Silver nanoparticles from *Prosopis glandulosa* and their potential application as biocontrol of *Acinetobacter calcoaceticus* and *Bacillus cereus*

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**ABSTRACT**

In the present study the characterization and properties of silver nanoparticles from *Prosopis glandulosa* leaf extract (AgNPs) were investigated using UV–Vis spectroscopic techniques, energy dispersive X-ray spectrometers (EDS), zeta potential and dynamic light scattering. The UV–Vis spectroscopic analysis showed the absorbance peaked at 487 nm, which indicated the synthesis of silver nanoparticles. The experimental results showed silver nanoparticles had Z-average diameter of 421 nm with higher stability (−200 mV). The EDS analysis also exhibited presentation of silver element. Additionally, the different concentrations of AgNPs (25, 50, 75 and 100 mg/mL) showed antibacterial activity against *Acinetobacter calcoaceticus* and *Bacillus cereus*. Finally, AgNPs from leaf extracts of *P. glandulosa* may be used as an agent of biocontrol of microorganism of importance medical. However, further studies will be needed to fully understand the antimicrobial activity of silver nanoparticles obtain from *P. glandulosa*.

1. Introduction

Nanoparticles (NPs) are a group of materials with distinctive features and extensive applications in different fields of science and medicine.[1] Metal nanoparticles have been extensively studied and different approaches have been engaged for the preparation of these compounds. Among these metal nanoparticles, silver nanomaterials exhibit broad spectrum biocidal activity toward bacteria, fungi, and viruses.[2] This has motivated its use in a large number of biomedical applications.[3] In this sense, the nanoparticles may penetrate inside the cell causing damage by interacting with phosphorus and sulfur containing compounds such as DNA and protein. Another possible contribution to the bactericidal properties of silver nanoparticles is the release of silver ions from the particles.[4] At the present, various chemical and physical methods are known for preparation of silver and other metal nanoparticles. However, these methods are very costly and toxic to the environment.[5] On the other hand, the biological method provides a feasible alternative for the synthesis of nanoparticles. In this sense, the use of plants as the production assembly of silver nanoparticles has drawn attention, because of its rapid, eco-friendly, economical protocol and providing a single step technique for the biosynthetic processes.[6] Several plant extracts have been used to produce nanoparticles, such as *Cissus quadrangularis* or *Ficus benghalensis*. In recent years, plant-mediated biological syntheses of nanoparticles have been gaining importance due to its simplicity and great potential with natural reductants.[9] The genus *Prosopis* L., is characteristic of arid and semi-arid zones, and its widespread distribution includes ecosystems in Asia, Africa, and the Americas.[10] In Mexico, the majority of the populations of the genus *Prosopis* are found in the north and center of the country, where they have formed forest extensions that are adapted to desert climate.

Species such as *Prosopis glandulosa* Torr., dominate the vegetation mosaic in Baja California Mexico, primarily in an ecosystem known as mezquital, which is characterized by a hyperarid climate with almost no rainfall (0.6 mm year−1).[11] These populations of the genus *Prosopis* have been the focus of scientific interest mainly because of their physiological and ecological adaptations to their hyperarid environment. However, studies about the use of *P. glandulosa* for the biosynthesis of AgNPs as a green chemistry method are scarce. In the present study, we report the easy synthesis of silver nanoparticles by an environmental friendly procedure...
involving the in situ reduction of Ag by P. glandulosa extracts and the evaluation of their antimicrobial activity against Acinetobacter calcoaceticus and Bacillus cereus.

2. Material and methods

2.1. Biosynthesis of silver nanoparticles (AgNP) from P. glandulosa

To prepare the AgNP from P. glandulosa, healthy leaves of this plant were collected in Baja California, Mexico. The collected leaves were surface sterilized with 0.5% NaOCl (Clorox) for 1 min each, followed by an extensive rinse with deionized sterile water. For sample preparation: P. glandulosa fresh leaves were cut into small pieces and dried using oven an electric oven for 12 h at 50 °C. Then 30 g of dried leaf of P. glandulosa was mixed with 300 mL of deionized water and heated at 70 °C for 30 min. About 50 mL of aqueous P. glandulosa leaf extract was then filtered thrice through Whatman No. 1 filter paper and centrifuged at 4000 rpm for 10 min to remove particulate matter and to get clear solutions which were then refrigerated (4 °C) for further use. For AgNPs synthesis 0.4 mL of aqueous P. glandulosa leaf extract were added into 0.16 mL of aqueous solution of 10 mM silver nitrate and heated to 60 °C for 30 min.

The color change was observed which stands as a preliminary identification of the formation of AgNPs.[12] Additionally, the AgNPs were purified by centrifugation at 10,000 rpm for 15 min to remove excess silver ions. The centrifugation process was repeated three times to remove all silver colloids with deionized water. After the sediment (AgNPs) was transferred to freeze dryer and the powder was used in antibacterial Assays.

2.2. Characterization of AgNPs from P. glandulosa

To determine the time point of maximum production of silver nanoparticles, the absorption spectra of the samples previously prepared with P. glandulosa leaf extract and silver nitrate were scanned in UV–Vis (vis) spectra, between wavelengths of 400–500 nm in a spectrophotometer (Thermo Scientific BioMate 3 Spectrophotometer, USA), having a resolution of 1 nm and using water as the blank.

2.3. Scanning electron microscopy and energy dispersive X-ray

A scanning electron microscope (SEM) (JEOL 6010; JEOL, Tokio, Japan) was employed to characterize the size and morphology of the AgNPs at an accelerating voltage of 10 kV according to the following procedure: a drop of AgNPs sample was transferred on to carbon coated copper grids. Then the film on the SEM grid was allowed to dry by putting it under a mercury lamp for 3 min and analyzed for size determination,[13] For EDS analysis, the AgNPs were dried and drop coated on to carbon film. EDS analysis was then performed using the oxford instrument Thermo EDS.

2.4. Zeta potential and dynamic light scattering

In order to find out the stability and size distribution of AgNPs obtained from P. glandulosa. Zeta potential was measured by using a Nanotrac Wave (Microtrac) according to,[14] Two thousand micro liter of sample was transferred in the clear disposable zeta cell for the measurement of zeta potential. Measurements were made by means of Dynamic Light Scattering (DLS) in the range of 0.1–1000 μm at 25 °C, using laser wavelength of 780 nm and a scattering angle of 90°. The DLS data were analyzed by the Microtrac FLEX operating software.[15]

2.5. Antibacterial assays

The antibacterial activity of the synthesized AgNPs was determined using the disk diffusion method. Two types of bacteria, including Gram-negative bacteria (Acinetobacter calcoaceticus) and Gram-positive bacteria (Bacillus cereus) were tested. Nutrient agar medium plates were prepared, sterilized and solidified. After solidification bacterial cultures were swabbed on these plates (each strain was swabbed uniformly onto individual agar plates). Sterile paper disks were placed on the agar plates. The plates were divided into three groups and each group included two types of bacteria (A. calcoaceticus and B. cereus); in the first group, 20 μL of sterile distilled water were applied to the disks as control. In the second group, 20 μL of different concentration of AgNPs, previously diluted with deionized water (25, 50, 75 and 100 mg/mL) was added to each disk. In the third group, 20 μL of leaf extract of P. glandulosa were applied to the disks (control). All the plates were incubated at 32 °C for 24 h. After incubation was completed, the zone of inhibition, which appeared as a clear area around the disks, was measured.

3. Results

3.1. Biosynthesis of AgNPs-P. glandulosa

The green synthesis of AgNPs through P. glandulosa extracts were carried out and confirmed by visual observation. After the addition of the plant extract to the aqueous AgNO₃ solution, the color of the solution reaction changed from yellowish to a reddish brown color within 30 min of incubation at 60 °C (Figure 1), indicating the formation of Ag-NPs. On the other hand, the UV–Vis spectroscopy is an important preliminary technique to ascertain the formation and stability of metal nanoparticles in aqueous suspension. Thus formation of AgNPs by reduction of aqueous Ag⁺ during exposure to the aqueous extract of P. glandulosa were followed
and characterized by UV–Vis spectroscopy. As shown in Figure 2, the surface plasmon resonance of the AgNPs was centered at approximately 487 nm.

**3.2. SEM and EDS analysis**

SEM has been employed to characterize the size, shape and morphology of synthesized silver nanoparticles. SEM provided further insight into the morphology and size details of the silver nanoparticles. SEM image (Figure 3) recorded at high magnifications of the Ag nanoparticles synthesized by treating AgNO₃ solution with *P. glandulosa* leaf extract. In general, the SEM image shows that the majority of nanoparticles were in spherical shape with varying size as visually seen from the adjacent photographs. It can be seen that some of the particles are well dispersed and many of them have formed aggregates. On the other hand, the EDS analysis gives qualitative as well as quantitative status of elements that may be involved in formation of nanoparticles. The elemental profile of synthesized nanoparticles using extract of *P. glandulosa* shows higher counts at 3 keV due to silver, confirms the formation of silver nanoparticles (Figure 4).

**3.3. Zeta potential and DLS**

Particle size, size distribution and zeta potential were important characterizations of the silver nanoparticles because they govern the other characterizations, such as saturation solubility and dissolution velocity, physical stability, or even biological performances.[16] As shown in Table 1, zeta potential was found to be −200.0 mV for synthesized AgNPs. On the other hand, the size distribution of the synthesized AgNPs is depicted in Figure 5. It is observed that the particles obtained are polydisperse mixtures in the range of 32–600 nm with the average mean size (diameter) of 421 nm.
The elemental analysis of the silver nanoparticles revealed highest proportion of silver followed by C, Cl and O. This result is consistent with the results reported by [18] who found that higher counts of AgNPs synthesized using banana peel extract at 3 keV. On the other hand, our results showed that zeta potential was found to be −200.0 mV for synthesized AgNPs from *P. glandulosa*. Similar result were observed by [20,21] that mentioned that a zeta potential higher than 30 mV or lesser than −30 mV is indicative of a stable system. In this context, colloidal suspension of Ag nanoparticles synthesized using *Ceriops tagal* leaves extract and *Malus domestica* fruit extract were highly stable with a zeta potential of −34.18 mV.[22] The values of zeta potential for the AgNPs obtained in the present study indicate a long term stability of the colloids, which could be attributed to the presence of bioactive components present in the aqueous extract that cover nanoparticles stabilizing them. On the other hand, in the present study the average size of the particles obtained from *P. glandulosa* was of 421 nm. These findings are in agreement with previous studies realized by Palanisamy et al. [23], who reported that the particle size of the synthesized silver nanoparticles using *Emblica officinalis* leaf extract was in the range 139–595 nm with average size 367 nm. The mechanism behind the activity of AgNPs from *P. glandulosa* against *A. calcoaceticus* and *B. cereus* is not yet fully explored and there are some common mechanisms behind up to date. However, there are various theories suggested about the action of AgNPs on microbes to cause the antimicrobial effect. One possibility of growth-restriction may be a chance of the generation of free radicals by AgNPs positioned at surface which may have been thrashed lipid membrane followed by destruction of microorganisms.[24,25] Another fact is that the DNA has sulfur and phosphorus as its major components; the nanoparticles can act on these soft bases and destroy the DNA which would definitely lead to cell death.[25]

### 3.4. Antimicrobial activity of synthesized silver nanoparticles

In the present study, the antibacterial activity of the synthesized AgNPs from *P. glandulosa* against *A. calcoaceticus* and *B. cereus* was investigated. Our results showed that all concentrations of AgNPs from *P. glandulosa* present a clear zone of inhibition against tested bacteria with respect to leaf extract of this plant used as control and the zone of inhibition increased with respect to the concentration of biosynthesized AgNPs used in the medium culture (Figure 6 and Table 2). In contrast, the leaf extract of *P. glandulosa* did not exhibit antimicrobial effect against tested bacteria (Figure 6).

### 4. Discussion

In the present study, the silver nanoparticles exhibit reddish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles.[17] Initially the reduction of Ag⁺ ions leads to the formation of silver atoms (Ag) which may follow by agglomeration into oligomer cluster. These clusters eventually lead to the formation of colloidal Ag particles.[18] Similar findings were reported by [19] who used aqueous extract of fresh leaves of *P. juliflora* with 0.01 M AgNO₃ aqueous solution for the synthesis of AgNPs and they revealed that metabolites such as terpenoids or flavonoids could be responsible for the reduction and capping of AgNPs.

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**Table 1. Zeta-potential analysis of silver nanoparticles synthesized from *P. glandulosa*.

| Average particle size | Mobility | Charge  | Zeta potential | Polarity | Conductivity |
|-----------------------|----------|---------|----------------|----------|--------------|
| 421 nm                | −15.63 u/s/v/cm | −0.855 fc | −200 mV | Negative | 543 us/cm |

**Table 2. Inhibition of microorganisms by AgNPs from *P. glandulosa*.

| Microorganisms | Zone of inhibition (mm) | 25% | 50% | 75% | 100% |
|---------------|-------------------------|-----|-----|-----|------|
| *Bacillus cereus* | 5 ± 0.11ᵃ | 6 ± 0.24ᵇ | 7 ± 0.07ᵇ | 10 ± 0.07ᵇ | 13 ± 0.02ᵇ |
| *Acinetobacter calcoaceticus* | 6 ± 0.31ᵇ | 6 ± 0.30ᵃ | 10 ± 0.14ᵃ | 12 ± 0.11ᵇ |

Notes: Results are expressed as mean ± standard deviation of values from triplicate experiments. Values with the same letter (a or b) within each line are equal according to the Tukey test at *p* ≤ 0.5.

The elemental analysis of the silver nanoparticles revealed highest proportion of silver followed by C, Cl and O. This result is consistent with the results reported by [18] who found that higher counts of AgNPs synthesized using banana peel extract at 3 keV. On the other hand, our results showed that zeta potential was found to be −200.0 mV for synthesized AgNPs from *P. glandulosa*. Similar result were observed by [20,21] that mentioned that a zeta potential higher than 30 mV or lesser than −30 mV is indicative of a stable system. In this context, colloidal suspension of Ag nanoparticles synthesized using *Ceriops tagal* leaves extract and *Malus domestica* fruit extract were highly stable with a zeta potential of −34.18 mV.[22] The values of zeta potential for the AgNPs obtained in the present study indicate a long term stability of the colloids, which could be attributed to the presence of bioactive components present in the aqueous extract that cover nanoparticles stabilizing them. On the other hand, in the present study the average size of the particles obtained from *P. glandulosa* was of 421 nm. These findings are in agreement with previous studies realized by Palanisamy et al. [23], who reported that the particle size of the synthesized silver nanoparticles using *Emblica officinalis* leaf extract was in the range 139–595 nm with average size 367 nm. The mechanism behind the activity of AgNPs from *P. glandulosa* against *A. calcoaceticus* and *B. cereus* is not yet fully explored and there are some common mechanisms behind up to date. However, there are various theories suggested about the action of AgNPs on microbes to cause the antimicrobial effect. One possibility of growth-restriction may be a chance of the generation of free radicals by AgNPs positioned at surface which may have been thrashed lipid membrane followed by destruction of microorganisms.[24,25] Another fact is that the DNA has sulfur and phosphorus as its major components; the nanoparticles can act on these soft bases and destroy the DNA which would definitely lead to cell death.[25]
However, further studies are needed to confirm their potential of AgNPs from *P. glandulosa* in the biocontrol of the microorganisms evaluated.

5. Conclusions

The biosynthesis of silver nanoparticles using *Prosopis glandulosa* is a simple, environmentally friendly, low-cost and non-toxic approach. The fabricated nanoparticles showed antimicrobial activity against *B. cereus* and *A. calcoaceticus* at different concentration. Our findings indicate that AgNPs from *P. glandulosa* may have potential benefits as biocontrol agents for human pathogens. However, further studies are needed to confirm their potential, determine their effect on human pathogens and identify the bioactive compounds in *P. glandulosa*.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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