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Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd

Floral research doi:10.1016/S2222-1808(15)61018-0

Biological synthesis of silver nanoparticles by using *Viola serpens* extract

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ARTICLE INFO

Article history:
Received 28 Jan 2016
Received in revised form 10 Feb 2016
Accepted 25 Feb 2016
Available online 10 Mar 2016

Keywords:
Silver nanoparticles
Viola serpens
Eco-friendly
Characterization

ABSTRACT

Objective: To formulate a biological approach for the biological synthesis of silver nanoparticles using aqueous extracts of leaves of *Viola serpens* which is considered as a eco-friendly method as it does not include any harmful chemicals.
Methods: The synthesized silver nanoparticles were characterized by using UV-vis spectroscopy analysis, scanning electron microscopy analysis and X-ray diffraction analysis.
Results: Scanning electron microscopy analysis study revealed that synthesized silver nanoparticles were of an average size of 80–90 nm. Crystalline nature of synthesized silver nanoparticles was confirmed by X-ray diffraction analysis.
Conclusions: The leaves of *Viola serpens* can be a potent source for the biological synthesis of silver nanoparticles. The outcome of the study can lead to the development of a novel drug for biomedical field.

1. Introduction

Researchers are nowadays utilizing the biological methods for synthesizing metal nanoparticles in order to meet the growing need of eco-friendly and safe nanoparticles. Such nanoparticles are synthesized by using plant extracts which could be a more safe method as compared to other conventional approaches of silver nanoparticles synthesis. Several procedures are available for silver nanoparticles synthesis, such as thermal decomposition[1], electrochemical[2], microwave assisted process[3] and green chemistry[4]. The most critical limitation of such methods is that these include toxic chemicals and are non eco-friendly. The biological molecules suitable for the metal nanoparticles synthesis were found to be reliable, safe and eco-friendly. Nanotechnology is an emerging science of synthesizing and utilizing nanoparticles. Few methods of nanoparticles synthesis make use of hazardous chemicals and are unsafe. So, developing an environmentally friendly procedure to make antimicrobial agents avoid using toxic chemicals is gaining importance. Biogenic processes utilizing plant extract as a reducing agent have emerged as a easy and reliable alternative method as compared to chemical procedures.

Because of their unique properties, such as catalytic activity, optical properties, electronic properties, antibacterial properties, and magnetic properties *etc*, metal nanoparticles are choice of scientist for their novel synthesis[5]. The biological synthesis of metal nanoparticles is a very wide area of research and nowadays is developing the interest of researchers for their novel biological procedures of synthesis due to its simplicity and potential applications in various fields which was implemented in the development of novel technologies. The biogenic approach for the eco-friendly and safe metal nanoparticles synthesis is an interesting topic of research in modern material science over the past few years[6].

Viola serpens (*V. serpens*) is a plant related to family Violaceae. The different parts of various *Viola* species are utilized for formulation of medicinal plant extract based antibacterial agents. A number of species of the Violaceae family contain phytochemicals and are rich in cyclotides[7].

Although this plant involves in many medicinal purposes, still

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The journal implements double-blind peer review practiced by specially invited international editorial board members.

there are no findings that show the mechanism of bioreduction by using *V. serpens* extract for the biological synthesis of silver nanoparticles. This study highlights the role of *V. serpens* extract as reducing agent in the bioreduction which converts silver ions into silver nanoparticles. Characterization of biologically synthesized silver nanoparticles was done through UV-vis, scanning electron microscope (SEM) and X-ray diffraction techniques.

2. Materials and methods

2.1. Collection and drying of leaves

Leaves of *V. serpens* were collected from hilly area of Dehradun and identified by Botanical Survey of India, Dehradun. Then *V. serpens* leaves were treated thoroughly three times with water followed by distilled water. Then these leaves were dried in air and fine powder was made by using pestle and mortar. The powdered material was packed in separate container until extraction was done.

2.2. Preparation of V. serpens leaves extract

Fifteen grams of fresh powdered plant material was weighed and mixed with 100 mL sterile autoclaved water and boiled for 15 min. Then material was filtered through Whatman No. 1 filter paper and extract was prepared. The prepared extract was maintained at 4 °C for further investigations[8].

2.3. Synthesis of silver nanoparticles by V. serpens leaf extract

Silver nitrate ($AgNO_3$) was obtained from grey scientific chemicals. All glass wares were washed with sterile distilled water and dried in an oven before use. And 1 mmol/L $AgNO_3$ solution was used for the bio-reduction reaction for the synthesis of biological nanoparticles. Ten milliliter of prepared plant extract was added dropwise into 200 mL of aqueous solution of 1 mmol/L $AgNO_3$ for reduction into silver ions and kept for 15–30 min at 60–65 °C. This extract was used as a reducing agent for 1 mmol/L of $AgNO_3$. Then synthesized silver nanoparticles were further characterized[8].

2.4. Purification of biosynthesized silver nanoparticles

The centrifugation of fully reduced solution of $AgNO_3$ was done at 5 000 r/min for 30 min. The particles settled down and were thoroughly washed with distilled water for 2 or 3 times to remove the extract from it and dried in hot air oven. The prepared silver nanoparticles were then stored for further purposes[9].

2.5. Characterization of synthesized nanoparticles

2.5.1. UV-vis spectroscopy

UV-vis spectral analysis was done by using a Shimadzu UV-vis spectrophotometer and the sample was scanned between 200 and 800 nm at a scanning speed of 300 nm/min. The sterile distilled water was used as a blank reference.

2.5.2. pH analysis of synthesized silver nanoparticles

Initially, pH of 1 mmol/L aqueous $AgNO_3$ solution was 3.8, then change in pH was observed, indicating silver nanoparticles synthesis by using extracts of *V. serpens*. pH was determined using digital pH meter systronics.

2.5.3. SEM analysis of biosynthesized silver nanoparticles

The morphological characterization of synthesized silver nanoparticles was done using high resolution SEM analysis (SEM-Zeiss EVO 40). The sample was prepared by a simple drop coating with suspended silver solution onto an electric clean glass, allowing the solvent (water) to evaporate. The sample was left to dry at room temperature.

2.5.4. X-ray diffraction analysis

The X-ray diffraction results were obtained by X-Pert Pro Diffractometer using step scan technique and with Cu-Ka radiation (1.500 Å, 40 kV, 30 mA) in 1–2 h configuration. The sample of biosynthesized nanoparticles were coated on to the glass slide followed by drying and finally sample was analysed by X-ray diffractometer.

3. Results

Present research work utilized the *V. serpens* leaves aqueous extract as a reducing agent for reducing $AgNO_3$ solution to silver ions (Figure 1).

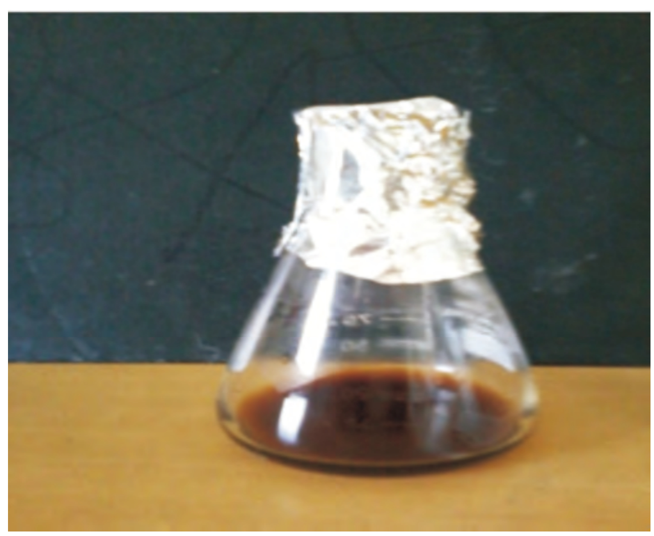

Figure 1. *V. serpens* leaves aqueous extract.

3.1. Synthesis of silver nanoparticle

Figure 2 depicts that silver nanoparticles were synthesized by using a biological method through reduction of $AgNO_3$ solution by plant extract. After addition of plant extract of *V. serpens* to $AgNO_3$ solution

(1 mmol/L), a change in color was observed from light yellow to dark brown. The brown colour indicated the biological synthesis of silver nanoparticles as shown in Figure 2.

Figure 2. Biological synthesis of silver nanoparticles.

3.2. UV-vis spectra analysis

The biologically synthesized silver nanoparticles were characterized by using UV-vis spectrophotometry. The peak observed at 426 nm (Figure 3) indicated the reduction of silver ions which further confirmed the formation of biosynthesized silver nanoparticles.

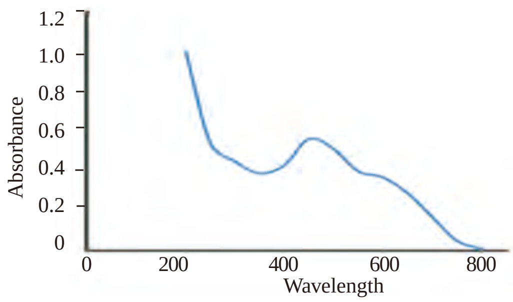

Figure 3. UV-vis absorption spectra of silver nanoparticles synthesized from *V. serpens*.

3.3. pH analysis

When plant extract was added dropwise into the aqueous $AgNO_3$ solution, a change in color was observed immediately which led to reduction in the pH, which may be an indication of synthesis of silver nanoparticles. In this study, it was observed that the pH changed from high acidic to low acidic.

3.4. SEM analysis of silver nanoparticles

Thin sample films were prepared on a carbon coated copper grid by just dropping a very little amount of the sample on the grid. Blotting paper was used to remove extra solution and then the films on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min. SEM image described that the relatively spherical shape of nanoparticles was synthesized by *V. serpens* leaves extract. It was depicted that relatively cubic and spherical silver nanoparticles were synthesized with diameter range 80–90 nm (Figure 4).

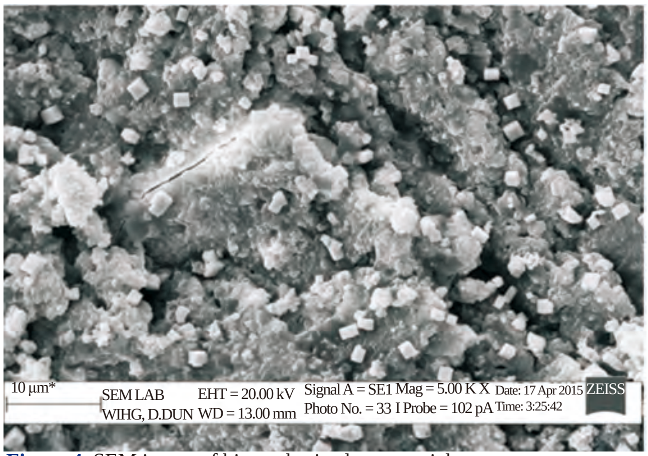

Figure 4. SEM image of biosynthesized nanoparticles.

3.5. X-ray diffraction studies

The X-ray diffraction analysis of biosynthesized silver nanoparticles was recorded and typical X-ray diffraction pattern was shown in Figure 5.

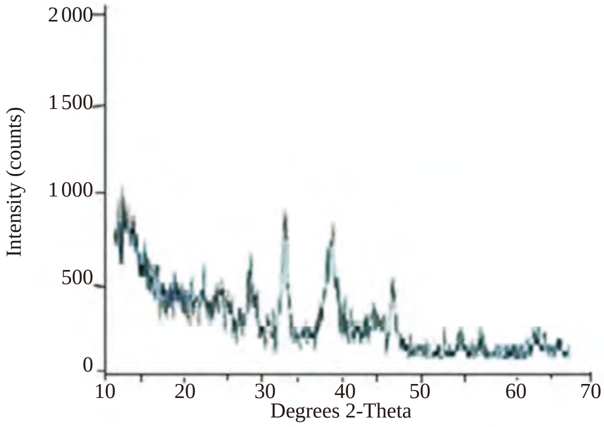

Figure 5. Graph denoted the X-ray diffraction analysis of biosynthesized nanoparticles.

4. Discussion

The flourishing of biological approach for synthesis of nanoparticles is evolving into a fruitful branch of nanotechnology. Previous studies reported that silver nanoparticle can be synthesized by plants such as *Catharanthus roseus*[10], *Phyllanthus amarus*[11], *Eichhornia crassipes*[6], and *Tinospora cordifolia*[8]. The present research dealed with the biological synthesis of silver nanoparticles by using the leaves extract of *V. serpens*. The approach seems to be cost effective approach and alternative to conventional techniques of synthesizing silver nanoparticles. Also, this approach appears to be eco-friendly, non toxic and safe in contrast with chemical and physical approaches for synthesizing silver nanoparticles.

When *V. serpens* leaves extract was added to the aqueous solution of $AgNO_3$, change of color from transparent to reddish brown was recorded, which indicated the synthesis of silver nanoparticles and this change in color has been previously observed by several researchers[8,11,12]. These researchers suggested that color change appeared due to the surface plasmon resonance of deposited silver nanoparticles. Confirmation of synthesis of silver nanoparticles was done by using UV-vis spectral analysis. It is generally recognized that UV-vis spectroscopy could be used to examine size and shape of nanoparticles. The peak was observed at 426 nm in current study and similar findings were reported earlier[8] which reported the peak obtained at 420–425 nm and 413 nm[13].

The size and shape of synthesized silver nanoparticles was characterized by SEM analysis. Present study depicted that relatively cubic and spherical silver nanoparticles were formed with diameter range 80–90 nm. In a similar study, biogenic synthesis of silver nanoparticles was reported earlier to depict the size and morphology of synthesized nanoparticles of size range 30–100 nm[14]. Another SEM study reported the spherical silver nanoparticles of size range 35–40 nm[15].

X-ray diffraction analysis is basically used for depicting the chemical composition and crystal structure of a material; therefore, in order to detect the presence of silver nanoparticles in plants tissues X-ray diffraction analysis was done. Earlier similar study was done by investigators[13,15]. The X-ray diffraction results clearly depicted that biologically synthesized silver nanoparticles were crystalline in nature. Similar findings were reported earlier[15]. Also one study suggested that the herbal formulations in present scenario are safe in contrast to the synthetic ones that are regarded as unsafe to human and environment[16]. Earlier a simple biological and low-cost approach was reported for synthesis of stable silver nanoparticles by reduction of $AgNO_3$ solution with a biological method using *Morus nigra* leaf extract as a reducing agent[17]. In the same way, in our study we have reported a low-cost, safe and simple biological approach for synthesis of silver nanoparticles.

It has been demonstrated that *V. serpens* leaves extract is capable of synthesizing stable silver nanoparticles, which is a cost effective and environment friendly approach, proving a simple and an efficient method for synthesis of nanoparticles. The synthesized nanoparticles were of cubic and spherical shape and the estimated sizes were in the range of 80–90 nm. The biologically synthesized silver nanoparticles in our study can be better alternative to synthetic ones.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors are thankful to Wadia Institute of Himalayan Geology for providing facilities to carry out SEM, X-ray diffraction analysis of synthesized silver nanoparticles. The authors also wish to acknowledge Sharda University for providing facilities for carrying out research work.

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