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ORIGINAL ARTICLE

Sustainable synthesis of silver nanoparticles using macroalgae *Spirogyra varians* and analysis of their antibacterial activity

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Abstract In this research, silver nanoparticles (SNPs) were synthesized through bio-reduction of silver ions using the *Spirogyra varians*. The procedure used is simple and sustainable making it suitable for economic production of SNPs. The structure and morphology of SNPs were characterized by UV–visible spectroscopy, X-ray diffraction (XRD) pattern, scanning electron microscopy (SEM) and Fourier Transform Infra-Red (FTIR). These nanoparticles indicated an absorption peak at 430 nm in the UV–visible spectrum. The crystallite average size was estimated about 17.6 nm and SEM image confirmed synthesis of relatively uniform nanoparticles. The antibacterial effect of SNPs was also tested on several micro-organisms by measuring the inhibition zone, MIC and MBC. The results confirmed that SNPs can act as a powerful antibacterial agent against various pathogenic bacteria.

1. Introduction

Nanostructures, the materials with at least a dimension in the size of 1–100 nm, exhibit outstanding properties that significantly differ from those of corresponding bulk solid due to their small size [1]. Over the past decade, a large number of physical, chemical, and hybrid methods have been developed to produce nanostructures of a broad class of materials [2–6]. Nevertheless, some of these procedures have undesired impacts on the environmental and social life. For instance, they need toxic solvents and/or generate hazardous byproducts, and/or involve high energy consumption. Accordingly, it is worthwhile to seek alternative sources of clean and renewable materials as well as a novel process to produce new materials including nanostructures [7–9]. In this respect, it is also expected to make effort to develop sustainable procedures for the synthesis of nanoparticles. Heteropoly acids (HPAs), polysaccharides, Tollens, irradiation, and biological methods have been introduced for the green synthesis of nanoparticles [10,11]. Biological methods using microorganisms [12,13],

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enzymes [14], and plant or plant extracts [15,16] also seem to be well positioned to address the challenge of preparing nanoparticles. Recently various biological procedures have been developed for the production of metal nanoparticles due to their imperative advantages over chemical and physical methods. Besides being sustainable, biological procedure is able to produce large quantities of nanoparticles accompanied with lower costs compared to the other methods [17].

Silver nanoparticles have recently attracted great attention because of their wide range of applications including in the field of high sensitivity biomolecular detection and diagnostics [18], antibacterials [19,20] and therapeutics [21], catalysis [22], biosensors [23], and in plant growth metabolism [24]. Based on the antibacterial properties of SNPs, various nanosilver products, including nanosilver-coated wound dressings, contraceptive devices, surgical instruments, and implants have been developed [25,26]. To the best of our knowledge there are a few reports on production of SNPs by using marine algae [27,28]. This work aimed to apply a biological technique for the synthesis of SNPs and examine its antibacterial properties against various pathogenic bacteria. In this regard, a bioresource, *Spirogyra varians* was used for bioconversion of silver ions to nanoparticles.

2. Materials and methods

2.1. Sample collection and preparation

The green algae *S. varians* were collected from the sweet water areas of Kerman, Iran and were brought to the laboratory and washed with distilled water several times to remove the impurities. The clean algae were dried at room temperature in the shade for a week and powdered using a mortar and pestle.

2.2. Preparation of seaweed extracts

Dried powdered *S. varians* (5 g) was mixed with 100 ml distilled water in the Erlenmeyer flask. The mixture was then centrifuged at 4000 rpm for 10 min at 4 °C. Finally, the extract was collected and stored at 4 °C for further uses.

2.3. Synthesis of silver nanoparticles

Silver nitrate ($AgNO_3$) of analytical grade (AR) was purchased from Merck. About 10 ml of the aqueous extract of *S. varians* was added into 90 ml of aqueous solution of 1 mM Silver nitrate $AgNO_3$. The mixture was exposed to a range of controlled temperatures for 20 min. Appearance of red color in solution indicated the formation of SNPs. The solution was then kept in dark for further analysis.

2.4. UV–vis spectra analysis

The reduction of pure silver ions was recorded by measuring the UV–vis spectra of the solution at room temperature with a Perkin Elmer Lambda 25 UV–vis spectrometer at the wave-length of 350–550 nm.

2.5. Powder X-ray diffraction (XRD)

The red solid product was separated by repeated centrifugation at 12,000 rpm for 10 min followed by redispersion of the pellet of SNPs into deionized water three times. The solid was then dried in an oven at 60 °C. The X-ray diffraction (XRD) pattern was obtained with a PW 1800 Philips diffractometer using Cu-Ka radiation ($k = 0.1541$ nm), and the data were collected from 10° to 80° (2θ) with a scan speed of 4 min^{-1}. XRD analysis was also applied to determine the particle size using Scherrer's formula:

$$d = \frac{k\lambda}{\beta \cdot \mathrm{Cos}\theta_{\max}} \quad (1)$$

where, d is the average crystal size, λ is the X-ray wavelength (0.1541 nm), β is the full-width at half-maximum (FWHM) and θ is the diffraction angle [29].

2.6. SEM analysis of silver nanoparticles

Scanning electron microscopy (SEM) analysis (Model: MIRA\\TESCAN) was performed in order to investigate the structure and morphology of the sample.

2.7. Antibacterial activity of Ag-NPs

The antibacterial activity of SNPs was evaluated against the following pathogenic strains: *S. aureus*, *B. cereus*, *S. typhimurium*, *E. coli*, *L. monocytogenes*, *P. aeruginosa* and *Klebsiella*. These cultures were grown on appropriate medium at 37 °C for overnight incubation and maintained at 4 °C in a refrigerator. Agar well diffusion method was used to study the antibacterial efficiency of prepared SNPs. The pure cultures of bacterial pathogens were sub-cultured on an appropriate medium. Wells of 5 mm diameter were made on Muller Hinton agar (Merck). Each strain was swabbed uniformly onto the individual plate. About 60 µl of the SNPs colloidal solution was poured into each well on the plates using a micropipette. For comparison, plate of the same diameter with 60 µl *cephalothin* (30 mcg) was used. After incubation at 37 °C for 24 h the zones of bacterial inhibition were measured. The assays were performed triplicate. MIC and MBC of SNPs were determined for various microbes using the macrodilution broth susceptibility test. MIC value corresponded to the concentration that inhibited 99% of bacterial growth and the MBC value corresponded to the concentration where 100% of the bacterial growth was inhibited, compared to the positive control (no treatment). The units for MIC and MBC were chosen as mg (silver)/ml. The MIC and MBC were examined visually, by checking the turbidity of the tubes. Mueller–Hinton broth (Merck) was used in the macrodilution method. A standardized suspension of approximately 10^6 CFU/ml was obtained by inoculating the culture in Mueller–Hinton broth and incubating the tubes at 37 °C for 24 h. A serial dilution of the SNPs colloidal solution was prepared within a desired range.

3. Results and discussion

3.1. Characterization of SNPs by UV–visible spectroscopy

UV–visible spectroscopy is one of the most widely used techniques for structural characterization of SNPs. Reduction of the silver ion to SNPs during exposure could be monitored by the visual change of silver nitrate and *S. varians* extract solution color from yellow to red at the beginning of reaction and after reaction (Fig. 1). The UV–vis spectra of the SNPs obtained at three different temperatures are depicted in Fig. 1. A prominent and sharp absorption band is observed at 420–430 nm, similar to those reported in the literature [30,31]. This absorption band is characteristic of SNPs as a result of the excitation of surface plasmon vibrations in the nanoparticles [16]. The smoother and broader absorption peaks at reaction temperatures of 30 °C compared to the one observed at 80 °C indicate particles with smaller size. In other words, at elevated temperatures, the peak absorbance appears more intense with decreased width. This observation may be attributed to the reduction potential of the silver ions [0.8 V] for the thermodynamically favored formation of the SNPs [32].

3.2. Characterization of SNPs by XRD

The sample of SNPs could be also characterized by X-ray diffraction analysis of dry powders. The diffracted intensities were recorded from 10° to 80° at 2 theta angles (Fig. 2). Four different and important characteristic peaks were observed at the 2θ of 38.1°, 44.3°, 64.5°, and 76.4° that correspond to (1 1 1), (2 0 0), (2 2 0), and (3 1 1) planes, respectively. All the peaks in XRD pattern can be readily indexed to a face-centered cubic structure of silver as per available literature (JCPDS, File No. 4-0783). The average crystal size of the silver crystallites was calculated from the FWHMs of the diffraction peaks, using the Scherrer equation. The size of the crystallite in different planes of silver was determined as 23.5, 20.3, 14.4, and 12.2 nm with the mean value of all peaks as 17.6 nm. The broadening of the Bragg peaks indicates the reduction in grain size.

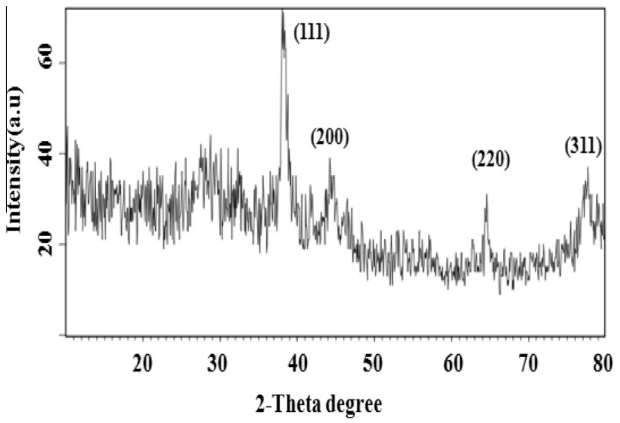

Figure 2 XRD pattern of as-synthesized SNPs.

3.3. Characterization of SNPs by SEM

Scanning electron microscopy has provided further insight into the morphology and size details of the synthesized nanoparticles. Fig. 3 shows the SEM image of SNPs. It seems that the sample consisted of a large quantity of dispersive nanoparticles with the average size of about 35 nm. As it is clear in the SEM image, the nanoparticles are relatively uniform and seemed as quasi-spheres.

3.4. FTIR spectra

The FTIR spectra of *S. varians* aqueous extract and bio-synthesized SNPs, shown in Fig. 4, indicate the presence of amino, carboxylic, hydroxyl and carbonyl groups. Display of strong broad O–H stretch carboxylic bands in the region 3423 cm^{-1} and carboxylic/phenolic stretching bands in the region 2927 cm^{-1} was observed. The peaks appearing in the region 1645 cm^{-1} are attributed to the stretching vibration of the [NH]C═O group that is characteristic of proteins shifted from 1645 cm^{-1} after the synthesis of Ag-NPs. The peaks appearing in the region 1515 and 1429 cm^{-1} might represent quinine OH bonds.

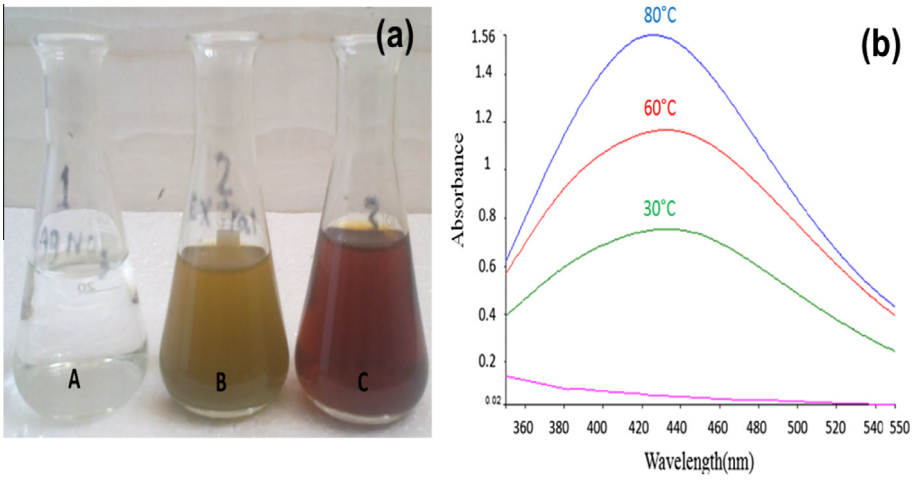

Figure 1 (a) Color changes for silver nitrate solution (A), solution of silver nitrate and *S. varians* extract at the beginning of reaction (B) and after reaction (C). (b) UV–visible spectrum of *S. varians* extract with and without SNPs during different wavelengths at different temperatures.

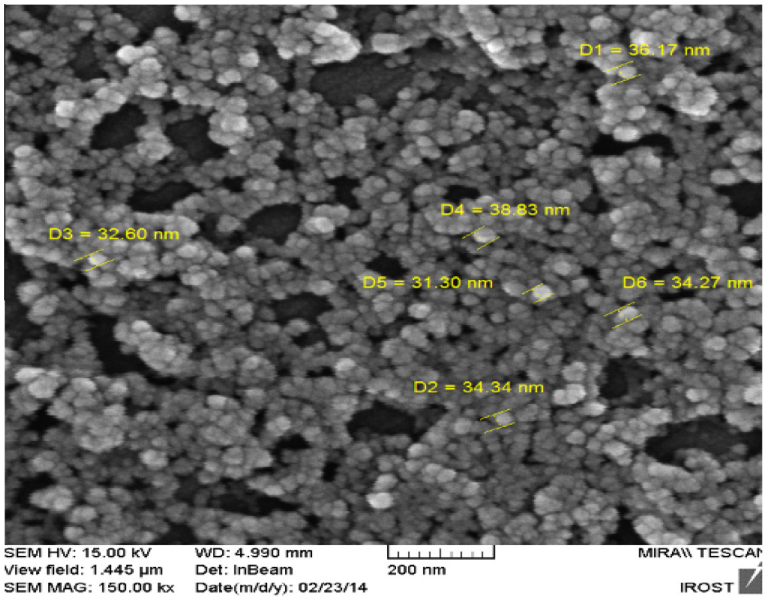

Figure 3 SEM image of silver nanoparticles.

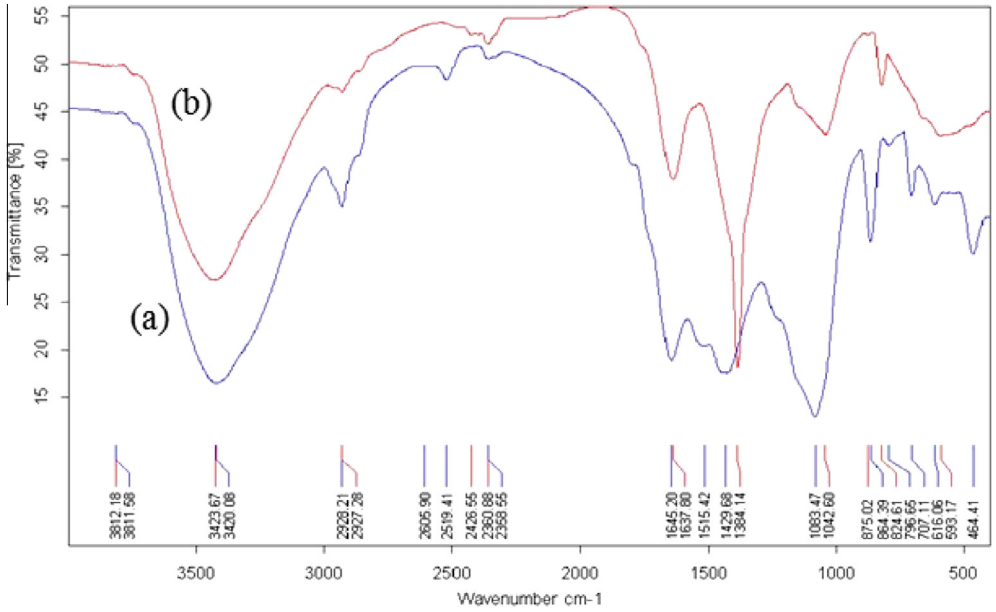

Figure 4 FTIR spectra of *S. varians* aqueous extract (a) and bio-synthesized SNPs (b).

3.5. Antibacterial activity of SNPs

The results of antibacterial activity of SNPs produced in this research are reported in Fig. 5 and Table 1. The diameter of the inhibition zone depends on the species of bacteria [33]. The antibacterial effect of SNPs on *B. cereus*, *P. aeruginosa* and *Klebsilla* was more significant compared to standard antibiotic. Minor antibacterial effect was reported for *S. typhimurium*. Furthermore, SNPs had excellent antibacterial effect on *S. aureus*, *L. monocytogenes* and *E. coli*. The mechanism of the bactericidal effect of SNPs is not very well-known. A suggested idea is SNPs after penetration into the bacteria can inactivate their enzymes, generate hydrogen peroxide and cause bacterial cell death [34]. The results of MIC and MBC tests, shown in Table 1, revealed a lower MIC and MBC value for *Escherichia coli* compared to the other tested pathogens. This observation can be justified according to the differences in bacterial cell walls. The cell walls of Gram negative bacteria are thinner Gram positive bacteria's and thus gram negatives are more sensitive to SNPs than Gram positives. Anyway, the results reveal a strong antimicrobial activity of SNPs synthesized in this research.

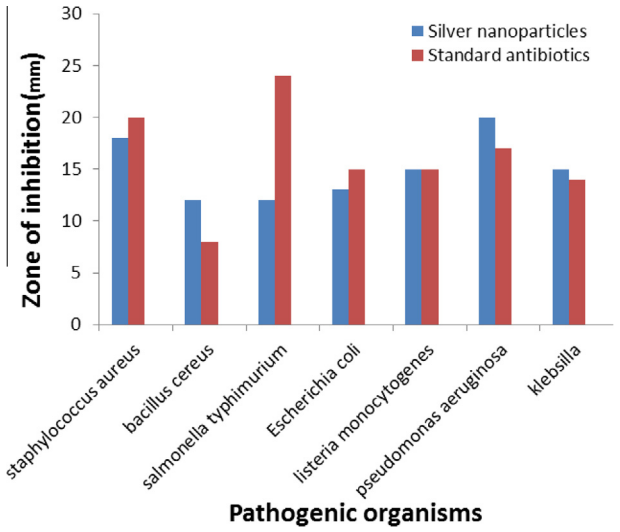

Figure 5 Graphical representation of antimicrobial effect of silver nanoparticles with bacteria and standard antibiotic.

Table 1 MICs and MBCs for test bacteria.

Test bacteria	MIC [mg/ml]	MBC [mg/ml]
Staphylococcus aureus	0.5	1
Bacillus cereus	0.25	0.5
Salmonella typhimurium	0.5	1
Escherichia coli	0.25	0.25
Listeria monocytogenes	0.5	1
Pseudomonas aeruginosa	0.5	0.5
Klebsiella	0.5	1

4. Conclusion

In summary, the bio-reduction of aqueous silver ions to silver nanoparticles using *S. varians* has been demonstrated. The procedure applied is simple, economic and ecofriendly. These characteristics are marvelous, through sustainable development of nanomaterial production. Besides that, smaller size, size uniformity and appropriate dispersion of the nanoparticles were other important aspects of this study. Investigation on the antibacterial effect of nanosized silver against pathogenic bacteria reveals high efficiency of silver nanoparticles as a strong antibacterial agent. This green and sustainable technology toward the synthesis of nanoparticles can be developed for the large-scale production of other nanomaterials.

Acknowledgments

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