

Green synthesis of silver nanoparticles

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ABSTRACT

This research reports obtaining an *Ocimum basilicum purpurascens* extract (OBPE) and its use as a reducing agent in the green synthesis of silver nanoparticles (AgNps) by the metal salt reduction method. FTIR characterized the extract to determine the functional groups acting as reducing agents. The AgNps obtained presented an average hydrodynamic diameter of 224 ± 7.6 nm and a ζ -potential of -17.6 ± 0.61 mV. The synthesis is carried out under mild conditions (temperature below 100 °C and atmospheric pressure); the precursor reagents and the residues generated are not pollutants. Therefore, the synthesis method is considered to be environmentally friendly. In addition, the AgNps obtained present desirable physicochemical characteristics for biomedical and soil remediation applications.

Keywords: *Green Synthesis, Metallic Nanoparticles, Natural extracts.*

INTRODUCTION

Silver is an element that has gained significant interest in the industry due to its attractive characteristics such as conductivity, chemical stability, catalytic activity, and especially its potent antimicrobial activity (Sánchez-Moreno & Álvarez-Rodríguez, 2017). Due to these properties, there has been significant interest by researchers in AgNp synthesis; and can be widely used in the nanotechnology industry in different application areas and products such as in electronics, catalysis, environmental remediation processes, as tools in biomedical devices, also their photoactivity promotes antimicrobial activity against bacteria and fungi (Ávalos et al., 2013; Sánchez-Moreno & Álvarez-Rodríguez, 2017).

Nanoparticles can be synthesized from different materials; the most common are made from carbon, silicon, titanium, and other. Many nanoparticles are also made from metals and metal oxides, containing elements such as cadmium, cerium, copper, germanium, gold, iron, lead, selenium, silver, zinc, or zirconium. In general, the main classes of nanoparticles in commercial use are fullerenes, single and multi-walled carbon nanotubes, quantum dots, metal oxides, and polymers (Powell & Kanarek, 2006). In the specific case of AgNps, they are applied in different fields such as electronics, clothing, paints, cosmetics, bactericides, bio fungicides, and biomedical applications in the medical-pharmaceutical and food industry (Ávalos et al., 2013). In environmental improvement, AgNps help restore contaminated sites and treat wastewater, detecting contamination levels and eliminating hazardous substances that can be toxic, corrosive, reactive, explosive, flammable, and infectious biological. Sensors detect and monitor contaminants such as pesticides, antibiotics, or microbes in different environmental matrices (Sancho-Villamor et al., 2020).

An example of these applications is the case of Orizano-Fabian & Benites-Alfaro (2018), where the percentage of reduction when applying AgNps to agricultural soil with total coliform content was 76 %, evidencing the reduction of the microorganism and the efficiency of the treatment. The treatment efficiency used in this research was based on elaborating the synthesis of AgNps using the camphor substrate (*Cinnamomum camphora*). Once the nanoparticle was obtained with a size of 37 nm, it was applied to the soil with total coliform content; the application was carried out in three doses and three different times to evaluate the reduction and efficiency. The final result was a reduction in the total coliform content of the soil, as shown in the results, demonstrating the efficiency of the AgNps treatment.

Within the environmental issue, the most important specific objectives are the prevention of pollution with the design of new less or non-polluting industrial processes, the remediation, and restoration of contaminated sites with efficient and low-cost methods, and the prediction in advance of the environmental impact of new products on the market and the early detection of pollutants at the source with susceptible methods to avoid damage (Vazquez-Duhalt, 2015). Nanotechnology is currently playing a critical role in meeting these objectives. For example, it has participated in the improvement of environmental processes through nanotechnology-based technology in household light bulbs that has decreased energy consumption in the United States by 10 %, with savings of more than 100 billion dollars annually and, above all, reducing carbon

emissions by 200 million tons each year (Alexander, 2006). Nanoparticles are widely used, and there are more than 1,000 commercial nanostructured products (Sánchez-Moreno & Álvarez-Rodríguez, 2017).

In the medical field, AgNps detect the presence of toxic substances in the human body. They are used for wound dressings, contraceptive devices, surgical instruments, and bone prostheses to prevent bacterial growth (Cheng et al., 2004; Cohen et al., 2007). In addition, AgNps are evaluated for the treatment of diseases that require a maintained drug concentration in blood or with specific targeting to cells or organs (Panyam & Labhasetwar, 2003), as is the case with HIV-1 virus, since it has been shown that in vitro treatment with AgNps interacts with the virus and inhibits its ability to bind to host cells (Elechiguerra et al., 2005).

The consumption of AgNps has been affected since the 1940s by the production of penicillin as an antibiotic. However, the resistance of microorganisms to antibiotics revitalized the development of AgNps, given their antimicrobial activity against more than 650 pathogens, including bacteria, fungi, and viruses (Ciobanu et al., 2013). As a result, the use of AgNps has increased in several areas.

Specific materials have been used to be coated with AgNps; Ciobanu et al. (2013) report the synthesis of hydroxyapatite nanocrystals doped with silver ions ($\text{Ca}_{10-x}\text{Ag}_x(\text{PO}_4)_6(\text{OH})_2$), with antimicrobial properties against bacterial strains of *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Providencia stuartii*, *Citrobacter freundii* and *Serratia marcescens* (Ciobanu et al., 2013). They also reported that the inhibitory activity increased in each sample with increasing silver concentration. Likewise, Zhang et al. (2016) tested the synthesis of AgNps by treating silver ions with *Allophylus cobbe* leaf extract as a methodology. The synthesized nanoparticles were used alone or in combination with antibiotics to evaluate their antibacterial and antibiofilm effect using agar disc diffusion, biofilm inhibition, and reactive oxygen species generation techniques against *Pseudomonas aeruginosa*, *Shigella flexneri*, *S. aureus*, and *Streptococcus pneumoniae*. The data found indicate that in combination with antibiotics, there is a significant antimicrobial and biofilm inhibitory effect at low concentrations (reference 0.1-1.0 $\mu\text{g/ml}$) of AgNps; thus, the results suggest that AgNps can be a therapeutic adjuvant for the treatment of infectious diseases caused by bacteria (Zhang et al., 2016).

AgNps have been used in pharmaceuticals and medical devices. A study involving drug-resistant pathogens such as multidrug-resistant *Pseudomonas aeruginosa*, ampicillin-resistant *Escherichia coli*, and erythromycin-resistant *Streptococcus pyogenes* determined that a nanoparticle suspension is capable of inactivating the antibiotic resistance panel of Gram-positive and Gram-negative bacteria, respectively. In addition, the AgNps can exert bactericidal rather than bacteriostatic mechanisms and inhibit bacterial growth from the first contact of bacteria with AgNps (Ciobanu et al., 2013).

Particular importance has been placed on the use of AgNps, due to the early detection and treatment of cancer, as well as the effectiveness of anti-cancer drugs and their relation to the possibility of reaching target cells in sufficient concentration without damaging healthy tissue and cells (Rai et al., 2014). Furthermore, it is mentioned

that AgNps can be used as therapeutic delivery agents to the biological cancer target, nano biosensors to predict the disease and thus minimize cancer growth and reduce the cost of treatments (Rai et al., 2014).

There are several chemical methods for the synthesis of nanoparticles; they usually are the most used since they can be taken on a large scale within the industry; this is due to the high reaction rate they present, as well as reduced polydispersity and controlled nanometric size (Sánchez-Moreno & Álvarez-Rodríguez, 2017). Chemical reduction is the most commonly used method in synthesizing AgNps, using silver nitrate as substrate due to its low cost and chemical stability compared to other salts. In addition, reductants and termination agents such as surfactants are required for their synthesis to control their growth (Cardoso, 2016). On the other hand, these methods use strong reductants such as sodium borohydride (NaBH_4), potassium borohydride (KBH_4), elemental hydrogen, and carbon monoxide. Toxic compounds and another procedure must be performed to eliminate their toxicity; they also present residues such as organic solvents and metal oxides (Orizano-Fabian & Benites-Alfaro, 2018) and require surfactant stabilizers to avoid agglomeration and sedimentation of nanoparticles. Due to the above, in recent years, there has been a growing need and interest in designing environmentally friendly processes for the synthesis of AgNps, where reducing agents and termination agents of natural or ecological origin, such as peptides, proteins, carbohydrates, microorganisms, algae, plant extracts, among others, are used to achieve the reduction of metal ions (Cardoso, 2016; Sánchez-Moreno & Álvarez-Rodríguez, 2017). In these methods, the intention is to substitute chemical agents with one of these mentioned alternatives; the bacterium *Shewanella oneidensis* was used as a metal reducing agent for the biosynthesis of AgNps; using a solution of AgNO_3 as the precursor, they obtained spherical and practically monodisperse AgNps, in size range 2-11 nm (Sánchez-Moreno & Álvarez-Rodríguez, 2017). The synthesis of nanoparticles using the green synthesis method with bacteria is cheaper, more straightforward, reproducible, and requires less energy than chemical synthesis (Cardoso, 2016). Another study (Kp & Savithramma, 2013) reported that AgNps could also be synthesized from *Cadaba fruticosa* leaves using AgNO_3 as the precursor. The biologically synthesized nanoparticles were very potent against microbes.

Although chemical synthesis techniques for AgNps are currently the most widely used and allow high yields and desirable characteristics such as controlled particle size, homogeneity, and monodispersity, they are coupled with toxic and hazardous by-products. On the other hand, the green synthesis methods are undoubtedly the most environmentally friendly. However, carrying their industrial production on a large scale is difficult. In addition, the AgNps obtained by this green synthesis method are of higher quality and chemical stability, which is why alternatives are still being sought to achieve this step and a high yield. Therefore, in the present work, we present the green synthesis of AgNps using an OBPE as a reduction and stabilizing agent for its potential use as an antioxidant, antibacterial, antifungal agent, an auxiliary agent in soil remediation.

MATERIALS AND METHODS

Silver nitrate AgNO_3 169.87 g/mol (99 %) was purchased from Sigma-Aldrich. Tridistilled water was subjected to Milli-Q equipment to obtain water with a resistivity of 18.25 $\text{M}\Omega/\text{cm}$, which was used in all experiments.

COLLECTION OF OBP LEAVE

The *Ocimum basilicum purpurascens* (OBP) seeds used to germinate the first generation of plants were obtained from plants provided by the nursery of the Universidad Estatal de Sonora Unidad Académica Hermosillo. The plant was cultivated, and with its seeds, a new generation of plants was obtained, thus for three generations. From the first generation, the plants were grown without using chemical additives or any other substance containing synthetic materials, such as pesticides, herbicides, and artificial fertilizers, to avoid any undesired agent in the synthesis of AgNps. The OBP plant used is the one shown in Figure 1.

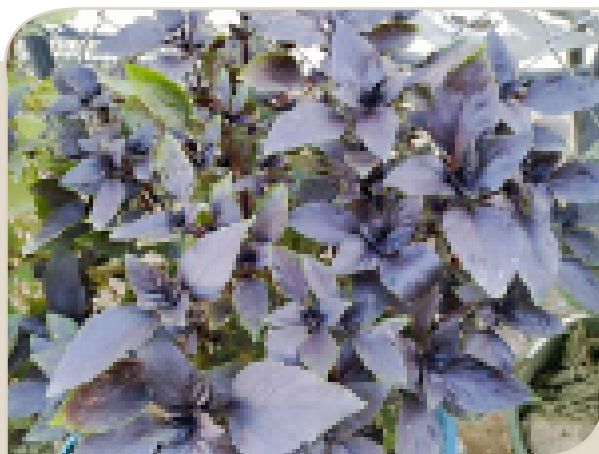


Figure 1. *Ocimum basilicum purpurascens* third generation plant

OBTAINING THE EXTRACT

Ten grams of tender leaves of OBP will be taken and cleaned, washing with abundant water, without using any detergent or soap. Then, the leaves of the purple basil will be divided into smaller pieces. Finally, the resulting pieces of leaves will be placed in 50 ml of ultra-pure water. During this process, the purple color of the leaves will be lost, and they will take a green color, and therefore the water becomes purple, as shown in Figure 2 (Syama Sundar et al., 2014).

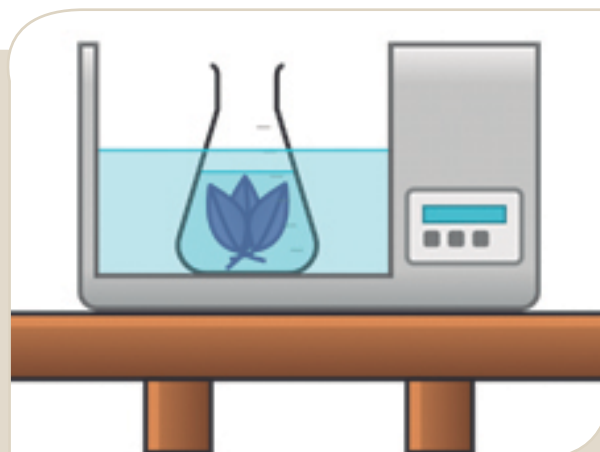


Figure 2. Schematic diagram of the preparation of *Ocimum basilicum purpurascens* extract

The next step is to strain the water with the leaves obtained in a filter. This filtering will be by the decantation method in order to eliminate impurities and only obtain the OBPE, which will be dark-colored between brown and purple Figure 3, then a part of the extract obtained will be lyophilized, and another part will be stored in a refrigerator at a temperature of -4°C to be used later.



Figure 3. *Ocimum basilicum purpurascens* extract

GREEN SYNTHESIS OF NANOPARTICLES

A one-step green synthesis was performed with variations to the reported method (Syama-Sundar et al., 2014). 0.5, 1, and 2 mL of OBPE were added to 45 mL of AgNO_3 (1 mM) solution. The solution was under magnetic stirring at 250 RPM and remained for 10 min after adding OBPE (Figure 4).

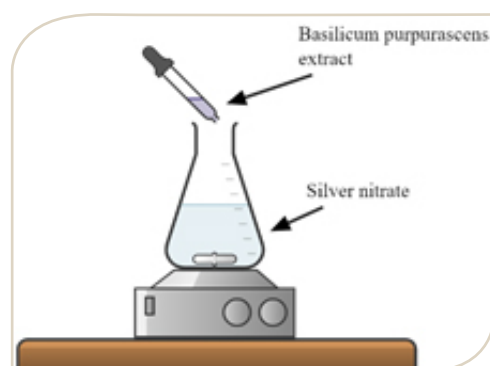


Figure 4. One-step green synthesis of AgNps with OBPE



Figure 5. AgNps₁, AgNps₂ y AgNps₃ samples with 0.5, 1, and 2 mL of the reducing agent, respectively

A change from colorless to a brown solution was observed. The solution obtained was centrifuged at 12000 RPM for 15 minutes. The supernatant was suspended in 20 ml of ultrapure water. The samples were labeled as AgNps₁, AgNps₂, and AgNps₃ for concentrations of 0.5, 1, and 2 mL of OBPE, respectively.

CHARACTERIZATIONS

FT-IR spectra were collected on a Spectrum Two spectrometer (Perkin-Elmer, MA, USA). IR spectra were collected 16 times (spectral resolution of 4 cm^{-1}). In addition, hydrodynamic diameters (D_h) and π -potential measurements were obtained by dynamic light scattering (DLS) on Zetasizer Nano ZS equipment (Malvern Instruments Ltd., Worcester-shire, UK).

RESULTS AND DISCUSIÓN

DHAND π -POTENTIAL

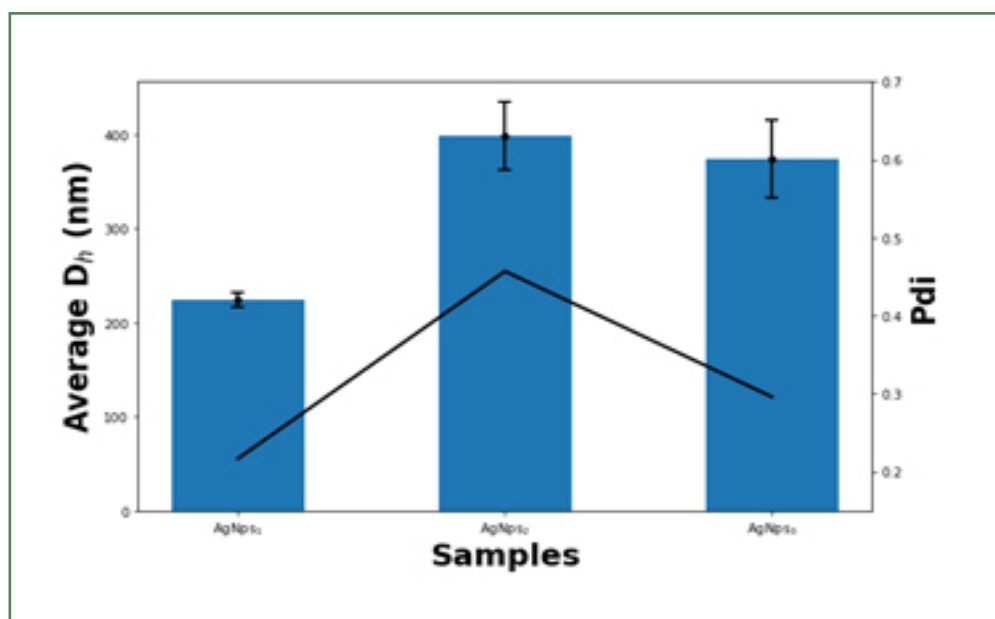


Figure 6. D_h of the samples AgNps₁, AgNps₂, and AgNps₃

Figure 6 shows the D_h and polydispersity index (Pdi) samples obtained. If we compare AgNps₁ with AgNps₂ the steric restriction may cause an increase of D_h . The functionalization of the particle surface restricts mobility, preventing the creation of a more significant number of nucleation centers, and affecting increased particle size and Pdi. On the other hand, the π -potential increases due to a lower number of negatively charged functional groups (Figure 7). The above could be attributed to the fact that these groups functionalize the particle surface. Nanoparticle synthesis using high molecular weight reducing agents has been previously reported, and an increase in size is observed due to a spatial restriction of the zerovalent metal atoms (Esquivel et al., 2018; Guzmán et al., 2018).

In contrast, the AgNps₃ sample presented a smaller size than AgNps₂, and the above could be attributed to the increase in the OBPE concentration as it was overcome by a predominance of the reducing character over the steric restriction effect. This size reduction effect upon increasing the concentration of the reducing agent has been widely reported (Bindhu & Umadevi, 2015; Guzman et al., 2018).

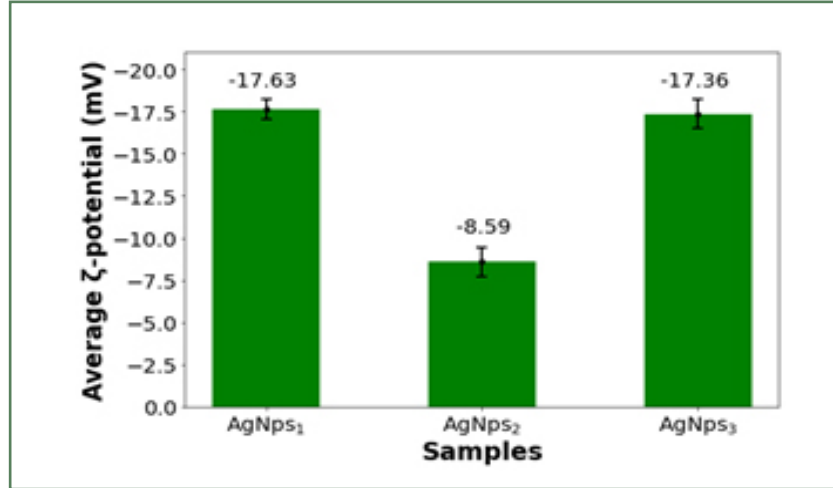


Figure 7. ζ -potential and Pdi of the samples AgNps₁, AgNps₂, and AgNps₃

FTIR SPECTRA

Analysis of the FTIR spectrum revealed strong peaks at 3400 and 3210 cm^{-1} , indicating O-H absorption (Figure 8). A signal is observed around 1600 cm^{-1} related to C=O stretching of a carboxylic acid (Hernández-Téllez et al., 2021). The bands at 1400 cm^{-1} reveal the C=C (ring) stretching of aromatic compounds. The above agrees with the results of other researchers indicating the presence of several primary and secondary metabolites, including phenolic content, glycosides, terpenoids, tannins, and flavonoids as chemical constituents in basil extracts (Pirtarighat et al., 2019). In addition, the primary metabolites found in *Purgennn Son Linalool*, geranial, geranyl acetate, and methyl cinnamate (Saruchi et al., 2022) and further suggest that the bioactive molecules are known silver ion reducing agents that are responsible for the formation of AgNps (Pirtarighat et al., 2019; Cheng et al., 2004).

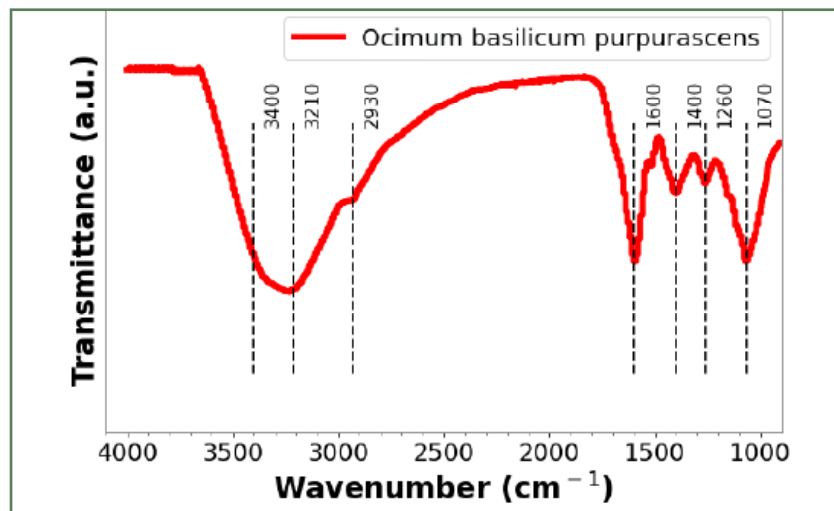



Figure 8. FTIR spectra of the *Ocimum basilicum purpurascens* extract.

CONCLUSIONS

This study reports the one-step green synthesis of AgNps using an extract of *Ocimum basilicum purpurascens* as a reducing agent. The synthesis is of short duration (10 minutes), uses mild conditions, and produces no environmentally toxic residues. The DLS analysis reveals sizes of 224 nm for the AgNps₁ sample; its high surface area can be used as an auxiliary agent in soil remediation. The AgNps₃ sample presents a size of 374 nm and a π -potential of -17.36 mV, which is a good candidate for biomedical applications as an antioxidant, antifungal, loading, and drug release. In addition, the extract on its surface provides potential characteristics that enhance its biocompatibility and ease of being functionalized for different applications. 

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