

# Green Synthesis of Silver Nanoparticles Using *Pluchea sericea* a Native Plants from Baja California, Mexico and their Potential Application as Antimicrobials

Ali Abdelmoteleb<sup>1</sup> · B. Valdez-Salas<sup>2</sup> · Monica Carrillo-Beltran<sup>2</sup> · Dagoberto Duran Hernandez<sup>1</sup> · D. González-Mendoza<sup>1</sup>

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**Abstract** The use of natural plant extracts in the synthesis of nanoparticles provides advancement over chemical and physical methods, due to its cost effectiveness and environment friendly nature. In this study, silver nanoparticles (AgNPs) have been synthesized with simple and green technique using *Pluchea sericea* plant leaf extract as reducer as well as stabilizer. The characterization and properties of AgNPs were investigated using UV–visible spectroscopic techniques, energy dispersive X-ray spectrometers (EDS), zeta potential and dynamic light scattering. The UV–visible spectroscopic analysis showed the absorbance peak at 487 nm, which indicates the synthesis of silver nanoparticles. The experimental results showed silver nanoparticles having Z-average diameter of 59.20 nm with higher stability (−70.9 mV). The EDS analysis also exhibits presentation of silver element. Additionally, the different concentrations of AgNPs (25, 50, 75 and 100 %) showed antibacterial activity against *Acinetobacter calcoaceticus*. Finally, AgNPs from leaf extracts of *P. sericea* may be used for antimicrobial activity against *A. calcoaceticus*. However, further studies will be needed to fully understand the antimicrobial activity of AgNPs and to determine if the microorganism can develop resistance toward these nanoparticles.

**Keywords** Silver nanoparticles · Plants extract · Biosynthesis · Antibacterial activity · Nanotechnology

## 1 Introduction

Nanoparticles (NPs) are a group of materials with distinctive features and extensive applications in different fields of science and medicine (Matei et al. 2008). Moral metal nanoparticles have been extensively studied and different approaches have been engaged for the preparation of metal nanoparticles (Raveendran et al. 2003). Among these metal nanoparticles, silver nanoparticles have attracted intensive research interest because of their important applications as antimicrobial, catalytic, and antifungal activity (Yang and Cui 2008; Ji-Ho et al. 2009). Silver has been used as an antimicrobial agent for centuries; the recent resurgence in interest for this element particularly focuses on the increasing threat of antibiotic resistance caused by the abuse of antibiotics (Yıldız and Ibtisam 2010). However, there are some limitations in using Ag ions or Ag salts as antimicrobial agents. Probable reasons include the interfering effects of salts. This type of limitation can be removed using silver in nano form. It is generally recognized that silver nanoparticles may attach to the cell wall, thus disturbing the cell wall permeability and cellular respiration. The nanoparticles may also 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 particles (Yıldız and Ibtisam 2010). It is important and necessary to prepare AgNPs with safely methods. Various chemical and physical methods are known for the preparation of silver and other metal

✉ D. González-Mendoza  
danasaf@gmail.com

<sup>1</sup> Instituto de Ciencias Agrícolas de la Universidad Autónoma de Baja California (ICA-UABC), Carretera a Delta s/n, Ejido Nuevo León 21705, Baja California, Mexico

<sup>2</sup> Instituto de Ingeniería de la Universidad Autónoma de Baja California, Calle de la Normal s/n y Boulevard Benito Juárez, 21100 Mexicali, Baja California, Mexico

nanoparticles. These methods are very costly and toxic to the environment (Kalaiarasi et al. 2013). Silver nanoparticles are fabricated by the reduction of silver ions to neutral silver atoms. Silver ions are reduced by the use of reducing agents (Kaushik et al. 2010). Biosynthesis of nanoparticles is nothing but the bottom up approach of nanoparticles synthesis. Phytochemicals present in the plants possess anti-oxidant or reducing properties which are responsible for the reduction of metal compounds. Methods used for the biosynthesis of metal nanoparticles are eco-friendly, biocompatible, nontoxic and clean (Sharma and Yangard 2009). In recent years, plant-mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and great potential with natural reductants (Sivalingam et al. 2012).

In this sense, the genus *Pluchea* belongs to one of the most diverse botanical family, Asteraceae. *Pluchea* counts about 80 species of small herbs and shrubs and a large number of these taxa (30–40) thrive in tropical regions. *Pluchea sericea* (Nutt.) Caville, commonly known as ‘cachanilla’ is an evergreen wild shrub growing in sandy or saline soils of the Baja California, Sonora and Chihuahua deserts in Mexico (Villaseñor and Villareal 2006). In Mexico, the study of *P. sericea* has focused principally in the evaluation and identification of secondary metabolites (e.g., flavonoids and phenolic compounds) with application in the pest control (Ail-Catzim et al. 2015). However, studies about the use of *P. sericea* for the biosynthesis of AgNPs as a green chemistry method are scarce. In this study, we report the easy synthesis of silver nanoparticles by an environmental friendly procedure involving the in situ reduction of Ag by *P. sericea* extracts and the evaluation of their antimicrobial activity against *Acinetobacter calcoaceticus*. This organism is a Gram-negative coccobacillus that has emerged from an organism of questionable pathogenicity to an infectious agent of importance to hospitals worldwide. This organism has the ability to accumulate diverse mechanisms of resistance, leading to the emergence of strains that are resistant to all commercially available antibiotics (Van Looveren and Goossens 2004).

## 2 Materials and Methods

### 2.1 Biosynthesis of Silver Nanoparticles (AgNPs) from *Pluchea sericea*

To prepare the AgNPs, fresh and healthy leaves were obtained from *P. sericea* plants grown at Mexicali, (32°24′34″N and 115°11′16″W) Baja California, Mexico. Leaves sterilization was done in the laboratory by one rinse with 0.5 % NaOCl (Clorox) for 3 min each, followed by an

extensive rinse with deionized sterile water. Then, the leaves were dried for 24 h at room temperature. The extract solution was prepared by heating 20 g leaves in flask with 200 mL of distilled water for 30 min at 60 °C. For AgNPs synthesis, 0.2 mL of *P. sericea* leaf extract were added into 0.8 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 (Azizi et al. 2013).

### 2.2 Characterization of AgNPs from *P. sericea*

An aliquot of the sample (1 mL) of the suspension were collected when finish of incubation period (30 min) to monitor the completion of bioreduction of Ag<sup>+</sup> in aqueous solution, followed by dilution of the samples with 3 mL of deionized water, and subsequent scan in UV visible (vis) spectra, between wavelengths of 400–500 nm in a spectrophotometer (Thermo Scientific BioMate 3 Spectrophotometer, USA), having a resolution of 1 nm according to Saxena et al. (2010).

### 2.3 Scanning Electron Microscope (SEM) and Energy Dispersive Analysis of X-ray Spectroscopy (EDS) Analysis of Silver Nanoparticles

A scanning electron microscope (SEM) JEOL 6010 was employed to characterize the size and morphology of the AgNPs at an accelerating voltage of 10 kV. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper, and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min. 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 (Ramalingam et al. 2013).

### 2.4 Zeta Potential and Dynamic Light Scattering (DLS) Measurements

Surface zeta potentials were measured using the laser zeta-meter (C:\Microtrac\FLEX 11.0.0.4\Databases\NANO-PARTICULAS.MDB). The mean size and its zeta potential of the particles was obtained present in the sample. Dynamic light scattering (or photon correlation spectroscopy) is an important technique generally used to realize the size distribution pattern of very small particles present in suspension or solution. Measurements were made by means of dynamic light scattering (DLS) in the range of 0.1–1000 µm according to Singh et al. (2010).

## 2.5 Antibacterial Assays

The antibacterial assays were done on *A. calcoaceticus* (Gram –) by standard disc diffusion method (Bao et al. 2011). Nutrient agar medium was used to cultivate bacteria. Fresh overnight cultures of inoculums (100  $\mu$ L) of each culture were spread on to nutrient agar plates. 20  $\mu$ L of synthesized AgNPs solution with four different concentrations (25, 50, 75 and 100 %) were prepared in distilled water, and was added to a 7 mm sterile filter paper discs and allowed to dry.

AgNPs containing discs were placed in plate and a control plate (discs with only *P. sericea* leaf extract) was separately prepared. The plates were incubated at  $30 \pm 2$  °C for 24. After incubation, the formation of inhibition zone was checked and the diameter of zone was measured.

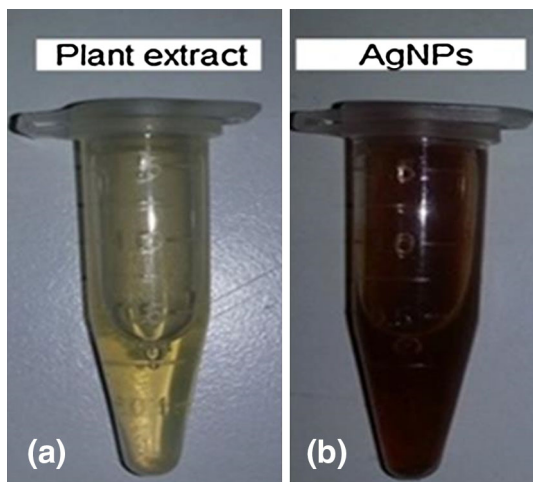
## 2.6 Statistical Analysis

Data were processed by analysis of variance with  $p < 0.05$ , with Tukey's test, using the Statistical Analysis System version 6.12 (SAS Institute 1997).

## 3 Results and Discussion

### 3.1 Synthesis of Silver Nanoparticles

The preliminary confirmation for the formation of AgNPs was the visual observation of color change of the aqueous solution of *P. sericea* leaf extract. Before the addition of silver nitrate the culture was in yellow color and at 30 min of reaction, the color of the solution was changed to brown (Fig. 1a, b, respectively). The color change may be due to the excitation of the surface plasmon resonance (SPR)

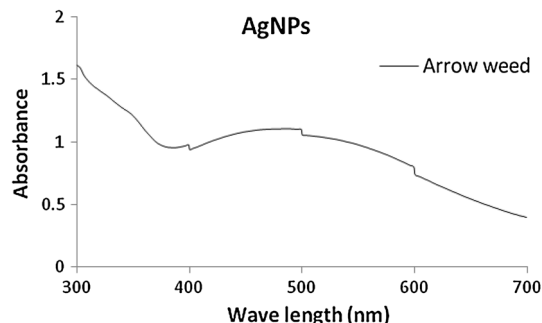


**Fig. 1** Synthesis of silver nanoparticles using plant extracts of *P. sericea*. **a** Extracts of *P. sericea* without AgNO<sub>3</sub>. **b** Extracts of *P. sericea* with AgNO<sub>3</sub>

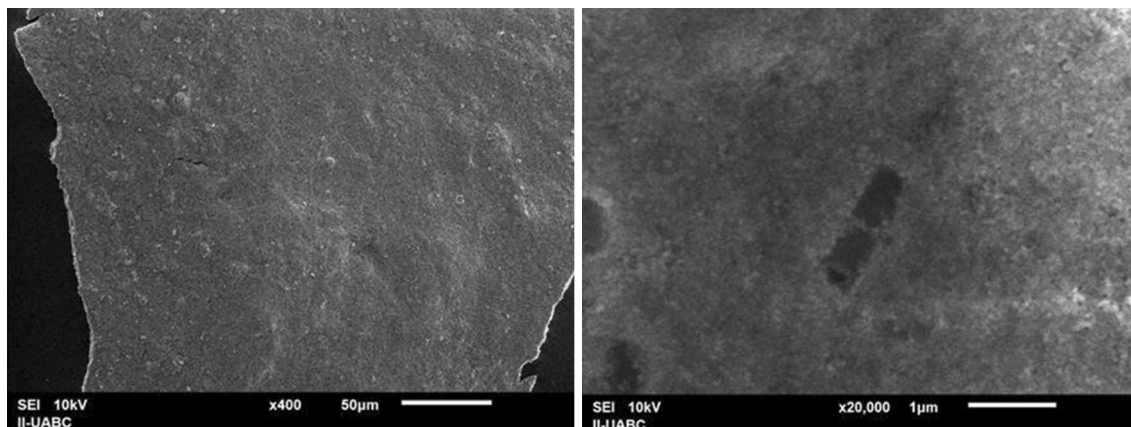
effect and the reduction of AgNO<sub>3</sub> (Azizi et al. 2013). This property is largely governed and dependent upon the particle type, size, shape as well as the nature of the surrounding medium (Dhand et al. 2016). In this sense, our result suggested that the biomolecules in plant extract may be responsible for the reduction of AgNO<sub>3</sub> and stabilization of AgNPs. In this context, Muthukrishnana et al. (2015) reported that presence of triterpenes and methoxy groups in the leaf extract of *Ceropegia thwaitesii* may play key role in reduction and stability of AgNPs. Therefore, the extract of *P. sericea* that is rich in polyphenols, such as flavonoids, triterpenes and sesquiterpenes molecules (Ail-Catzim et al. 2015), could act as reducing and stabilizing agents for AgNPs production. On the other hand, the UV–vis spectra obtained from *P. sericea* leaf extract and AgNO<sub>3</sub> show characteristic absorption band of AgNPs at 487 nm (Fig. 2). This was confirmed with the previous reports where spherical AgNPs showed the absorption bands at around 400–500 nm in the UV–visible spectra (Prathna et al. 2011). The results showed resemblance with the results of Baharara et al. (2014) who reported the synthesis of silver nanoparticles from *Achillea biebersteinii* flower extract with a strong absorption peak centered at 460 nm.

### 3.2 SEM and EDS Analysis

The morphology and size of silver nanoparticles analyzed using SEM is shown in Fig. 3. 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. In the microphotographs, it has also been seen that the synthesized silver nanoparticles were surrounded by other variety of compounds present in the extract (e.g., flavonoids, triterpenes and sesquiterpenes molecules) which may act as capping agent and provide stability to the silver nanoparticles. On the other hand, the EDS analysis gives qualitative as well as quantitative status of elements that may be involved in the formation of nanoparticles.



**Fig. 2** UV-vis spectra of the synthesized silver nanoparticles

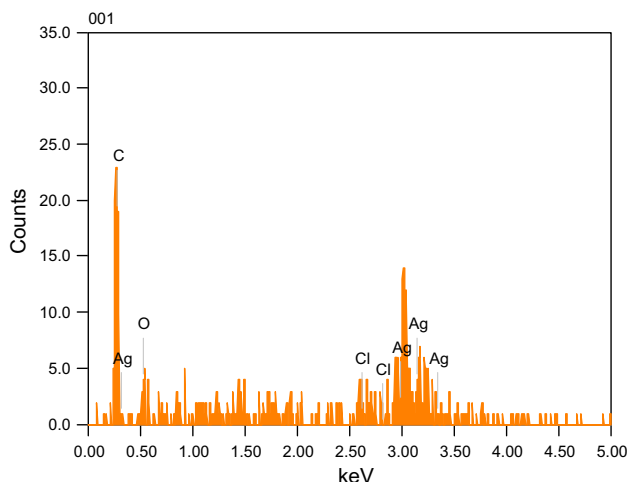


**Fig. 3** SEM image of silver nanoparticles from *P. sericea*

Figure 4 shows elemental profile of synthesized nanoparticles using leaf extracts and confirms the formation of silver nanoparticles. Figure 4 also showed higher counts at 3 keV due to silver nanoparticles. Generally, metallic silver nanocrystals show typical optical absorption peak approximately at 3 keV due to surface plasmon resonance (Baharara et al. 2014). The elemental analysis of the silver nanoparticles shown in the figure revealed highest proportion of silver followed by C, Cl and O. The peaks are from the biomolecules bound to the surface of the silver nanoparticles and the graphite pad used as sample support. This finding was in accordance with Baharara et al. (2014) who reported that higher counts of AgNPs at 3 keV.

### 3.3 Zeta Potential and Dynamic Light Scattering (DLS)

Particle size, size distribution and zeta potential were important characterizations of the silver nanoparticles



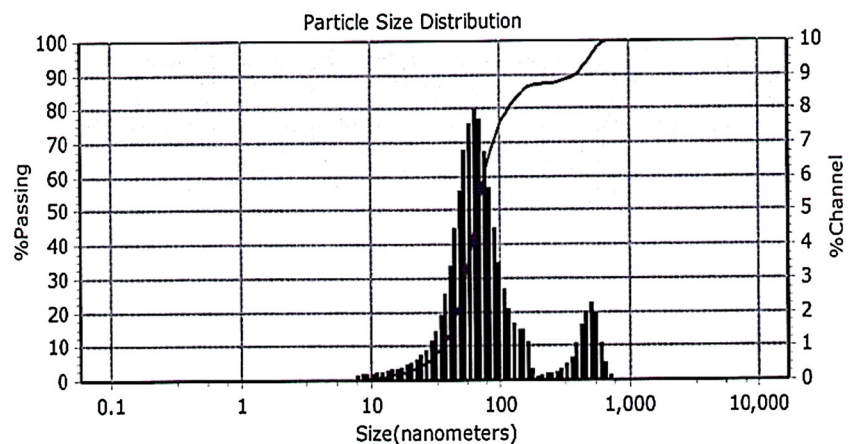
**Fig. 4** EDS image of silver nanoparticles produced from *Pluchea sericea*

because they govern the other characterizations, such as saturation solubility and dissolution velocity, physical stability, or even biological performances (Vishal and Agrawal 2011). The zeta potential value of the colloidal solution was determined to be  $-70.9$  mV. The negative surface charge could be due to the adsorption of bioactive components present in the aqueous extract onto the nanoparticles surface. The stability of the colloidal system is determined by the magnitude of the zeta potential ( $\zeta$ ). If the particles in suspension have a large negative or positive zeta potential then they will tend to repel each other and there will be fewer tendencies for the particles to come together and aggregate. The particles in suspension with  $\zeta$ -potentials more positive than  $+30$  mV or more negative than  $-30$  mV are considered stable (Duman and Tunç 2009).

However, if the particles in suspension have low zeta potential values then there will be no force to prevent the particles coming together and aggregating. From Table 1 it is clear that the colloidal suspension was highly stable with a  $\zeta$ -potential of  $-70.9$  mV. These results are in agreement with those obtained by Umoren et al. (2014) who found that zeta potential of silver nanoparticles synthesized using red apple (*Malus domestica*) fruit extract was  $-65.07$  mV. From the zeta potential value, it is evident that the nanoparticles prepared by leaf extract were found to stable without adding a different physical or chemical capping agent. Higher zeta potential indicates greater stability of the synthesized silver nanoparticles (Jebakumar and Sethuraman 2012). The size distribution of the synthesized AgNPs is depicted in Fig. 5. It is observed that the particles obtained are polydisperse mixtures in the range 20.42–119.4 nm. The average size of the synthesized silver nanoparticles using *P. sericea* leaf extract is around 59.20 nm. In this concern, Umoren et al. (2014) showed that the particle size of the synthesized silver nanoparticles using red apple fruit extract was in the range 50–300 nm with average size 150 nm.

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

Average particle size mobility	Charge	Zeta potential	Polarity conductivity
59.20 nm–5.54 u/s/v/cm	–0.03313 fc	–70.9 mv	Negative 582 us/cm

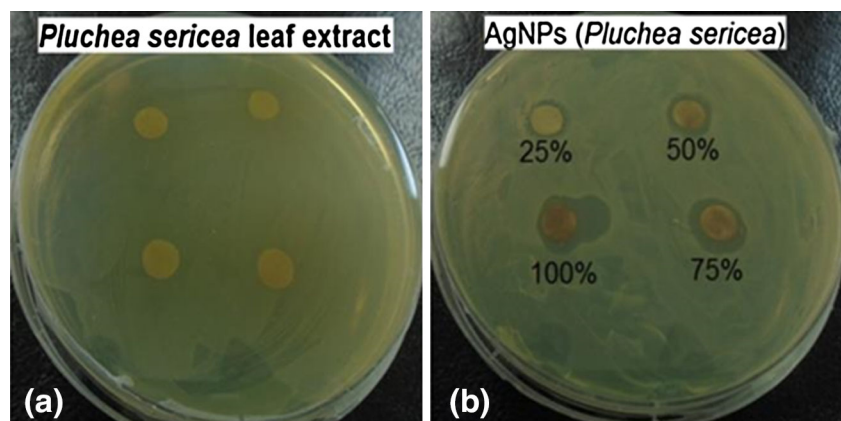
**Fig. 5** Particle size distribution of silver nanoparticles from dynamic light scattering measurements

### 3.4 Antibacterial Activity

*Acinetobacter* species are receiving increasing attention as significant opportunistic pathogens, usually in the context of serious underlying disease (Dijkshoorn et al. 2007). Disc diffusion is a distinguished and accustomed method to determine how is toxicity effect of a material in solution against to bacterial colonies (Parashar et al. 2011). In this sense, our results showed that all concentrations of AgNPs from *P. sericea* present a clear zone of inhibition against tested bacteria compared with *P. sericea* leaf extract used as control (Fig. 6). The results of antibacterial activity of prepared AgNPs evaluated from the disc diffusion method are given in Table 2. The result showed that AgNPs solutions, low (25 and 50 %) and high (75 and 100 %), doses from extract of *P. sericea* had an antimicrobial activity ( $p < 0.05$ ) against *A. calcoaceticus* (zone of inhibition range 8.0–9.0 mm, respectively) with respect to control. Similar results were observed in *Proteus vulgaris*-MTCC

1771, *Bacillus subtilis* (MTCC 10619), and *A. calcoaceticus* treated with AgNPs obtained from *Abelia grandiflora* and *Urtica dioica* Linn (Sharma et al. 2014; Jyoti et al. 2015). Generally, inhibition of microorganism increased in a dose dependent manner in the presence of nanoparticles (Sharma and Yangard 2009). Nevertheless, in this study, the low and high dose rates were much closed.

Therefore, the differences registered in 25 and 50 % doses of AgNPs were not significant. Analogous effect was observed when used high doses of AgNPs. On the other hand, the exact mechanism which silver nanoparticles employ to cause antimicrobial effect is not clearly known and is a debated topic. In this sense, several reports suggested that the synthesis of AgNPs could produce Ag ions which will damage the cell membrane, interrupt the metabolic activity, and subsequently lead to denaturation of protein, and finally cell death. AgNPs could also produce reactive oxygen species (ROS) (e.g., singlet oxygen, hydroxyl radical, and peroxide radical) which are toxic to

**Fig. 6** Antibacterial activity of synthesized AgNPs against *Acinetobacter calcoaceticus*: **a** *P. sericea* extract; **b** AgNPs from *P. sericea*

**Table 2** Inhibition of *Acinetobacter calcoaceticus* by nanoparticles from *P. sericea*

Microorganism	Zone of inhibition (mm) silver nanoparticles			
	25 %	50 %	75 %	100 %
Nanoparticles from <i>P. sericea</i>	8 ± 0.23 <sup>a</sup>	8.5 ± 0.42 <sup>a</sup>	9 ± 0.85 <sup>b</sup>	9 ± 0.73 <sup>b</sup>
<i>P. sericea</i> leaf extracts (control)	NA	NA	NA	NA

Results are expressed as mean ± standard deviation of values from triplicate experiments. Treatments with the same letter a or b are not significantly different (Tukey  $p < 0.05$ ),  $n = 3$

NA not appearing

the bacteria (Danilcauk et al. 2006; Kim et al. 2007). Finally, this new green method for the synthesis of AgNPs was established based on the use of plant extracts as reductants. The first step in this new synthesis was the selection of the plant extract with the best reductant characteristics. For this purpose, *Pluchea sericea* leaf was selected. This plant is abundant in Mexicali valley, therefore, they can be considered as low cost residues. This feature makes it very attractive for economic, availability and reuse issues. The biosynthesis of AgNPs using *P. sericea* leaf is a simple, environmentally friendly, low-cost and non-toxic approach compared with chemical and physical methods that are very costly and toxic to the environment (Kalaiarasi et al. 2013).

#### 4 Conclusions

This study demonstrates that AgNPs can be synthesized through a green approach that is an inexpensive, pollution free, and eco-friendly method using *P. sericea* leaf extract as a bio-reducing agent. Additionally, the AgNPs from *P. sericea* showed antimicrobial activity against *A. calcoaceticus* bacteria at two different concentrations (25 and 75 %). Our findings indicate that AgNPs from *P. sericea* 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. sericea*.

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