



ISSN: 2525-815X

Journal of Environmental Analysis and Progress

Journal homepage: www.jeap.ufrpe.br/

10.24221/JEAP.4.3.2019.2523.216-222



Green synthesis of silver nanoparticles using leaf extract from *Tabebuia roseoalba* and *T. pentaphylla*

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ARTICLE INFO

Received 03 Jun 2019

Accepted 29 Jul 2019

Published 31 Jul 2019

ABSTRACT

Metal nanoparticles are nanostructures that can be applied to biotechnology because they present different biological activities. Among them, the silver nanoparticles (AgNPs) are known to present antimicrobial activity allowing their application in several areas such as medicine and industry. The biological synthesis of AgNPs is ecologically correct and advantageous techniques. The objective of this study was to evaluate the synthesis of AgNPs through the green synthesis using extracts of leaves of *Tabebuia roseoalba*, and *T. pentaphylla* grew *in vivo* and *in vitro*. The nanoparticle synthesis solution was colorimetrically evaluated, and the nanoparticles were physically characterized. The results obtained demonstrate that both extracts of both *Tabebuia* species tested can synthesize AgNPs, however only when cultured under *in vivo* conditions. These data suggest that photosynthesis under natural conditions promotes the production of metabolites that are essential to green synthesis.

Keywords: Nanobiotechnology, AgNPs, Nanosilver, leaves extract.

Introduction

Nanobiotechnology is an area of nanotechnology-related to the creation, use, and enhancement of nanostructures in biotechnological processes. It can be said in a simplified form that this is the manipulation of matter at the atomic and molecular levels. In this context, the synthesis of metal nanoparticles (MNPs) is inserted (Tarafdar et al., 2013). Notable metal nanoparticles such as silver, gold, platinum, and palladium have aroused considerable interest because of their small size and large surface area about their volume (Geonmonond et al., 2018). At this nanometric scale, these particles present very different characteristics of larger particles present in the same material on a larger scale.

Noble metal nanoparticles can be applied in the field of medicine, biology, physics, chemistry, and materials science (Chandra et al., 2014). Gold nanoparticles, for example, are present in combined imaging techniques and photothermal tumor therapy (Khoshgard et al., 2014). Platinum nanoparticles can be found in the manufacture of electrochemical sensors (Yang et al., 2006). In

contrast, a study shows that nanoparticles of palladium have the electrocatalytic capacity (Chen & Ostrom, 2015). Among the MNPs, silver nanoparticles (AgNPs) can also be applied in various areas such as integrated circuits, sensors, filters, fibers, cell electrodes, and antimicrobial agents. Thus, AgNPs has captured the interest of many researchers (Iravani, 2011). The antimicrobial property of AgNPs makes these nanostructures applicable in different areas of medicine, industry, livestock, and other areas where it is necessary to combat the disordered proliferation of microorganisms. Also, studies have shown that surface-immobilized AgNPs can be used as support for the immobilization of enzymes, as well as assigning an antimicrobial property to a particular surface (Khan et al., 2011).

For the synthesis of nanoparticles, physical, chemical, or biological methods are used. Physical and chemical methods consume more energy and can also be toxic, yet the biological method is characterized by being an ecologically correct technique and more advantageous (Iravani, 2011). Also, nanoparticles synthesized through the

biological path most often have biocompatibility, biodegradability, and lower production costs (Jiang et al., 2018).

Previously, the production of nanoparticles through the biological pathway has been carried out using fungi, bacteria, cyanobacteria, and plants. The green synthesis is one of the routes used for the biological synthesis of MNPs *in vitro*, which consists in the use of plant extracts to produce MNPs by the process of bioreduction, in this process the silver ions are reduced in metallic silver, which in turn succeed in the production of nanoparticles (Kuppusamy et al., 2016).

Considering the importance of AgNPs, the objective of this study is to evaluate the potential of the foliar extract of arboreal species of the Atlantic Forest of the genus *Tabebuia* as catalysts of the synthesis and characterization of AgNPs through the green synthesis, using extracts of the plants maintained *in vivo* and *in vitro*.

Material and Methods

Plant material

Leaves of *Tabebuia roseoalba* (Tr) and *T. pentaphylla* (Tp) were obtained from two forms of plant growth: I - young leaves collected from adult plants located at the Campus of the Centro de Tecnologias Estratégicas do Nordeste (CETENE), II - leaves collected from plants grown in tissue culture (under heterotrophic conditions).

Vegetable extract

Two different types of extracts were performed: *in vivo* and *in vitro* according to the collections I and II above mentioned. The leaves fresh were properly washed, and 2.5g of the plant samples were macerated in 20 mL of deionized water using mortar and pestle and later filtered on filter paper. The solutions were titrated to 100 mL and stored at 4 °C.

Synthesis of AgNps

The synthesis of nanoparticles was based on the Okafor et al. (2013) methodology. Initially, was prepared a silver nitrate solution in 1 mM concentration in deionized water. The solution was stored in an opaque container to avoid contact with light. The solutions, for the synthesis of AgNps, were prepared using 9.75 mL of silver nitrate solution and 250 µL of each of the plant extracts, obtaining the final volume of 10 mL. The tubes containing the synthetic solutions were maintained at 37.1°C in a water bath for 1 h, protected from light, until AgNPs formation.

The syntheses were identified as: I - *in vivo* synthesis (using silver nitrate and *in vivo* extract of material) and II - *in vitro* synthesis (using silver

nitrate and *in vitro* extract of material and Tr and Tp according to each of the species used.

Antioxidant PVP

The antioxidant power of polyvinylpyrrolidone (PVP) was evaluated in order to prevent aggregation of AgNPs formed. Three concentrations of PVP were tested. In this, the extracts were mixed, in the ratio of 1:9 (v/v), with AgNO₃ solution (1mM) and added with 0, 34, 67 and 100 mg.mL⁻¹ of PVP. The blends were held at 37°C for 1h protected from light. After the synthesis was stored for 30 days at 25 ± 2°, C protected from light, and the process of formation of the AgNPs was evaluated at one, eight, and 15 days.

Photostability

After the synthesis, the solutions containing the nanoparticles were maintained for 15h at 25 ± 2°C in the presence of light for evaluation of Photostability of the AgNPs observing the alteration of the coloration visually.

Physical characterization

UV-Vis

UV-vis Diffuse Reflectance spectra of the powders were obtained using a LAMBDA 650 UV-vis spectrometer (PerkinElmer) equipped with an integrating sphere.

X-ray diffraction

A thin film of the AgNPs was made by overlapping 20 layers each of 100 µL solution of AgNPs formed on a heated plate (100°C) of silicon. The test was performed with the tube fixed at an angle of incidence of 2°. The samples were analyzed in X-ray Diffractometer Bruker D8 Advance Davinci under radiation γ in copper tubes. The scans were made with angulation: 10°-70° (Nickel filter) voltage: 40 KV current: 40 mA and a time constant was 5s.

Results

The formation of AgNps promoted a change in the coloration of the synthetic solutions that initially presented orange coloration and after the incubation time presented as dark yellow/brown (Figure 1). The UV-Visible spectrophotometry analysis, shown in Figure 2, indicates that the AgNPs were formed since the synthesis is characterized as having the absorbance peak at the wavelength of 400-500 nm. Comparing the formation of nanoparticles between leaf extracts *in vitro* and *in vivo* through UV-Vis, the surface plasmon resonance of AgNPs is visible at 420-440 nm only in leaf extract *in vivo*, thus

defining the formation of nanoparticles using the green synthesis. The extract from *in vivo* grown plants of *T. roseoalba* produced a higher number of nanoparticles when compared to *T. pentaphylla*. However, the formation of AgNPs from *in vitro* culture extracts was not evidenced by the results obtained, as observed in Figure 2CD.

The results of X-ray diffraction analyses are presented in Figure 3. According to the graphs, the control treatment of the two plant species presented peaks in the expected range for the formation of silver nanoparticles (200-380 nm). It is possible to determine the synthesis of silver nanoparticles with leaf extracts from both tested species. However, the best concentration of PVP for the formation of AgNPs was 34 mg.mL⁻¹ (Figure 3B). Higher concentrations of PVP negatively affected the synthesis of AgNPs as can be observed by the high level of noise in the obtained spectra. From the results obtained, the X-ray diffraction pattern is clear that the AgNPs formed using the *Tabebuia* sheet were essentially crystalline.

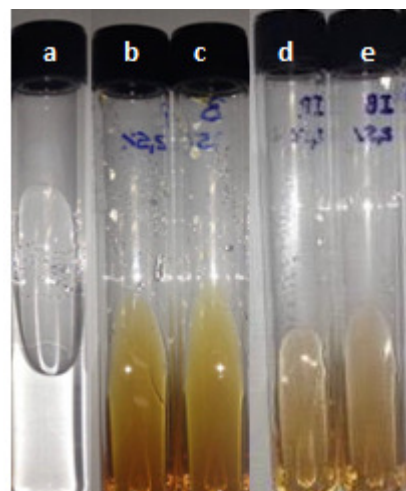


Figure 1. Synthesis of silver nanoparticles using silver nitrate solution added with plant extracts: Negative control, silver nitrate added (a), *Tabebuia roseoalba* (b) and *T. pentaphylla* (c) leaf extract *in vivo*, *T. roseoalba* (d) and *T. pentaphylla* (e) *in vivo*.

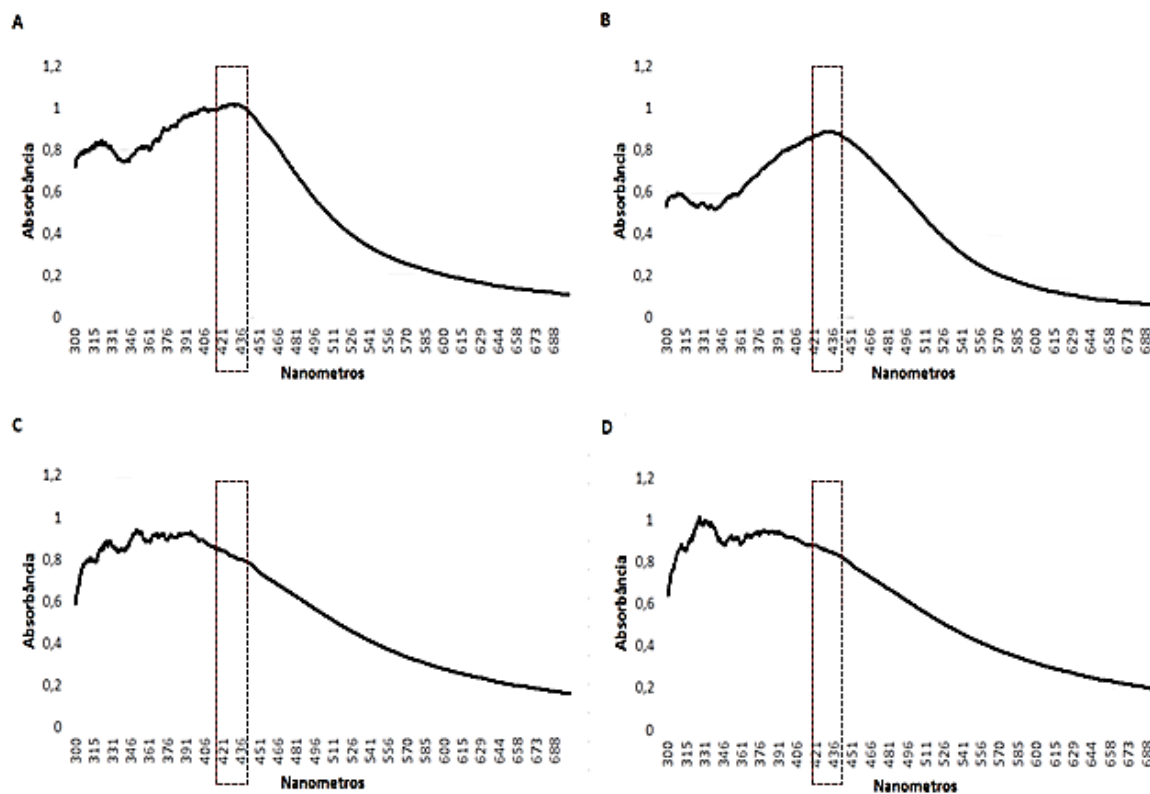


Figure 2. UV-Vis spectrophotometry analyzes: A. *Tabebuia roseoalba in vivo*; B. *T. pentaphylla in vivo*; C. *T. roseoalba in vitro*; D. *T. pentaphylla in vitro*.

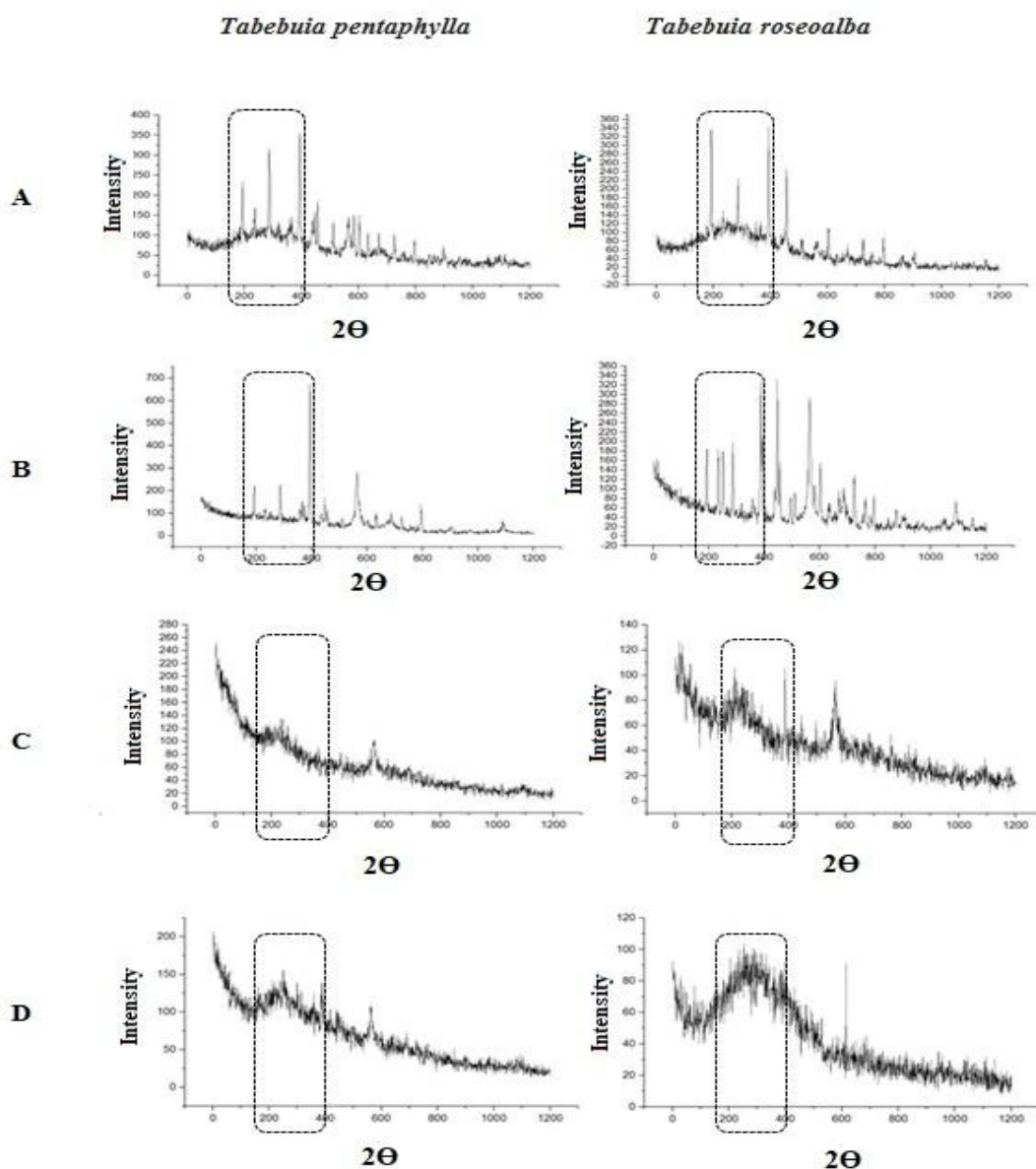


Figure 3. DR-X results of AgNPs green synthesis. A. Synthesis without PVP – control; B. adding 1 g; C. adding 2 g; D. adding 3 g of PVP.

Discussion

Due to its possible applications in different productive chains (pharmacological, energy, bioremediation process, etc.), the study of the synthesis of AgNPs has been gaining interest in recent years. As the chemical synthesis has the disadvantage of a large number of toxic residues generating, the study of metallic nanoparticles green synthesis, using natural compounds or plant components, has emerged as an alternative environmentally friendly methodology (Ahmed et al., 2016; Taheriniya & Azad, 2016). Using this strategic process, the toxicity of the chemicals generated in that synthesis process would be avoided. Green synthesis has been performed using compounds from different biological sources. Among them, extracts of fungi and plants have

been the most used to synthesize NPs without the need for additional reducing agents and stabilizers (Ahmed et al., 2016; Moghaddam et al., 2015). According to Prasad (2014), plants are better synthesizers for nanoparticles probable because plants can actively uptake and bioreduce metal ions from soils and solutions during the detoxification process. As the extracts of plants contain functional substances with proven action in bioreduction, these substances may be responsible for the synthesis and stabilization of metallic nanoparticles (Rajan et al., 2015). In the present study, it was verified that extract from *Tabebuia* species tested has potential use for nanoparticle synthesis.

Some of the main vantages of green synthesis are a low-cost production and

environmentally friendly process. As so, these processes have a potential advantage of being employed and are suitable in the large-scale production of nanoparticles (Olad et al., 2018). Therefore, this study focused on the biosynthesis of AgNPs with plant extracts of *Tabebuia* leaves (*Tabebuia* spp.) from different species. It has been previously described that the size and shape of the nanoparticles could influence by varying the pH, temperature, and ratio between the leaf extract and the silver nitrate solution (Kredy, 2018). However, it was observed in the present study that the condition of plant culture (*in vivo* or *in vitro*) was also an essential factor that can affect the synthesis of silver particles since the extracts made with leaves of plants grown *in vitro* were ineffective to produce AgNPs.

The colorimetry observed in the formation of the nanoparticles indicated that the original format of the synthesis would be spherical nanoparticles. It is believed that the weak binding of proteins in the solution with silver ions, leading to the isotropic growth of AgNPs (Tippayawat et al., 2016), may have generated the spherical form of AgNPs. The change in coloration in silver nanoparticle synthesis solutions is due to excitation of surface vibration of silver nanoparticles (Khalil et al., 2014; Rao & Tang, 2017). A color variation from reddish-brown to dark brown can occur according to the size of the nanoparticles produced (Albernaz, 2014). Differences between ions solutions before *Tabebuia* leaves extract additions were observed. However, the color of the solution obtained with *in vitro* *Tabebuia* leaves was lighter than the color observed in the solution of *in vivo* leaves. These results indicate that physiological differences between plants from the same species could interfere in the process reproducibility.

Although high temperatures and pressure facilitate the reduction processes (Liu et al., 2013), the synthesis of AgNPs from *Tabebuia* (*in vivo*) leaves extracts occurred rapidly and proved to be stable under ambient temperature and pressure conditions.

It is well described that the use of capping agents, such as Polyvinylpyrrolidone (PVP) can positively interfere in the nanoparticles synthesis, favoring anisotropic crystalline growth. According to (Koczur et al., 2015), well-dispersed silver nanoparticles can be obtained with PVP as a reaction dispersant between silver ions and glucose. According to these authors, PVP may have protected AgNPs from excessive ion accumulation, preventing weak growth and agglomeration. In the case of *Aloe vera*, a plan of AgNPs arose predominantly as the main peak using the PVP as a capping agent (Tippayawat et al.,

2016). The same result was observed in the formation of AgNPs with *Tabebuia* spp. extract (*in vivo* cultured plants). This result corroborates the relevance of using capping agents to improve nanoparticle synthesis.

However, as the plant condition of culture interfere drastically on leaves extract potential use to nanoparticle synthesis, and the most relevant physiological differences between *in vitro* plants compared to *in vivo* plants have low photosynthetic rates (Arigita et al., 2002). It was suggested that during the photosynthesis, essential molecules are produced for the process of AgNP synthesis.

Some other studies suggest that binding proteins can be involved in AgNPs formation and stabilization. Under suitable conditions, some substances found in leaf extracts (triterpene acids, flavonoids, sesquiterpene glycosides, polysaccharides, and proteins) may act as silver nitrate reducers for the formation of AgNPs (Rao & Tang, 2017). However, the precise class of substance that mediates AgNPs synthesis using *Tabebuia* leaves extract must be further investigated.

The use of X-ray diffraction analysis could be helpful to indicate AgNPs formation. In the present study, X-ray diffraction showed that the peak pattern observed in the silver nitrate sample differed from the peaks observed in the nanoparticle samples. The free silver ions may have been complexed with the organic substances present in the plant extract, thus providing the formation of nanoparticles. This organization of silver ions in the form of nanoparticles may interfere with the X-ray diffraction pattern, as seen in the spectra. The typical X-ray spectrum of nanomaterials consists of crystalline component peaks. The identification of the structure, composition, and degree of the texture of the crystalline phases can be inferred by the relative positions and intensities of the peaks. Peak width is related to the crystallinity of the material, i.e., grain size and grid distortion. According to Khalil et al. (2014), this result may suggest that the crystallization of the bioorganic phase occurs on the surface of silver nanoparticles.

The temperature and time of reaction are the main parameters in order to establish a nanoparticle synthesis routine process (Liu et al., 2014). The nanoparticle particle shape can interfere with antibacterial agent potential (Feng et al., 2000). Determining favorable synthesis conditions are essential to control morphology, size, and purity of nanoparticle formation. Another important characteristic, previously reported in the literature, is that the inorganic (metal) nanoparticles biosynthesis with plant extracts

improved its bactericidal effect (Yousefzadi et al., 2014). According to Tippayawat et al. (2016), the increase in bactericidal activity was possibly caused by synergistic antibacterial effects of AgNPs and naturally occurring chemicals in *Aloe vera*. As various natural ligands may interact with the microbial membrane, such as saponin, tannin, terpenoids, and flavonoids (Griffin et al., 1999; Sahu et al., 2013), it is possible that silver nanoparticles produced by green synthesis are potentially more efficient in the control of microbial contamination if combined with some of these compounds during nanoparticle formation. As so, the AgNPs produced by *Tabebuia* leaves extracts could be potentially used to control microorganism contamination (ex. During plant *in vitro* culture). However, this antimicrobial effect must be later further investigated.

Conclusion

The results obtained in this study show that organic compounds which originate from the *Tabebuia* leaf extract probable function as reductants and stabilizers for AgNPs green synthesis. A wide range of substances found in leaves extracts, such as triterpenic acids, flavonoids, sesquiterpenes glycosides, polysaccharides, and proteins can be act as silver nitrate reductants for the AgNPs formation under proper conditions.

Acknowledgments

This study was funded by Centro de Tecnologias Estratégicas do Nordeste (CETENE). Authors also express gratitude to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support and to CETENE for structural support.

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