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Biosynthesis of Ag nanoparticles using *Salicornia bigelovii* and its antibacterial activity

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Abstract
Background and aim: In recent years, the field of nanotechnology has become the most active area of research in modern material science. While many chemical- as well as physical methods are also used, green synthesis of nanoparticles is becoming the most evolved method of synthesis. In this study, we synthesized silver nanoparticles from the seed extract of Salicornia bigelovii.
Methods: This experimental study was conducted from December 2017 to January 2018 in Kerman University of Medical Sciences, Kerman, Iran. The effects of two concentrations (1m M and 4mM) on the synthesis of nanoparticles were studied. Characterizations were done using different methods including ultraviolet (UV) visible spectroscopy, transmission electron microscopy (TEM), X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FTIR). Antibacterial activity of Ag nanoparticles against Staphylococcus aureus and Escherichia coli was studied using microdilution method. The data were analyzed using Probit test in SPSS (Version 20, USA).
Results: Formation of the AgNPs was confirmed by surface plasmon spectra using UV–Vis spectrophotometer and absorbance peaks at 434 nm. The FTIR spectra showed the possible role of the functional group like carbonyl groups in reduction of silver ions to silver nanoparticles. The XRD analysis showed that the synthesized silver nanoparticles are of face-centered cubic structure. The TEM showed the formation of silver nanoparticles ranging in diameter from 1 to 50 nm. The minimal inhibitory concentration and minimal bactericidal concentration of AgNPs were determined for both S. aureus and E. coli 6.25 and 12.5 µg/mL, respectively.
Conclusion: An environmentally friendly approach is more affordable than chemical methods. Physicochemical approaches can be harmful to the environment and to human health. Thus, the green synthesis methods are simple, less expensive, and can cut consumption of energy; they can be used for synthesis of fixed nanoparticles with preferred shape and size, without the use of toxic chemical agents.
Keywords: X-Ray Absorption Spectroscopy, Biosynthesis, Silver, Nanoparticles

1. Introduction

The biosynthesis of nanostructures is a green and environmentally friendly method. Gold, platinum, zirconium, cadmium, titanium oxide, zinc oxide, iron oxide, etc. are nanoparticles produced in this way. These particles are practically used in medicine, cosmetics, pharmaceutical and biochemical sensors (1-6). The synthesis of materials at the Nanoscale (1 to 100 nm) has attracted the attention of many researchers due to the unique physicochemical

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properties (7-10). Materials at the Nanoscale, which are called nanostructured, take many forms. From a variety of nanostructures, nanoparticles are widely produced and used in different sciences (11-13). Silver nanoparticles are the most used nanoparticles. Various chemical and physical methods have been used for the synthesis of nanoparticles. But these methods are often ineffective and have harmful effects on the environment (14, 15). Another method recently used for the synthesis of nanostructures is the use of biological resources to produce nanostructures called biosynthesis (16-18). Today, the synthesis of nanoparticles has been carried out with bacteria, fungi, plants and even insects (19-21). In comparison with most physical and chemical methods, this approach is cost effective, safe and environmentally friendly, and has shown a high performance (22, 23). Nanoparticles (NPs) have been of great concern due to their electrical, optical, magnetic, engineering and energy properties (24-31). The bioreduction properties of some bioresources such as *Phoenix dactylifera*, *Sinapis arvensis*, Banana (32), *Fusarium solani* (33), olive (34), *Brassica oleracea* (35), *Streptomyces microflavus*, *Streptomyces somaliensis*, carob (36), cobweb (37) and worm (38) have been studied in the synthesis of silver nanoparticles. In this study, we used the seed extract of *Salicornia bigelovii* (Figure 1) for synthesis of silver nanoparticles. As an important useful plant in food industry, *Salicornia bigelovii* is halophyte, and is used for desertification. *Salicornia bigelovii* is from the Chenopodiaceae family whose seeds contain 18% protein and 24% oil of which 70% are linoleic acid (omega-6). The following information about the analysis and the results of UV-visible spectroscopy, XRD, TEM, FTIR of synthesized AgNPs is described.

Figure 1. The plants and seeds of Salicornia bigelovii.

2. Material and Methods

2.1. Biosynthesis

This experimental study was conducted from December 2017 to January 2018 in Kerman University of Medical Sciences, Kerman, Iran. Seeds of *Salicornia bigelovii*. Torr were obtained from BO Da Yi investment and development Co, Shanghai, China. First, they were washed with water for cleaning the dust and then disinfected with 70% alcohol for approximately 2 minutes. They were then washed 3 times for approximately 2 minutes with deionized water. In the next step, we added 5 grams of the seeds to 250 ml Erlenmeyer flasks, containing 100 ml of deionized water boiling for 15 minutes, and then brought to room temperature. Finally, the seeds were poured out and the remaining extract was filtered using paper Whatman No. 1. The extract was used as the reduction and stabilization for the synthesis of silver nanoparticles. The extracts were stored at 4 °C in the dark. The Silver nitrate ($AgNO_3$) used to synthesize silver nanoparticles were obtained from Merck, Germany. For this synthesis, silver nanoparticles were used for final concentration (1 and 4 mM) from silver nitrate solution. It was done so that 15 ml of the extract was added to 30 ml from 1 and 4 mM primary stock separately. $AgNO_3$ was not added to the control sample. Finally, the samples were kept in the dark at 28 °C.

2.2. Characterization of AgNPs

To prove the production of silver nanoparticles, we used Absorption spectra UV-visible (Scan Drop Company Analytik Jena, Germany). The formation and quality of the compounds were checked by X-ray diffraction (XRD) spectrum (PANalitical, X PERTPRO, Holland) with CuKα radiation λ=1.5405 Å (Bragg angles: $10° \leq 2\theta \leq 70°$). FTIR (Bruker Tensor 27 of Germany) of the samples was measured. After 24 h of reaction with the S. bigelovii seed extract, the silver nanoparticles were centrifuged at 10,000 rpm for 10 minutes, and the dry powder of the nanoparticle was obtained and used for analysis. The size and morphology of the synthesized nanoparticles in the seed extract were examined using Transmission Electron Microscopy (TEM), the product of Carl ZIESS, Germany. The sample was suspended in distilled water, dispersed ultrasonically (5 mins) to separate individual particles, and one or two drops of the suspension was deposited on to hydrocarbon coated copper grids and dried under infrared lamp

2.3. Bioassay antibacterial activity of AgNPs

Staphylococcus aureus and *Escherichia coli* were obtained from the Pasteur Institute, Tehran, Iran. The standard microdiffusion method using 96-well sterile microtiter plate was used to study the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). The bacterial suspension (1 day-old) was inoculated in the wells containing different concentrations of Ag nanoparticles. After 24 h of incubation of the samples at 37°C, optical densities at 600 nm was recorded (BioTek's PowerWave XS2, USA). MIC is the lowest concentration of the nanoparticles to inhibit the growth of bacterial cells. MBC was the concentration at nanoparticles which killed 99% of bacterial cells. The data were analyzed using Probit test in SPSS (Version 20, USA) (39).

3. Results

3.1. UV-visible

During the biosynthesis using the extract, the color of the reaction medium changed rapidly to dark brown (Figure 2-B) due to Surface Plasmon Resonance (SPR). Synthesis of AgNPs from two concentrations (1 and 4mM) of AgNO3 was confirmed by using UV–vis spectroscopy. The absorption spectra of AgNPs solution showed a Surface Plasmon Resonance with a peak at 434 nm. (Figure 2-B). The characterization results are for AgNPs which were obtained with 4 mM $AgNO_3$.

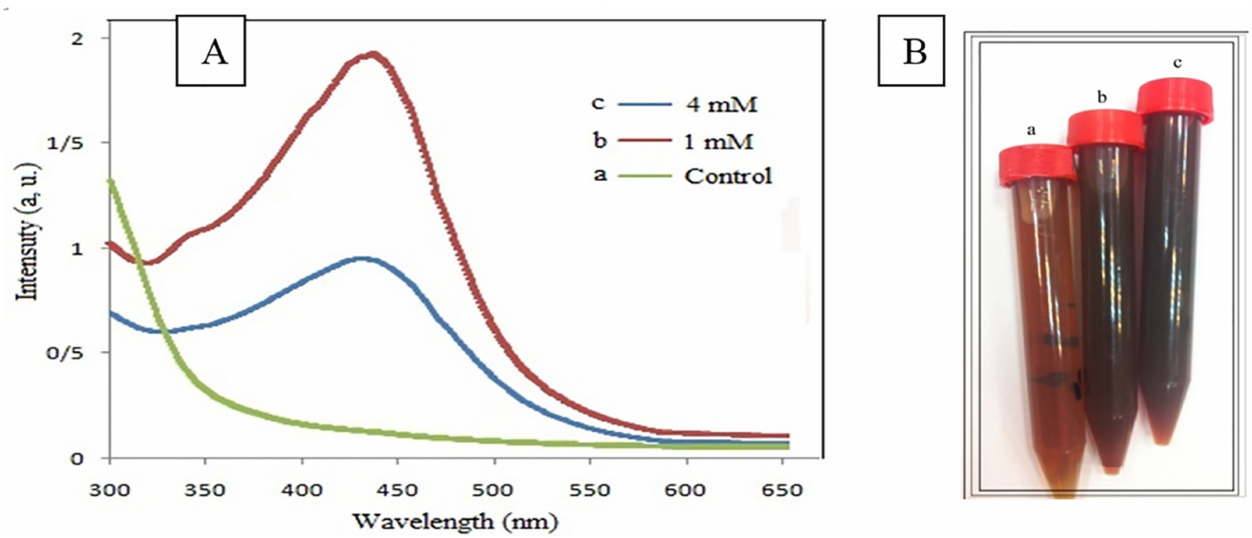

Figure 2. (A): Color change of the Salicornia bigelovii seed extract rapidly from light brown to dark brown after treatment with different concentrations of: 1(b) and 4 (c) mM silver nitrate; (B): UV-vis absorption of silver nanoparticles at 28 °C synthesized using different concentrations of: 0 (a), 1 (b) and 4 (c) mM silver nitrate.

3.2. XRD

Four main characteristic diffraction peaks for Ag which are denoted by (*) were observed at 38.176, 44.11, 64.543, and 77.311, which correspond to the (111), (200), (220), and (311) crystallographic planes of face centered cubic (FCC) Ag crystals, respectively (Figure 3).

3.3. TEM

TEM analysis was used to reveal the formation and the corresponding morphology of the silver nanoparticles. The TEM images are shown in Figure 4. The TEM image shows AgNPs ranging in diameter from 1 to 50 nm.

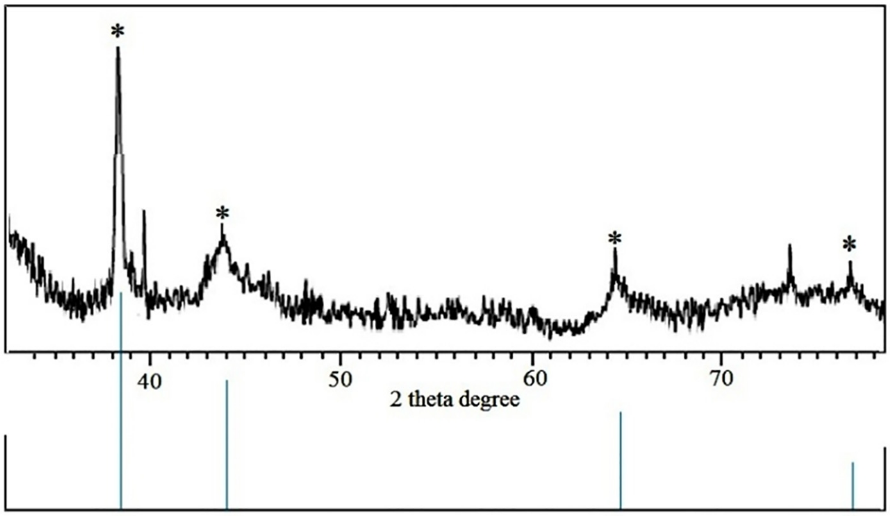

Figure 3. XRD pattern of silver nanoparticles synthesized using *Salicornia bigelovii*

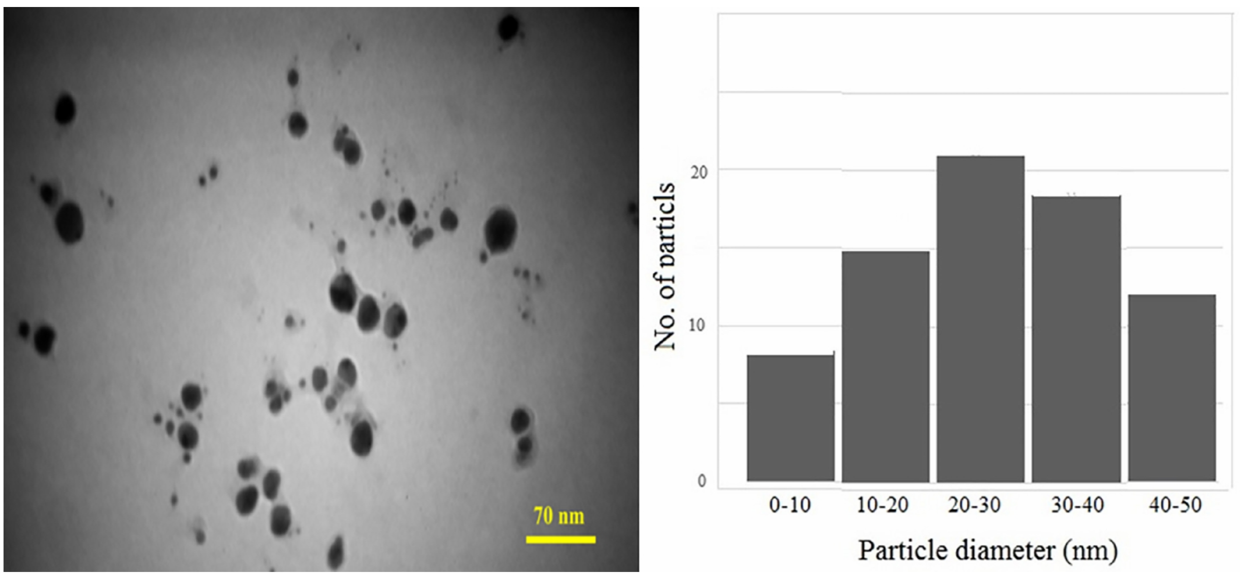

Figure 4. TEM images of silver nanoparticles synthesized at 30° C and 5 mL extract Salicornia bigelovii and plot size and frequency of the nanoparticles and histogram of particles size distribution.

3.4. FTIR

Vibration characteristics of chemical functional groups in a sample are identified using infrared spectroscopy. When infrared light interacts with matter, its chemical bonds will contract, stretch, and bend. Regardless of the structure of the rest of the molecule, a chemical functional group tends to adsorb infrared radiation in a specific wavenumber range, as a result. Hence, the correlation of the band wave number position with chemical structure is used for recognizing a functional group in a nanoparticle associated molecule in a sample. A number of strong bands were in FTIR spectra (Figure 5). It has been reported that proteins can provide a good protective environment for metal hydrosol during their growth processes (Mitra and Das 2008). The band at 1426 cm^{-1} is assigned to the methylene scissoring vibrations from the proteins. It is well known that proteins can bind to silver nanoparticle through either free amine groups or cysteine residues in the proteins (Gole et al. 2001) and therefore, stabilization of silver nanoparticles by the surface bound proteins is possible in the present green synthesis. The extract samples show strong absorption bands at 3750, 3443, 2927, 2367, 1636 and 1426 cm^{-1}. The bands at 1636 cm^{-1} are characteristic of amide I (Caruso et al. 1998). The amide band I is assigned to the stretch mode of the carbonyl group coupled to the amide linkage. The FTIR results indicate the presence of proteins and other biomolecules in the seed extract, and these biomolecules might participate in the formation of Ag nanoparticles. The sensitivity of *S. aureus* and *E. coli* to the AgNPs produced by ecofriendly method was tested by microdiffusion assay. The MIC and MBC of AgNPs were determined for both *S. aureus* and *E. coli* 6.25 and 12.5 µg/mL, respectively. Antibacterial tests showed a significant activity against the tested bacteria. The AgNPs showed a significant antibacterial effect against the tested bacteria, while the extract (Control negative) did not show antibacterial effect.

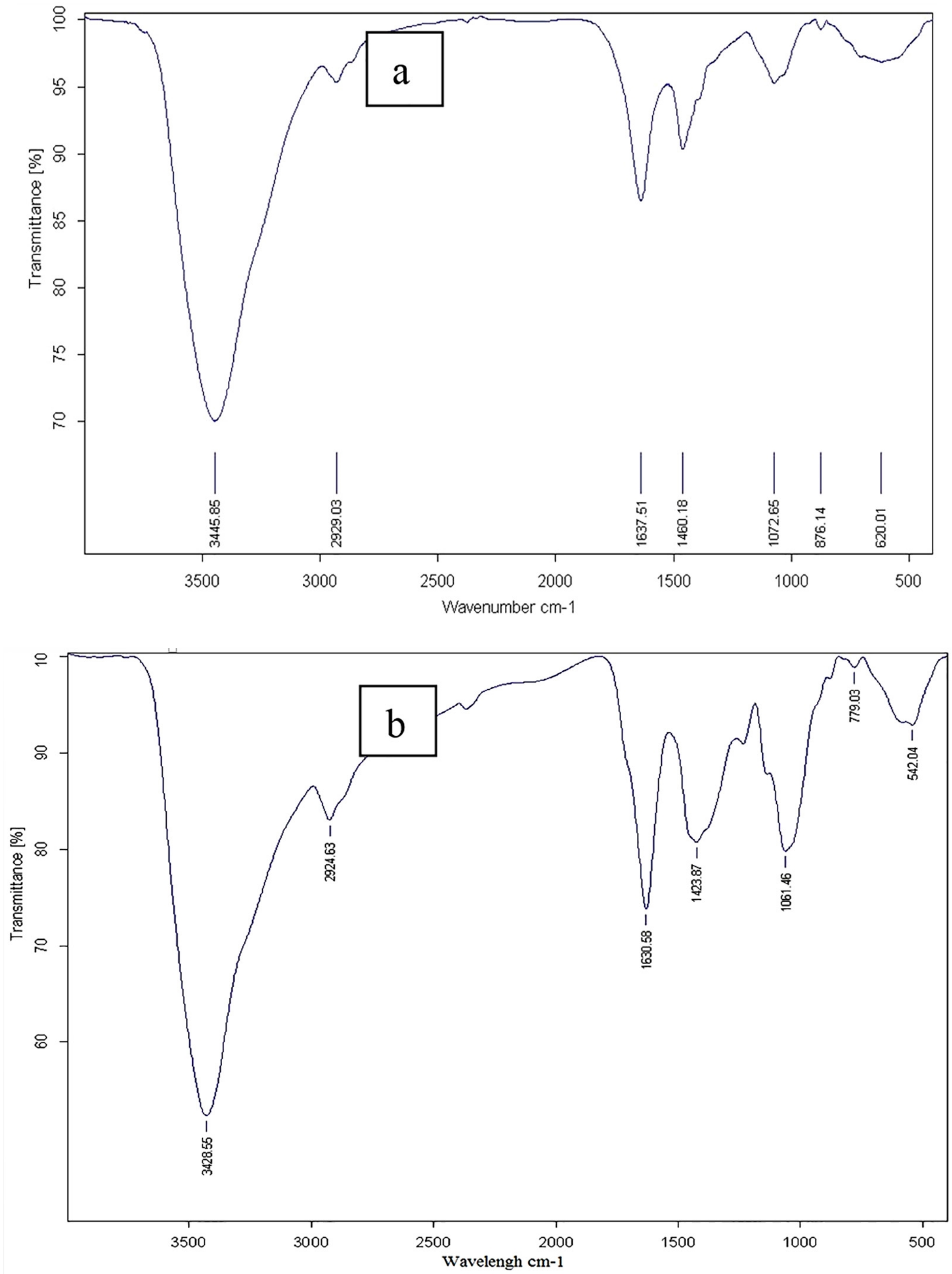

Figure 5. FTIR spectra a): before and b): after synthesis nanoparticles.

4. Discussion

Silver nanoparticles (1 to 50) nm were synthesized using a cheap, simple and environmental method. Antimicrobial effects of silver nanoparticles compared with *Salicornia* extract alone broth microdilution method were investigated. The MIC and MBC of AgNPs were determined for both S. aureus and E. coli 6.25 and 12.5 µg/mL, respectively.

Mirza Jani et al. studied the effects of silver nanoparticles on Staphylococcus aureus PTCC1431 and reported that minimum inhibitory concentration was 4 µg/ml (40). Wadi et al. studied the effect of silver nanoparticles on *Staphylococcus aureus*, *Staphylococcus aureus* which are resistant to methicillin (MSRA) and Candida glabrata and the minimum inhibitory concentration was reported 1.95, 1.95 and 15.63 µg/ml, respectively (41). Khamenei et al.. declared that minimum growth inhibitory concentration (MIC) and minimum concentration which destroyed more than 99 percent of *Staphylococcus epidermidis* bacterial cells were 8 and 32 µg/ml, respectively ((42). While in our research, minimum inhibitory concentration of silver nanoparticles was determined at 6.25 µg/ml.

5. Conclusions

Silver nanoparticles with a size range of 1 to 50 nm were synthesized using *Salicornia* extracts without using any foreign chemical composition. Synthesis using the natural source is very inexpensive, simple and environmentally friendly. Antimicrobial effects of silver nanoparticles compared with *Salicornia* extract alone broth microdilution method were investigated. A green approach would be less expensive than chemical methods. Physicochemical approaches could be hazardous to the environment and to human health. The green synthesis approaches are simple, more affordable, and reduce energy consumption, which can be used for synthesis of fixed nanoparticles with preferred shape and size, without the use of toxic chemical agents.

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Conflict of Interest:

There is no conflict of interest to be declared.

Authors' contributions:

All authors contributed to this project and article equally. All authors read and approved the final manuscript.

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