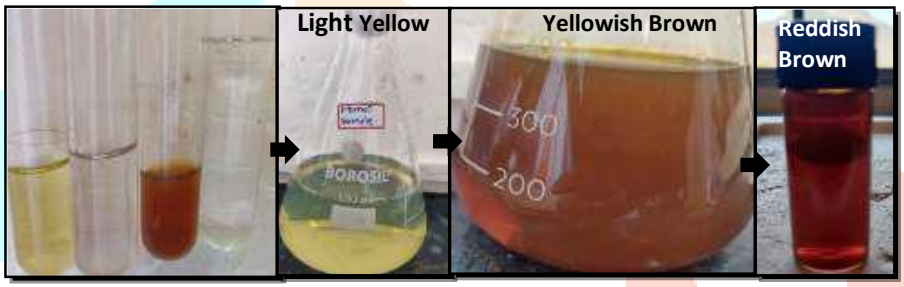

Figure 1: Colour Changes during AgNPs synthesis

The synthesized silver nanoparticles were further characterized through UV-Vis spectroscopy. A Plasmon resonance band was observed at 440 nm (Figure 2), which is consistent with previous reports in the literature (Obaid et al., 2017). Slight variations in the absorbance values suggest changes in the particle size (Tripathy et al., 2010). UV-Vis spectroscopy is a valuable tool for examining the size and shape of nanoparticles in aqueous suspensions, as acknowledged by Tripathy et al., 2010.

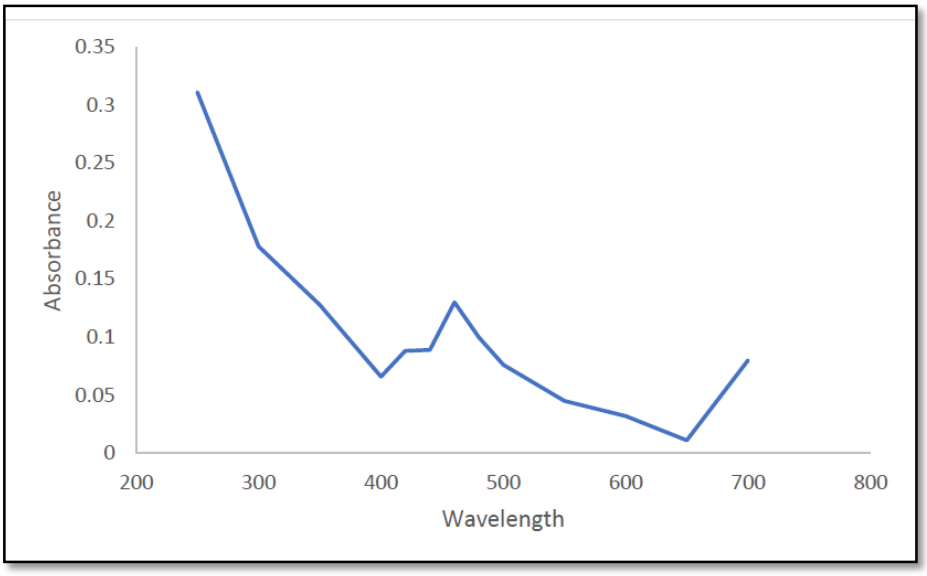

Figure 2: UV-vis spectra of AgNPs

Furthermore, the UV-Vis spectra showed an increase in the intensity of absorption peaks over regular intervals of time, and the color intensity of the solution became more pronounced with prolonged incubation. This indicates that the *A. indica* leaf extract acted as an efficient reducing agent, facilitating the rapid bio reduction of silver ions to form silver nanoparticles.

3.2 Antimicrobial activity of silver nanoparticles:

The use of elemental silver and its compounds as antimicrobial agents has a long history dating back to ancient times. Silver was employed to preserve water, often in the form of silver coins or vessels, as documented by Devi and Joshi in 2015. This historical use of silver for its antimicrobial properties demonstrates the enduring significance of silver-based materials in safeguarding public health and maintaining the purity of various substances.

3.2.1 Antibacterial activity of Silver Nanoparticles

We investigated the potential antibacterial properties of silver nanoparticles synthesized using *A. indica* leaf extract. Both the plant extract and the silver nanoparticles were promptly assessed for their antimicrobial effectiveness against two types of bacteria, namely, the Gram-positive *B. subtilis* and the Gram-negative *E. coli*. The observed zones of inhibition were 3 mm and 4 mm, respectively (Figure 3).

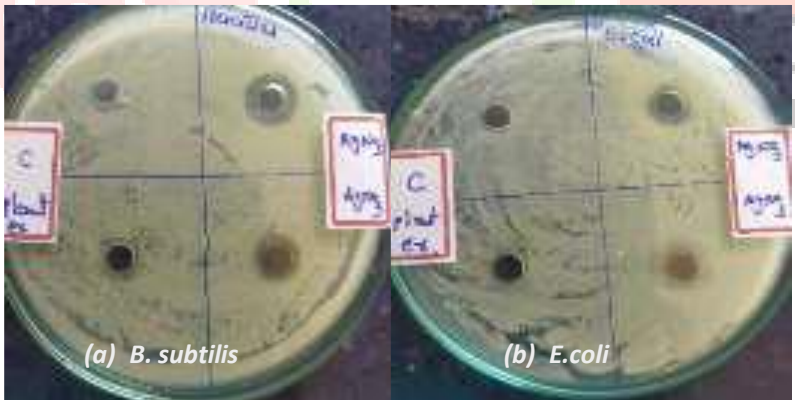

Figure 3: Antibacterial activity of AgNPs

Based on the size of the inhibition zones, it is evident that the synthesized silver nanoparticles exhibit notable antibacterial activity against both *E. coli* and *B. subtilis*. In contrast, the plant extract displayed a smaller inhibition zone of 2 mm, and the control group did not exhibit any antibacterial activity, as shown in Figure 3.

It is worth noting that while the plant extract alone was expected to possess antibacterial properties, it demonstrated relatively low activity in this experiment. This could be attributed to the extraction method used and the lower concentration employed during the experimentation. In contrast, the silver nanoparticles proved to be highly efficient in their antimicrobial properties. This enhanced performance can be attributed to their exceptionally large surface area, which allows for better contact with the cell walls of microorganisms, as suggested by Ibrahim in 2015.

3.2.2 Antifungal activity of Silver Nanoparticles

The antifungal activity of silver nanoparticles against *Capnodium spp.* was evaluated by observing the clear zone of inhibition on an incubated plate.

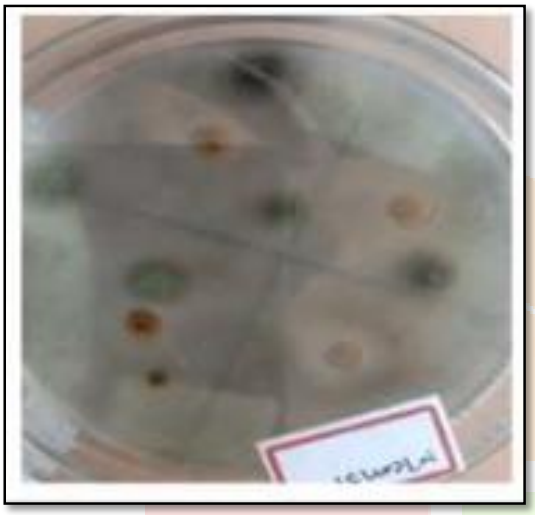

Figure 4: Antifungal activity of AgNPs

In the presence of silver nanoparticles, a clear zone with a diameter of 4 mm was observed, indicating the inhibition of fungal growth (Figure 4). In contrast, in the control plate, no zone of inhibition was observed. This result demonstrates the effectiveness of silver nanoparticles in inhibiting the growth of *Capnodium spp*.

4. CONCLUSION

The study presents a method for the synthesis of silver nanoparticles (AgNPs). In this method, *A. indica* leaves extract is used as a reducing agent. This suggests that the extract from *A. indica* leaves plays a crucial role in reducing silver ions to form AgNPs. The method you described seems to emphasize the use of environmentally friendly and non-toxic processes for AgNP synthesis, which is important in green chemistry. Furthermore, the study also investigates the antibacterial properties of the synthesized AgNPs. It is reported that these AgNPs display bactericidal (bacteria-killing) and fungicidal (fungus-killing) properties. This suggests that the AgNPs have potential applications as antimicrobial agents, which could be of significance in various fields, including medicine, agriculture, and water treatment. Thus present study hints at the possibility of extending the research to study the antibacterial activity of these AgNPs against resistant strains of bacteria. This is important because the emergence of antibiotic-resistant bacteria is a significant global health concern. Investigating the effectiveness of these AgNPs against such strains would be valuable and could lead to interesting results.

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