



Effect of Selected Parameters on the Biosynthesis of Silver Nanoparticles

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ABSTRACT

Recently, nanosilver as a result of its unique features in terms of antimicrobial potentials has been generally accepted in the field of nanomedicine. This study aimed at optimising the biosynthesis of nanosilver using *Nicotiana tabacum* leaf aqueous extract. In the study, effect of parameters such as AgNO_3 concentration (0.20, 0.40, 0.60, 0.80, 1.00 and 1.20 mmol dm^{-3}), volumes aqueous extract (10, 20, 30, 40, 50 and 60 cm^3) and temperature (30, 40, 50, 60, 70 and 80°C) were investigated in the synthesis of nanoparticles. The biosynthesized silver nanoparticles (AgNPs) were characterized by Fourier transforms infrared (FTIR) spectroscopy. The hydroxyl (-OH) and amine (-NH₂) functional groups of *Nicotiana tabacum* leaf extract were shown to be might involve in the biosynthesis of silver nanoparticles by FT-IR peaks at 3446.3 and 2877.63 cm^{-1} respectively. Result showed the biosynthesis of silver nanoparticles using aqueous extract of *Nicotiana tabacum* is a clean, in expensive and safe method and consequently should have high antibacterial activity.

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INTRODUCTION

The creation of innovative and effective antimicrobials to treat illnesses brought on by bacteria resistant to antibiotics has garnered attention in recent years (Salam *et al.*, 2023). Numerous investigations have demonstrated the effectiveness of using noble metal-containing nanoparticles, like copper, gold, and silver, to eradicate all varieties of bacteria (Skłodowski *et al.*, 2023). Researchers in the fields of food science, medicine, agriculture, and agricultural technology have recently paid close attention to silver nanoparticles due to their distinctive qualities and diverse applications (Mohammad *et al.*, 2022).

For the synthesis of silver nanoparticles, several methods have been devised, including chemical reduction, micro-emulsions, radiation, hybrid techniques, photochemical reduction and sono-

electrochemical, microwave-based systems, and recently, a green synthesis route (Masum *et al.*, 2019). However, the adoption of these physiochemical techniques is limited on a large scale due to the use of hazardous chemicals, high costs, high energy and time requirements, and challenges with waste management, even though some of them are long-lasting and scientifically viable (Tudi *et al.*, 2021).

The green synthesis of silver nanoparticles using a variety of microbes, plants, and algae, on the other hand, is a natural, biocompatible, and ecologically friendly process (Alsaiani *et al.*, 2023). However, using plant resources for nanosilver synthesis can be more advantageous than using bacteria or chemicals because there is no risk of bacterial or toxic chemical contamination, and it uses less energy with broader implications and is easier (Singh *et al.*, 2023). This has led to an increase in the

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demand for affordable, environmentally friendly, and green nanosilver synthesis methods that use non-toxic chemicals (Nkosi *et al.*, 2024). Additionally, metal ions are reduced to nano-metals by the presence of functional molecules, primarily phenol, in the green production of nanosilver based on plant extract mechanisms (Osman *et al.*, 2024). Previous research has shown that a variety of natural plants have been used to create nanosilver (Zuhrotun, Oktaviani, and Hasanah, 2023).

The plant *Nicotiana tabacum*, commonly referred to as cultivated tobacco, has a wide variety of development morphologies. These include broad, oval-shaped, simple leaves with a strong, hairy stem, as well as enormous clusters of tubular, white, cream, pink, or red blooms that can reach 3.5–5.5 cm in length (Leal *et al.*, 2023).

The plant sap and decoction of *Nicotiana tabacum* are used to treat headache, sinusitis, and conjunctivitis because of their unique therapeutic properties. In addition, it is used to manage inflammatory illnesses and to treat swellings, hernias, rheumatism, arthritis, gout, hemorrhoids, and gastrointestinal issues. This decision was also motivated by the possibility of influencing the shape control of nanoparticles because the leaf extract contains naturally occurring phytochemicals, including phenolic substances, flavonoids, tannins, quinines, and saponins (Zhang *et al.*, 2024).

In spite of the fact that this plant has been the subject of numerous pharmacognosy and phytochemistry studies, its potential as a biocompatible material for the synthesis of nanosilver has yet to be thoroughly explored. As a result, this study employed the *Nicotiana tabacum* leaf extract to create biodegradable, natural nanosilver with no risk of chemical toxicity, while preserving natural resources.

Materials and Methods

Materials

In order to obtain biosynthesized silver nanoparticles, fresh tobacco (*Nicotiana tabacum*) leaves were randomly collected from different locations in Tunga Awuje Paiko, Niger State

Nigeria and stored before use. Silver nitrate (AgNO_3) was obtained from Sigma Aldrich.

Methods

The leaves of *Nicotiana tabacum* were washed with tap water and then distilled water, air dried for one week and ground into powdery form by using mortar and pestle. The powdered samples were sieved and stored at room temperature in opaque polythene bag prior to use. To obtain the aqueous extract of *Nicotiana tabacum*, 10g of the powdered leaves were weighed into 250 cm³ beaker followed by the addition of 100 cm³ distilled water. The mixture was heated in water bath for 15 minutes at 80°C, left to cool and then filtered using Whatmann No.1 filter paper to obtain the filtrate as the aqueous extract.

However, 1.0 mmoldm⁻³ silver nitrate solution was prepared by dissolving 0.034 g of AgNO_3 salt using 50 cm³ of distilled water in a 100 cm³ volumetric flask and the solution was made up to the mark with distilled water.

Effect of volume of leaf extract on the synthesis of silver nanoparticles

The synthesis of silver nanoparticles was achieved through optimization. In this context, 10.00, 20.00, 30.00, 40.00, 50.00 and 60.00 cm³ of *Nicotiana tabacum* leaf extract were measured in six separate 250.00 cm³ conical flasks. To each conical flask, 20.00 cm³ of 1.00 mmoldm⁻³ AgNO_3 solution was added and stirred at 200.00 rpm for 10.00 minutes. After this, the flasks were incubated at room temperature for 24.00 hours and a colour change of silver nitrate from colourless to brown which signified the formation of silver nanoparticles was observed, after this, the maximum wavelength and absorbance of the silver nanoparticles were recorded using Shimadzu UV spectrophotometer (UV-1800). The optimum volume (volume that gave the highest absorbance) of extract was used for further optimization of temperature and concentration of silver nitrate solution.

Effect of temperature on the preparation of silver nanoparticles

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The effects of temperature on the biosynthesis of silver nanoparticles were studied by addition of optimum volume of the leaf extract obtained to six separate 250.00 cm³ conical flasks containing 20.00 cm³ of 1.00 mmoldm⁻³ silver nitrate solutions, heated to respective temperatures of 30, 40, 50 60, 70 and 80°C, stirred at 200.00 rpm for 10.00 minutes and left to age for 24.00 hours. A colour change of silver nitrate from colourless to brown which signified the formation of silver nanoparticles was observed. After this, the absorbance and maximum wavelengths were recorded using Shimadzu UV spectrophotometer (UV-1800) and the optimum temperature (temperature that gave the highest absorbance) was used for further optimisation of concentration of silver nitrate solution.

Effect of silver nitrate concentrations on the preparation of silver nanoparticles

The optimum values for temperature and volume were used for the optimization of silver nitrate concentration. In six conical flasks containing silver nitrate of concentrations 0.20, 0.40, 0.60, 0.80, 1.00 and 1.20mmoldm⁻³, optimum volume of the aqueous leaf extract was added, heated at optimum temperature and stirred at 200.00 rpm for 10.00 minutes. The flasks were allowed to cool, aged at room temperature for 24 hours. A colour change from

colourless silver nitrate to pale brown signifies the formation of silver nanoparticles was observed.

The maximum wavelengths and absorbances of the silver nanoparticles were recorded using Shimadzu UV spectrophotometer (UV-1800).The optimum silver nitrate concentration (concentration that gave the highest absorbance) was recorded.

Characterization of the Biosynthesized Silver Nanoparticles

The FTIR spectrum of the biosynthesized silver nanoparticles was measured in KBr pellets of sample over the transmission mode in the range of 400 – 4000 cm⁻¹ at resolution of 4 cm⁻¹ using a Frontier FT-IR Perkin Elmer, UK.

RESULTS AND DISCUSSION

FTIR spectroscopy is one of the essential techniques for characterizing the surface chemistry of synthesized nanoparticles because it shows the various organic functional groups like carbonyls and hydroxyls attached to the surface of nanoparticles and the other surface chemical residues (Kassem *et al.* 2023). So, FTIR discloses the likely bio-molecules present in the leaf extract, which aid the reduction of the silver ions and its interfacing with the silver nanoparticles.

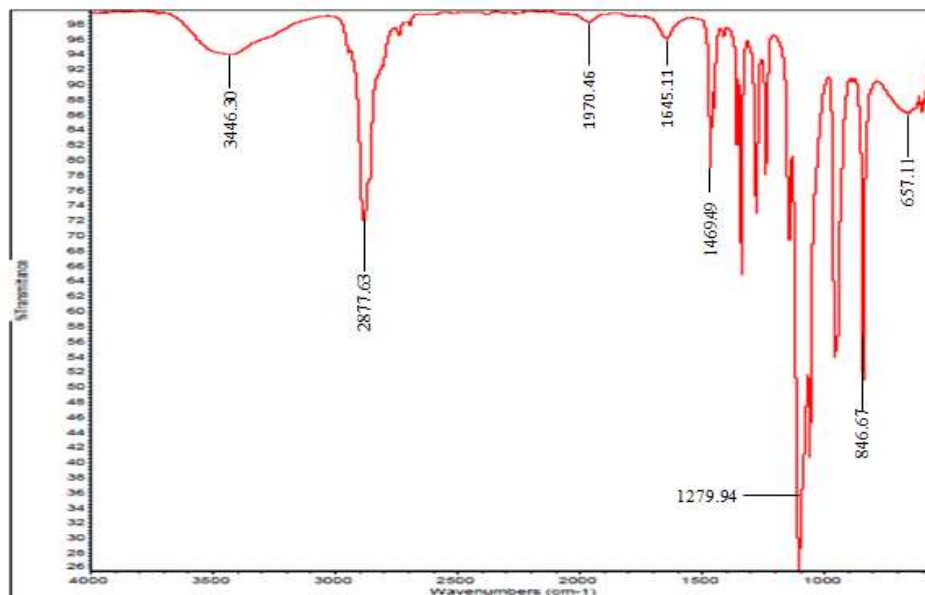


Figure 1: FTIR spectra of the synthesised silver nanoparticles

The FTIR spectrum of the silver nanoparticles synthesized using *Nicotiana tabacum* leaf extracts is shown in (Figure 1). The FTIR spectrum showed characteristic bands at 3446.3, 2877.63, 1645.11 and 846.667cm⁻¹ which corresponded to the strong stretching vibrations of hydroxyl (-OH) or amino (-NH₂), carboxylic (-COOH), carbonyl (-C=O) and alkyl amine (R-NH₂) of the phytochemical respectively, chiefly alkaloids and oxalic acid reported present in the *Nicotiana tabacum* leave extract by Prasad *et al.* (2011) in their biogenic synthesis of silver nanoparticles using *Nicotiana tabacum* leaf extract.

In this study, the formation of silver nanoparticles upon the addition of aqueous leaf extract of *Nicotiana tabacum* to the beakers containing aqueous solution of silver nitrate led to a change in the colour of the solution to pale brown. The appearance of pale brown colour was due to the excitation of surface plasmon resonance (SPR) typical of silver nanoparticles having absorbance values which were reported earlier in the visible range of 410 - 450 nm (Gowamma *et al.*, 2015).

Silver nanoparticles prepared using different volumes of aqueous leaf extracts (10 – 60 cm³) with 1 mmoldm⁻³ of silver nitrate were analysed by UV spectrophotometer and plasmon resonance band were observed at 425-455.5 nm (Figure 1). This is in agreement with the results (420 – 430 nm) reported by Hamouda *et al.* (2019). Figure 2 shows that increase in the volume of aqueous leaf extract leads to increase in the absorbance. The optimum volume for the synthesis of silver nanoparticles was attained at 30 cm³ volume of the aqueous leaf extract at maximum absorbance of 1.792 and wavelength 447 nm. Therefore, this volume (30 cm³) was taken to optimized subsequent parameters. The maximum absorbance of the nanosilver obtained using different volumes of plant extracts were not the same for all plants; for instance, 10 cm³ leaf extract of *P. guajava* used to prepare nanosilver showed the maximum absorbance at 460 nm (Sriram and Pandidurai, 2014), while 5 cm³ of insulin plant leaves showed highest absorbance peak at 420 nm (Ajithada *et al.*, 2014).

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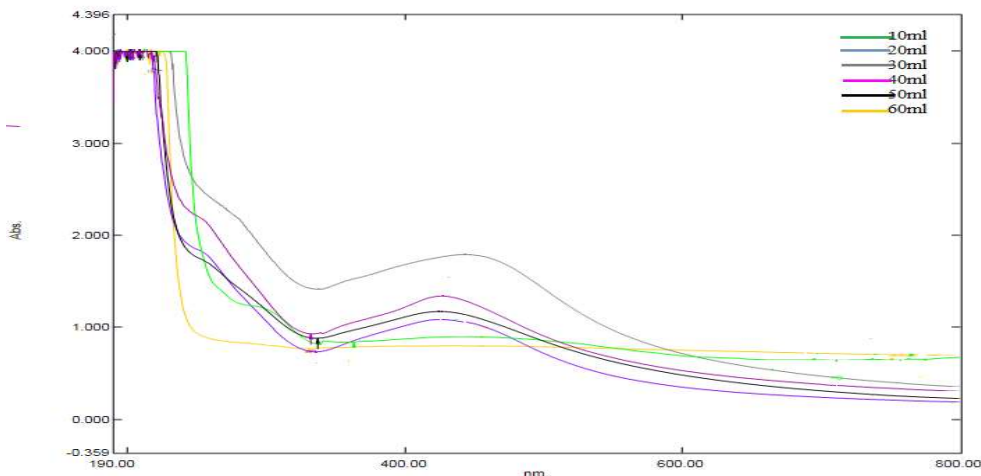


Figure 1: Optical absorption spectra of silver nanoparticles prepared using various volumes of *Nicotiana tabacum* leaf extract

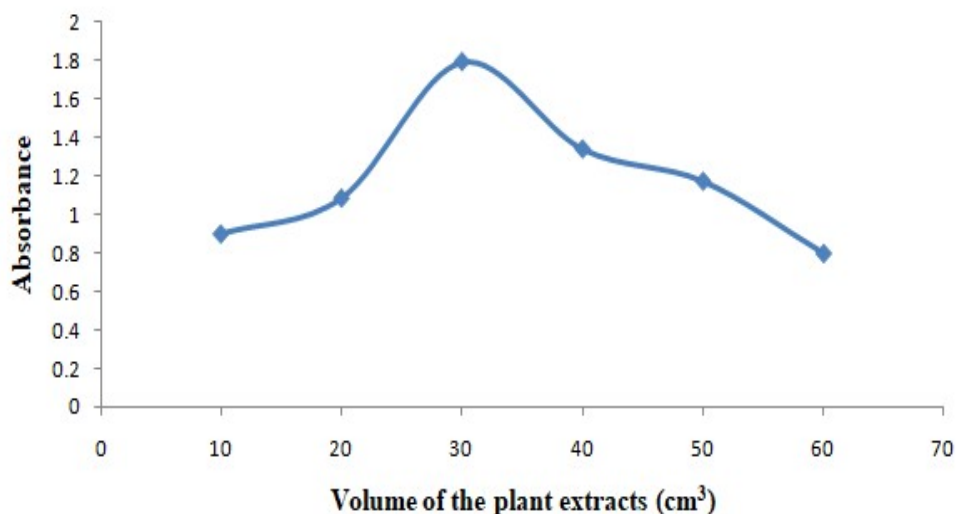


Figure 2: Variation of absorbance of the synthesized silver nanoparticles with respect to the changes in volumes of *Nicotiana tabacum* leaf extract

In the optimization of silver nanoparticles synthesis using 10 cm³ extracts of *Aspergillus Niger*, the highest absorbance peak obtained was at 420 nm (Khan *et al.*, 2016). The optimum volume obtained in this study is in agreement with the volume of plant extract reported showing absorbance of about 0.5 at maximum wave length of 450 nm for silver nanoparticles synthesised by aqueous extract of

saffron (*Crocus Sativus L.*) (Bagherzade *et al.*, 2016). It is slightly different from the λ_{max} . The differences in the volume of plant leaf extract used in the synthesis of silver nanoparticles may be attributed to the difference in the nature and amount of phytochemicals available in the extract (AbuDalo *et al.*, 2019).

Figure 3, shows the absorption spectra of silver nanoparticles at different temperatures in

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the range of 30 – 80°C. As the temperature changed, the absorbance of the prepared nanosilver also changes (Figure 4). This indicates that the yield of the synthesized nanoparticles increases with increasing temperature, which may be due to the higher

reaction rates at higher temperatures (Jiang *et al.*, 2011). Also, at higher temperatures, the kinetic energy of the molecules increases and silver ions get more consumed, thus leaving less possibility for particle size growth (Jain and Mehata, 2017).

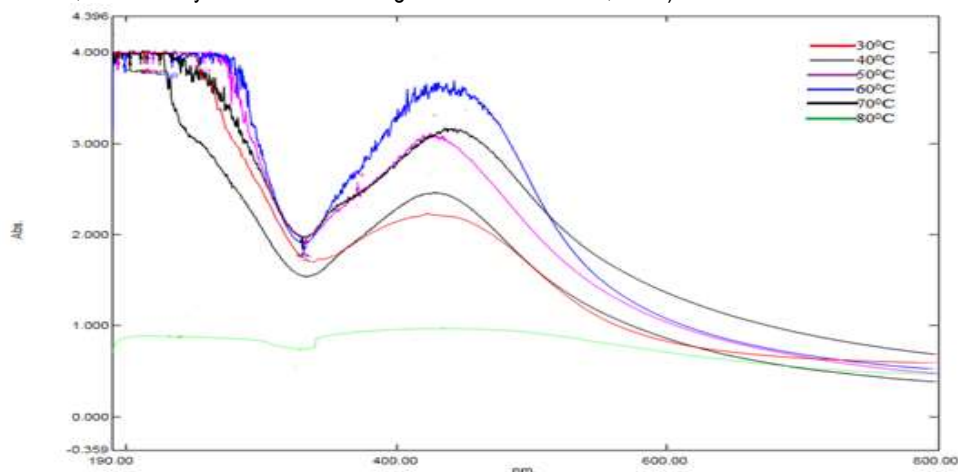


Figure 3: Optical absorption spectra of silver nanoparticles synthesised using various solution temperatures

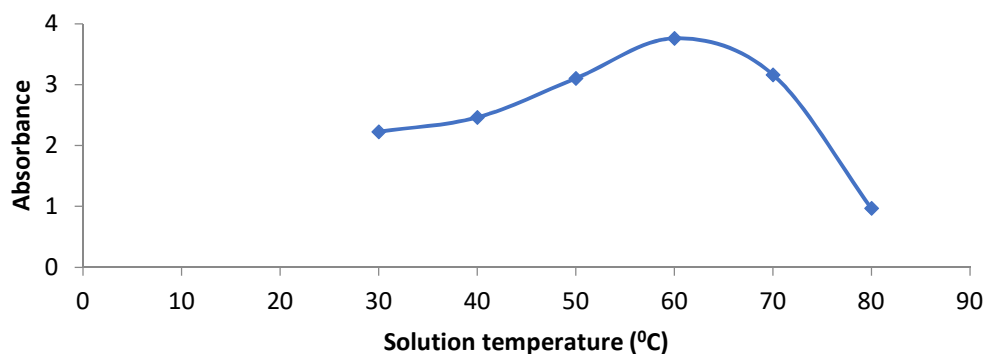


Figure 4: Variation of absorbance of the synthesised silver nanoparticles with respect to changes in solution temperatures

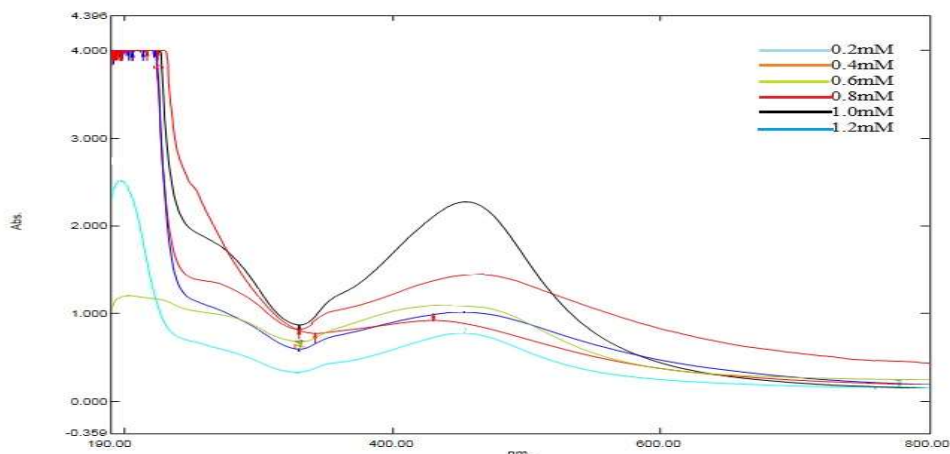


Figure 5: Optical absorption spectra of silver nanoparticles prepared using various concentrations of silver nitrate solution

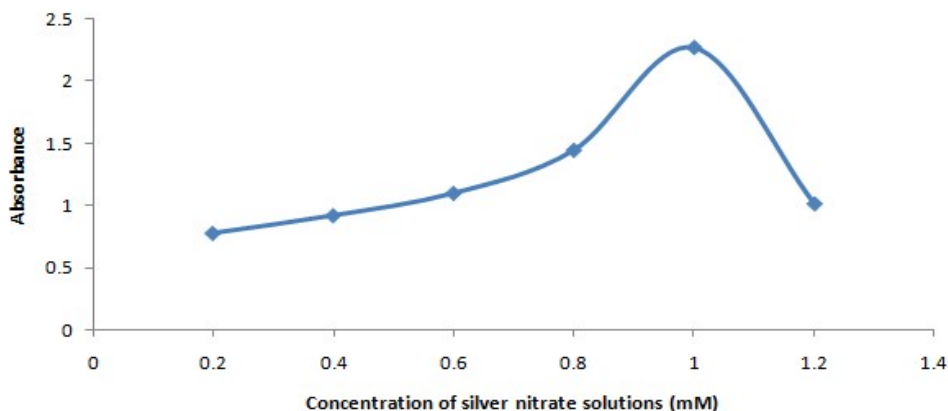


Figure 6: Variation of absorbance of the synthesized silver nanoparticles with respect to changes in the concentrations of silver nitrate solutions.

Figure 5 shows that by increasing the concentration of silver nitrate solution to 1 mmoldm^{-3} , the absorbance of the nanoparticles formed increased (Figure 6), however, by further increasing to 1.2 mmoldm^{-3} the absorbance decreased. This implies that the optimum concentration of silver nitrate for the synthesis was 1 mmoldm^{-3} and that above this concentration; the solution may have unfavourable effects on *Nicotiana tabacum* (Vanaja *et al.*, 2013). The result obtained from this study is lower than the 5 mmoldm^{-3} of silver nitrate solution obtained as optimum

concentration for the optimization of biological synthesis of silver nanoparticles by Korbekandi *et al.* (2013) using *Fusarium oxysporu*. However, 1 mmoldm^{-3} of silver nitrate solution used for the preparation of silver nanoparticles by Christopher *et al.* (2015) gave characteristic absorption peak at 440 nm in the UV-vis spectrum for *Aegle marmelos* leaf extract which is similar to the obtained result in this study.

CONCLUSION

The biosynthesis of silver nanoparticles was achieved by using *Nicotiana tabacum* also

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the process of synthesis was improved by optimization which is a beneficial mode. For the first time, the biosynthetic conditions of silver nanoparticles through *Nicotiana tabacum* were optimized by varying the values of the selected parameters. After performing the experiments, the optimized factors were volume (30 cm³), concentration of AgNO₃ (1 mmoldm⁻³) and temperature = 60°C. The optimization of biosynthesis of silver nanoparticles results to maximizing yield, reduction of by-products, and improvement of synthesis reproducibility.

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