Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 7 (2025)
https://www.jneonatalsurg.com

An Insight On Phoenix Sylvestris Synthesized Agnps For Its Potential As Antimicrobial Agent And Wastewater Management

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Cite this paper as: Reddi Papa Rao, D.K. Acharya, K. Swapnavahini, Sumanth Kumar Matha, (2025) An Insight On Phoenix Sylvestris Synthesized Agnps For Its Potential As Antimicrobial Agent And Wastewater Management. *Journal of Neonatal Surgery*, 14 (7), 803-807.

ABSTRACT

The emphasis of nanobiotechnology is on the environmentally benign creation of nanoparticles utilizing living creatures, such as bacteria, plant extracts, or plant biomass. Plants are among the many agents utilized in the synthesis of nanoparticles, and they have major applications. Plant-derived biomolecules cause Ag^+ ions to be reduced from silver nitrate to silver nanoparticles (AgNPs). In the current study, aqueous leaf extract from Phoenix sylvestris was utilized as a stabilizing and reducing agent during the manufacture of silver nanoparticles. The production of silver nanoparticles is shown by a color shift from translucent yellow to dark brown, indicating the confirmation of the synthesized nanoparticle. Utilizing UV-Vis absorption spectroscopy, the quantitative production of silver nanoparticles was observed. The generated AgNPs' Surface Plasmon Resonance was measured at 462 nm.The gram positive and gram negative bacteria were used in the antimicrobial tests. The results showed that the zone of inhibition of the produced silver nanoparticles was 20.52 mm for *Staphylococcus aureus*, 21.54 mm for *Proteus vulgare*, 20.37 mm for *Klebsiella pneumoniae*, and 21.86 mm for *Bacillus subtilis*. Using the leaf extract from *P.sylvestris*, the removal capacities for [Cr (VI)], [Fe (III)], and [Mn (II)] were found to be 41.6%, 42.4% and 3.3%, respectively. This demonstrates the stimulatory nature of AgNPs towards the removal of heavy metals from wastewaters.

Thus it may be considered that the plant-based approach is a safe, affordable, and ecologically beneficial biological way to produce silver nanoparticles.

Keywords: *AgNPs, Phoenix sylvestris, Green synthesis, leaf extract, anti-bacterial activity, heavy metals.*

1. INTRODUCTION

Currently, nanotechnology is a key area that facilitates the study of the modern material sciences. The creation and manipulation of matter at scales less than 1µm, typically between 1 and 100 nanometers (nm), is the domain of nanoparticles[1]. Nanoparticles exhibit entirely new or enhanced features and offer a broad range of applications depending on their particular characteristics such as size, distribution, and form.

Because of its bactericidal and fungicidal properties, silver nanoparticles have found numerous and significant applications[2]. Antimicrobial activity is due to Blocking of respiratory enzyme routes, modifications to microbial DNA, and changes to the cell wall[3]. In the past, hydrazine, sodium citrate, and sodium borohydride were used as chemical reducing agents to produce uniform solutions during the manufacture of metallic nanoparticles[4]. However, the chemical technique has certain drawbacks as they are toxic, combustible, and limited synthesis rate of the compounds used[5]. Currently, the usage of chemicals in green nanoparticle manufacturing is being utilized to both safeguard and enhance the environment. Three crucial considerations for the synthesis of nanoparticles have been proposed by Raveendran et al. (2003): the choice of solvent, the use of a reducing agent, and the use of non-toxic material for nanoparticle stabilization[5]. Biological agents have been used recently to reduce and stabilize metallic nanoparticles during green synthesis[6]. When compared to physical and chemical approaches, using biological entities like microbes[7], enzymes[8], plant extract, or plant biomass could be a great way to produce nanoparticles at a lower cost and with less environmental impact. Because plant-based nanoparticle synthesis eliminates the complex process of sustaining cell culture, it may be preferable to other biological processes[9]. It has been claimed that plants can be used to synthesize gold, silver, and copper alloy metal nanoparticles[10–14].Because of its unique qualities, which include strong conductivity, chemical stability, and catalytic and antibacterial activity, colloidal silver is very interesting[15–16]. During our current investigation, we discovered that, plant extracts from *Phoenix sylvestris* can be used to synthesize silver nanoparticles. The current study goals to synthesize silver nanoparticles from silver nitrate solution using an aqueous leaf extract from *Phoenix sylvestris*, and test the produced silver nanoparticles against both gram-positive and gram-negative bacteria and to extend its application for waste water management.

2. MATERIAL AND METHODS

2.1 Preparing Plant Leaf Extract: We gathered 10g of fresh, healthy *Phoenix sylvestris* leaves from the area near our university. Collected fresh leaves were cleaned, chopped coarsely, and then soaked in 100 milliliters of milliQ water. They were then boiled for five minutes in a conical flask. The obtained leaf extract was placed in a refrigerator at 4ºC for future experimentation after being filtered with Whatmann filter paper no. 1.

2.2 Silver nanoparticle synthesis: A 1 mM $AgNO_3$ solution was made, preserved in an amber-colored bottle, and utilized for all the experiments. 90 ml of 1 mM aqueous silver nitrate was combined with 10 ml of plant leaf broth, continuously stirred, and left to react at room temperature to produce Ag^+ ions. The reaction mixture's colour shift from translucent yellow to dark brown shows that *Phoenix sylvestris* leaves can be used to generate silver nanoparticles. The reduction of the Ag+ ions was monitored over time by UV-visible spectral analysis.

2.3 Characterization: The Shimadzu 2450 UV spectrophotometer (Kyoto, Japan) was used to confirm the production of silver nanoparticles using UV-Vis spectrum analysis. Silver's (Ag) Surface Plasmon Resonance (SPR) gives it special optical qualities. The optical absorbance between 300 and 800 nm was periodically scanned at a resolution of 1 nm to look at the rate at which plant leaf extract reduced the amount of silver ions. Following dilution of a small aliquot of 200 µL of the plant extract sample with 1 ml deionized water and use for UV-Visible analysis, the reaction between the metal ions and the leaf extract was seen.

2.4 Antibacterial Activity: Using the standard agar well-diffusion method[17], the antibacterial activity of the silver nanoparticles synthesized using *Phoenix sylvestris* was evaluated against gram-positive and gram-negative bacteria, including *Bacillus subtilis*, *Staphylococcus aureus*, *Proteus vulgare*, and *Klebsiella pneumonia*, respectively.On nutrient agar, the pure cultures of bacterial pathogens were subcultured. 100µl of newly cultured overnight cultures of the corresponding bacteria were placed on Petri plates that contained Nutrient Agar. Using sterilized cotton swabs, every strain was uniformly swabbed. Using gel puncture, 10 mm diameter wells were created on nutritional agar, and 60 µL of AgNP solution was added to each well. Following a 24-hour incubation period at 37°C, the various zones of inhibition were measured.

2.5 Metal cations

The metal cations studied were chromium [Cr (VI)], cobalt [Co (II)], copper [Cu (II)], manganese [Mn (II)] and zinc [Zn (II)]. The stock solutions Cr, Fe, Co, Cu, Mn and Zn (1000 ppm) were prepared from $K_2Cr_2O_7$, $CoCl_2$, $CuSO_4$, $MnCl_2$ and $ZnSO_4$ in deionized water. All AR grade reagents and deionized double distilled water were used for all the experiments. In all the experiments, the samples were filtered using Whatman filter papers (no. 41). The filtered solutions of the metals were estimated by using Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Agilent 7700, USA)[18].

2.6 Metal Analysis

In this experiment, a heavy metal solution of roughly 10 mL was mixed with 2 mL of AgNPs and it was incubated for 30 minutes[19]. The mixture was well-mixed and allowed to stand at room temperature for half an hour. Following equilibration, the slurry was filtered using Whatman 41 filter paper. ICP-MS was used to determine the metal concentration in the clear filtrate. The difference between the concentrations of metal in the aqueous solution and a control without the addition of AgNPs was used to compute the quantity of metal absorbed by AgNPs[18] .

3. RESULTS AND DISCUSSION

3.1 Synthesis and UV-Vis Spectral Analysis: An aqueous silver nitrate solution of *Phoenix sylvestris* was used to carry out the green synthesis of silver nanoparticles through the leaf extract. A non-toxic substance that serves as a stabilizing and reducing agent during synthesis is used in this approach. The reduction of aqueous metal ions with plant leaf extract is the reaction's mechanism. Plant extracts undergo color changes once the process is finished. Furthermore, it is often known that, depending on their size, silver nanoparticles exhibit either dark brown or yellowish brown[20]. The color changed within five to ten minutes of adding the aqueous leaf extract to the 1 mM aqueous $AgNO_3$ solution, and the change persisted for up to twenty-four hours.The activation of the silver nanoparticles' Surface Plasmon Resonance (SPR) vibrations produced the color shift that was observed[17]. Silver hydrosol was created when the aqueous silver ions (Ag^+) reacted with the herbal extracts and were reduced in solution. The color change would make it easy to monitor the reaction, and UV-Vis spectroscopy would further confirm it. The colloidal solutions of SNPs exhibited a UV visible spectrum of 462 nm (Figure 1), with the widening of the absorption peak indicating the polydispersed nature of the particles[18]. The process of photosynthesis and the availability of additional H^+ ions to convert silver nitrate into silver nanoparticles occur in the green leaves. It is hypothesized that the organic matrix contains silver binding proteins, which supply amino acid moieties that act as nucleation sites[21]. This is the molecular source for the production of these silver crystals. Silver nanoparticles can be synthesized extracellularly quickly and efficiently using a variety of plants. The ability to reduce Au (III) ions to generate gold nanoparticles Au (0) and silver nitrate to form silver nanoparticles Ag(0) has been demonstrated by leaf extracts of geranium[21–23], lemongrass[24–25], and cinnamon[26].

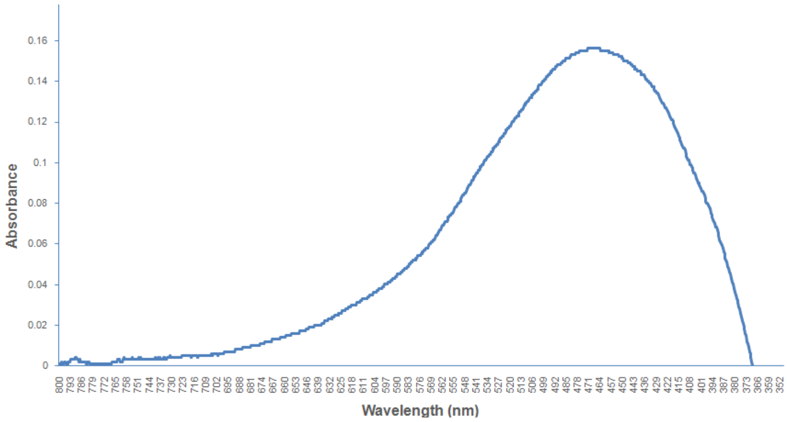

Figure 1: Synthesis of AgNPs with *Pheonix sylvestris* leaf extract

3.2 Antibacterial activity: Gram-positive bacteria *Bacillus subtilis*, *Staphylococcus aureus*, and gram-negative bacteria *Proteus vulgare* and *Klebsiella pneumonia* were the targets of the antimicrobial experiments. Following an overnight incubation period, the plates developed a zone of inhibition, which was measured with a zonal ruler. Table 1 shows the antimicrobial activity of the synthesized silver nanoparticle. The results showed that the zone of inhibition of produced silver nanoparticles was 20.52mm for *Staphylococcus aureus*, 21.54 mm for *Proteus vulgare*, 20.37 mm for *Klebsiella pneumonia*, and 21.86 mm for *Bacillus subtilis*. The electrostatic connection between the positively charged nanoparticles and the negatively charged cell membrane of the microbe is most likely where the antibacterial activity comes from[27].

Table 1: Antimicrobial activity of *Pheonix sylvestris* leaf extract

AgNPs Volume(µl)	**Zone of Inhibition (mm)**
Bacillus subtilis	21.86
Staphylococcus aureus	20.52
Proteus vulgaris	21.54
Klebsiella pneumoniae	20.37

3.3 Metal Removal: By utilizing inductively coupled plasma mass spectrometry, the degree of heavy metal removal was evaluated in all cases (ICP-MS). The absorption of [Cr(VI)], [Fe(II)], and [Mn(II)] was examined in this study utilizing metal ion solution concentrations and a fixed AgNP dosage to evaluate the efficacy of silver nanoparticles' adsorption capacities as metal-ion contaminant removal media. For every metal ion, a decrease in metal concentration was observed. The results obtained shows that even at a short period of incubation of 30 minutes, adsorption grows instantly[19]. The metal ion removal capability is highest for [Fe(II)] and later for [Cr(VI)]. A frequent point in all of these studies is that these nanomaterials showcase considerably higher adsorption capacity than any different traditional adsorbents in comparable experimental conditions[28] with high concentration of metals. This is basically due to the plenty surface areas of the nanomaterials as compared to other traditional sorbents[29].

4. CONCLUSION

According to the research, plant leaf extract has the potential to be a reliable and environmentally friendly feedstock for the effective synthesis of silver nanoparticles. It offers an easy and affordable way to create silver nanoparticles. The produced phytonanoparticles have demonstrated a broad spectrum of actions against the bacterial strains, indicating the high effectiveness of silver nanoparticles as potent antibacterial agents. As a result, these phytonanoparticles have the potential to be used in biological applications and to help develop medications for a variety of diseases. The synthesized Phyto-nanoparticles have exhibited heavy metal removal capability in the order [Cr (VI)] > [Mn (II)] > [Zn (II)] > [Cu (II) > [Co (II)].

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