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Synthesis of silver nanoparticles using seed exudates of *Sinapis arvensis* as a novel bioresource, and evaluation of their antifungal activity

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Abstract

Background: In general, silver nanoparticles (AgNPs) are particles of silver with a size less than 100 nm. In recent years, synthesis of nanoparticles using plant extract has gained much interest in nanobiotechnology. In this concern, this study investigates green synthesis of AgNPs from silver nitrate using *Sinapis arvensis* as a novel bioresource of cost-effective nonhazardous reducing and stabilizing compounds. A stock solution of silver nitrate (0.1 M) was prepared. Different concentrations of silver nitrate (1, 2.5, 4, and 5 mM) were prepared from the above solution, then added to 5 mL of *S. arvensis* seed exudates. The mixtures were kept in 25°C. The synthesis of AgNPs was confirmed by the change in mixtures color from light yellow to brown. The antifungal activity of synthesized AgNPs was investigated *in vitro*.

Results: The resulting AgNPs were characterized by UV-vis spectroscopy, X-ray diffraction (XRD), transmission electron microscopy (TEM), and Fourier transform infrared spectroscopy (FTIR). Formation of the AgNPs was confirmed by the change in mixture color from light yellow to brown and maximum absorption at 412 nm due to surface plasmon resonance of AgNPs. The role of different functional groups in the formation of AgNPs was shown by FTIR. X-ray diffraction was shown that the AgNPs formed in our experiments were in the form of nanocrystal, and TEM analysis showed spherical particles with an average size of 14 nm. Our measurements indicated that *S. arvensis* seed exudates can mediate facile and eco-friendly biosynthesis of colloidal-spherical AgNPs with a size range of 1 to 35 nm. The synthesized AgNPs showed significance antifungal activity against *Neofusicoccum parvum* cultures.

Conclusion: The AgNPs were synthesized using a biological source. This synthesis method is nontoxic, eco-friendly, and a low-cost technology for the large-scale production. The AgNPs can be used as a new generation of antifungal agents.

Keywords: Synthesis; AgNPs; Spherical AgNPs; Antifungal

Background

Silver nanoparticles (AgNPs) are particles of silver which are in the range between 1 and 100 nm. Nanostructure materials indicate unique physicochemical and biological environmental properties, including optical, magnetic, electronic, catalytic activity, and biological properties [1], which have increased their applications in medicine, agriculture, environment, and industry [2]. AgNPs have high potential as commercial nanomaterials [3] and an effective antimicrobial agent.

Several techniques for synthesizing AgNPs have been proposed. Generally, AgNPs are prepared by different kinds of chemical and physical methods, but majority of these techniques are both expensive and environmentally hazardous [4]. Furthermore, the synthesized nanoparticles may be unstable and tend to agglomerate rapidly and become useless unless capping agents are applied for their stabilization [5]. Diverse chemical and physical methods have been used to prepare AgNPs with various sizes and shapes, such as UV irradiation [6,7], microware irradiation [8], chemical reduction [9],

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electron irradiation [10], photochemical [11], and lithography methods [12]. However, most of these methods involve more than one step, high energy requirement, low material conversions, difficulty in purification, and hazardous chemicals [13]. The synthesis of nanoparticles by chemical methods may lead to the production of some toxic chemical compound that may have adverse effects on their applications [14].

The biological synthesis of nanoparticles can potentially eliminate these problems. Biological synthesis of nanoparticles is nontoxic, eco-friendly, and a low-cost technology for the large-scale production of well-characterized nanoparticles [14]. Therefore, there is a need to develop biological processes for nanoparticle synthesis. Recently, many live organisms such as bacteria, fungi, algae, and plants have been used for synthesis of nanoparticles [15-17]. The reduction of Ag^+ to Ag^0 took place by combinations of biomolecules such as proteins, polysaccharides, and flavonoids [18]. Green synthesis of AgNPs has become very important in the recent years. Green AgNPs have the potential for large scale applications in the formulation of dental resin composites, bone cement [19,20], water and air filters [21,22], clothing and textiles, medical devices and implants [23], cosmetics [4], and packaging [24]. Besides their antimicrobial properties, AgNPs and silver nanocomposites have other interesting characteristics which will further enable them to be used in catalysts, biosensors, conductive inks, and electronic devices [25,26]. They can be produced economically and in large industrial scale [14].

In this paper, we report biosynthesis of stable colloidal AgNPs using *Sinapis arvensis* seed exudates. This plant is an important medicinal crop in the southern regions of Iran. Antifungal activities of synthesized AgNPs were also investigated. Details of biosynthesis, physical characterizations, and antifungal activity of AgNPs are described.

Methods

Reagents

Silver nitrate and potato dextrose agar (PDA) medium were obtained from Merck, Darmstadt, Germany. Seeds of *S. arvensis* were obtained from Pakanbazr, Isfahan, Iran. Strain of the fungus *N. parvum* was prepared from the Department of Plant Protection, Shahid Bahonar University of Kerman, Iran.

Seed exudates preparation

The surface of *S. arvensis* seeds were disinfected using 30% sodium hypochlorite for 5 min and rinsed with sterile distilled water three times. In the next step, the seeds were placed in 70% alcohol for 2 min and then washed four times with sterile distilled water and then imbibed in deionized (DI) water. (1 g dry weight/10 mL DI water) After being incubated at 25°C for 48 h in the dark, seeds were removed from the soaking medium. The supernatant phase was collected and centrifuged at 4,500 rpm for 10 min to separate the liquid fraction from any large insoluble particles and filtered by Whatman filter paper no. 1 (Sigma Aldrich, St. Louis, MO, USA). During the experiment, pH was 4.5.

Biosynthesis of AgNPs

For this reason, a 50 mL stock solution containing 0.1 M silver nitrate was prepared. Different concentrations of silver nitrate (1, 2.5, 4, and 5 mM) were prepared from the above solution, then added to 5 mL of *S. arvensis* seed exudates and incubated at 25°C as described previously [27]. After treatment, the pale yellow color of reaction mixture was changed to brown indicating synthesis of AgNPs.

Characterization of AgNPs

UV-vis spectroscopy

The biosynthesis of AgNPs was monitored periodically using a UV-vis spectrophotometer (Scan Drop-type product, Analytik Jena, Germany) at different concentrations at room temperature. These measurements operated at a resolution of 1 nm and wavelength range between 300 and 650 nm [14].

X-ray diffraction

The formation and quality of compounds were investigated by X-ray diffraction (XRD) technique. For this purpose, AgNPs was centrifuged (at 13,000 rpm; 25°C) for 10 min, washed with DI water and re-centrifuged in three cycles. Then purified AgNPs were dried and subjected to XRD experiment. X-ray diffraction was performed by STOE Stadi P (STOE & Cie. GmbH, Darmstadt, Germany) ($\lambda = 1.54178$ Å). The scanning was done in the region of 2θ from 10° to 80° [17].

Transmission electron microscopy

Transmission electron microscopy (TEM) was performed by using of a Carl Zeiss (Jena, Germany, 80 kV) for determining the morphology and size of AgNPs.

Inductively coupled plasma spectrometry

Inductively coupled plasma spectrometry (ICP) Varian BV ES-700, Sydney, Australia, was used to determining the remaining concentration of silver ions after synthesizing AgNPs. Changing rate of metal ion to nanoparticles is calculated by the following equation:

$$Q = \left(\frac{C_0 - C_f}{C_0}\right) \times 100$$

where C_0 and C_f stands for initial and final concentration

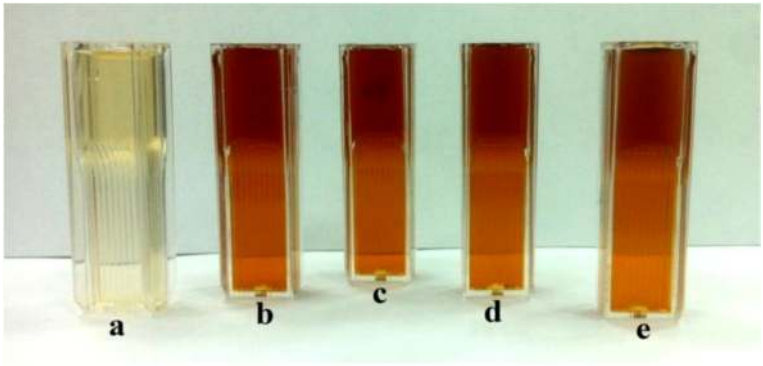

Figure 1 Reduction of Ag^+ to Ag^0. The color change of the *S. arvensis* exudates upon the formation of AgNPs from different concentrations of silver nitrate: **(a)** control, **(b)** 1 mM, **(c)** 2.5 mM, **(d)** 4 mM, and **(e)** 5 mM.

of silver ion (mg/L), respectively. *Q* is the conversion percentage of silver ion to AgNPs [28].

Antifungal activity of AgNPs

To determine the antifungal activity of AgNPs, mycelium growth inhibition test was used. Four concentrations of AgNPs (2.5, 5, 10, and 40 μg/mL) were prepared in PDA medium after autoclaving. 6-mm agar plugs of fresh culture of *N. parvum* prepared and transferred to centers of media containing different concentrations of synthesized AgNPs. Control plates contain no AgNPs. All plates incubated at 28°C. When in control, the fungus completely covered the entire surface of the medium, the mean radius of fungal growth in all plates measured and recorded [29]. All treatments were performed in triplicates.

The following formula was used to assess of the growth inhibition of mycelium:

$$\text{Mycelium growth inhibition } \% \;=\; \frac{R-r}{R} \times 100$$

R is the mean radius of control and *r* is the radius of samples treated with nanoparticle. Data processed with SAS statistical analysis using Duncan's test.

Results and discussion

Visual observation

Reduction of Ag^+ to Ag^0 was confirmed by color change of the reaction mixture from colorless to brown (Figure 1).

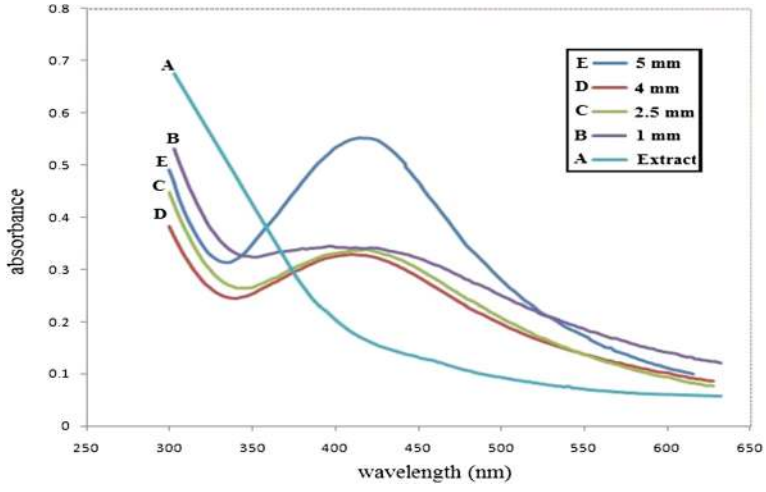

Figure 2 UV-vis absorption spectrum of AgNPs synthesized by treating 1, 2.5, 4, and 5 mM silver nanoparticles after 1 week.

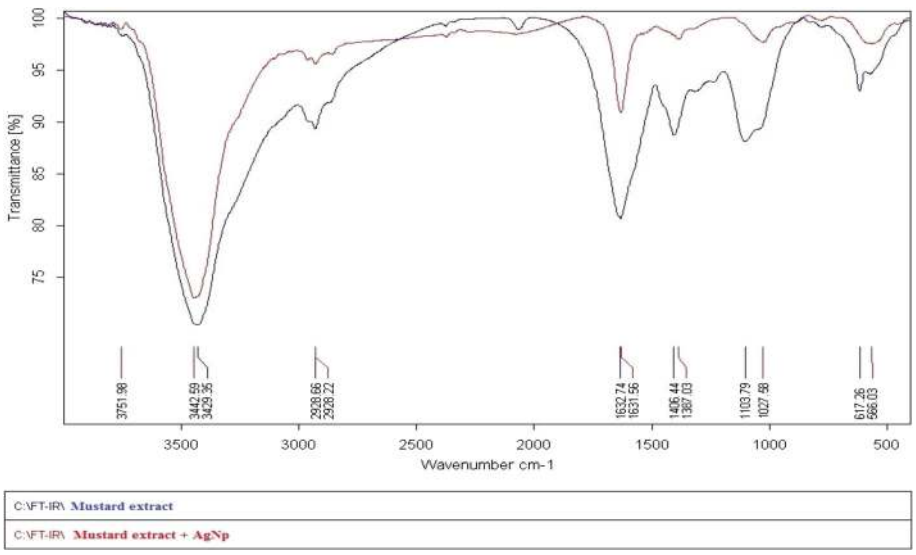

Figure 3 FTIR spectra of the *S. arvensis* exudates before and after the synthesis of AgNPs.

Ultraviolet visible scanning spectroscopy studies

It was observed that the maximum absorbance of reaction mixture occurs at 412 nm, indicating that AgNPs were produced. Figure 2 shows the UV-vis absorption spectra of synthesized AgNPs with different concentrations of silver nitrate.

FTIR analysis

Fourier transform infrared spectroscopy (FTIR) spectrum of biosynthesized AgNPs shows absorption peaks at 3,429, 2,928, 1,632, 1,406, 1,103, and 617 cm^{-1} (Figure 3). Strong absorption peak at 3,429 cm^{-1} is resulted from stretching of the N-H band of amino groups or is indicative of present O-H groups due to the presence of alcohols, phenols, carbohydrates, etc. The peak that appeared around 2,928 cm^{-1} is related to the stretching of the C-H bonds [30]. The peaks at 1,632 and 1,406 cm^{-1} are assigned for aliphatic amines. The absorption peak at 1,632 cm^{-1} is close to that reported for native proteins [31]. The peak at 1,406 cm^{-1} corresponds to C-C stretching vibrations for aromatic ring [32]. FTIR study indicates that probably the carboxyl (-C=O), hydroxyl (-OH), and amine (N-H) groups in seed exudates are mainly involved in the reduction of Ag^{+} ions to Ag^{0} nanoparticles.

XRD analysis

The formation of the nanocrystalline AgNPs was further confirmed by the XRD analysis as showed in Figure 4.

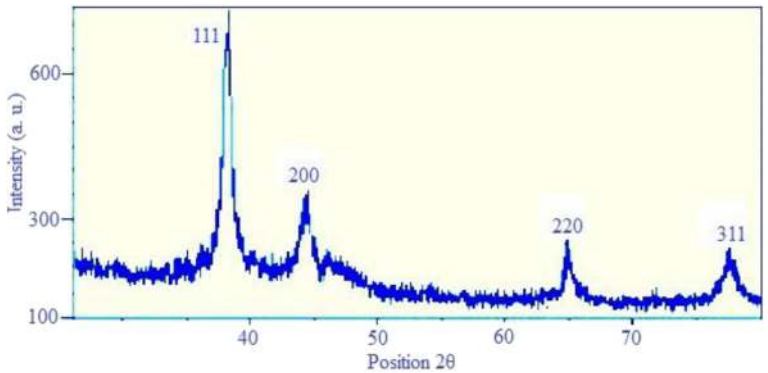

Figure 4 XRD pattern of AgNPs synthesized by treating silver nitrate with *S. arvensis* seed exudates.

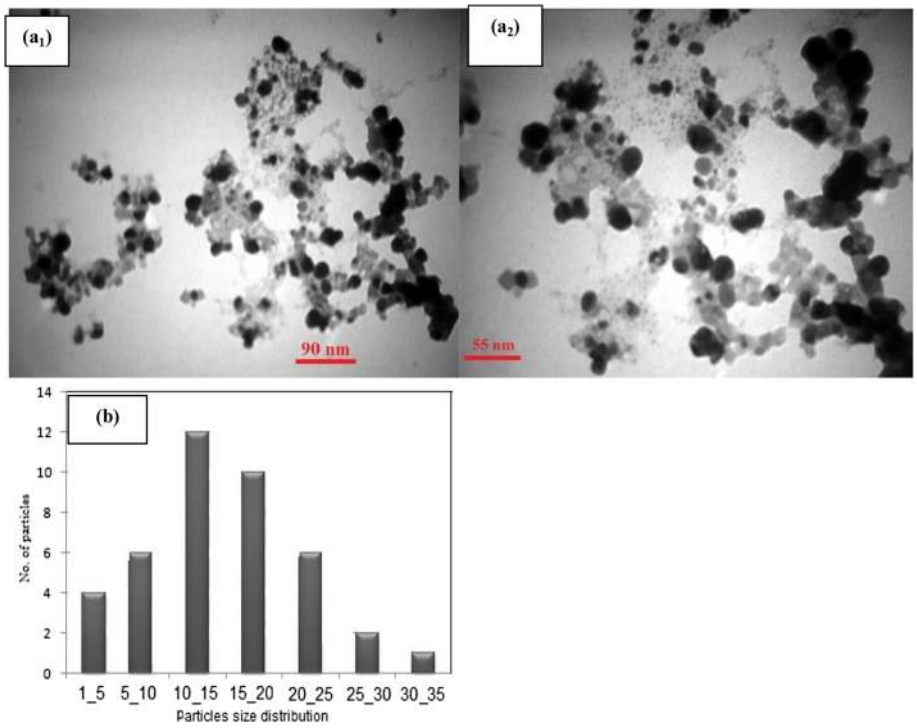

Figure 5 The TEM images of the prepared AgNPs. (a_1, a_2) TEM images of AgNPs that were synthesized by *S. arvensis* seed exudates in different scales. **(b)** Histogram of particle size distribution of the biosynthesized AgNPs.

Strong peaks were observed at 2θ values at 38.09°, 44.15°, 64.67°, and 77.54°, corresponding to (111), (200), (220), and (311) Bragg's reflection based on the face-centered-cubic (fcc) crystal structure of AgNPs [33]. The broadening of Bragg's peaks indicates the formation of AgNPs. The XRD pattern thus shows that the AgNPs formed by the reduction of Ag^+ ions by *S. arvensis* seed exudates are crystalline in nature.

Transmission electron microscopy analysis

TEM was used to determine the size and shape of nanoparticles. The TEM images of the prepared AgNPs at 55 and 90 nm scales are shown in the Figure 5a_1 and a_2. TEM images show that they have spherical shape. Particle size distribution histogram determined from TEM is shown in Figure 5b. AgNPs size is between 1 and 35 nm.

Table 1 ICP analysis

Q (%)	C_f (μg/mL)	C_0 (μg/mL)
0.955	23.85	535

Inductively coupled plasma spectrometry

ICP analysis revealed complete reduction of Ag ions within 50 days of the reaction, and more importantly, it showed that the conversion percentage of metal ion to metal nanoparticles is more than 95% (Table 1).

Antifungal activity of synthesized AgNPs

Inhibitory effects on fungal growth in a PDA medium containing 2.5, 5, 10, and 40 μg/mL concentrations of AgNPs were studied. The results showed a very significant effect of synthesized AgNPs on the mycelium growth of the fungus *N. parvum*. More than 83% mycelium growth inhibition of the fungus *N. parvum* was treated with a concentration of 40 μg/mL of AgNPs. The lowest level of growth inhibition was observed at a concentration of 2.5 μg/mL of AgNPs with the 15% mycelium growth inhibition. The growth inhibition for concentrations of 5 and 10 μg/mL, respectively, were 15% and 71%. Results clearly showed that with the increase in the concentration of AgNPs, the inhibitory effects on fungal mycelium growth increased (Figure 6).

Percentage of growth inhibition due to the effect of AgNPs was analyzed by statistical software SAS. Results confirmed a significant effect of AgNPs on fungal growth inhibition at 1% confidence interval.

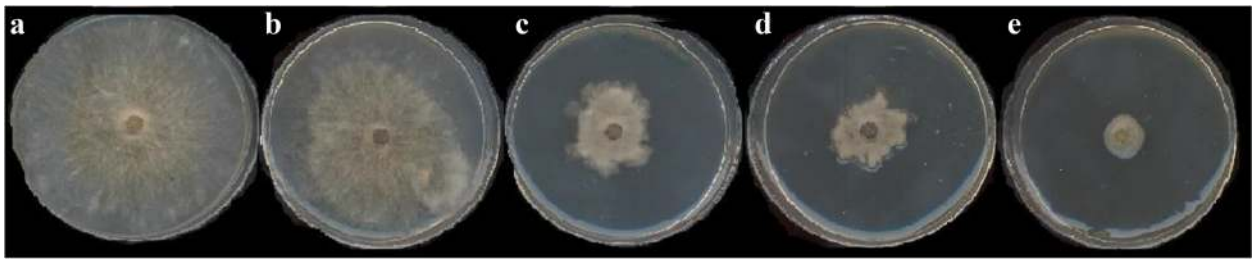

Figure 6 Inhibitory effects on fungal mycelium growth. Fungal growth on PDA medium containing control **(a)**. PDA medium containing **(b)** 2.5 μg/mL, **(c)** 5 μg/mL, **(d)** 10 μg/mL, and **(e)** 40 μg/mL of AgNPs.

Conclusions

Metallic nanoparticles are traditionally synthesized by wet chemical synthesis techniques where the chemicals used are quite often toxic and flammable. But in this study, the *S. arvensis* seed exudates were successfully used for the single-pot biosynthesis of spherical AgNPs in ambient conditions with the size range from 1 to 35 nm, as inferred from the TEM imaging. This was achieved without the use of external stabilizing or capping agents. We concluded that *S. arvensis* seed exudates are a bioreductant and capping agent as well as an easily available plant source playing an important role in the synthesis of highly stable AgNPs. X-ray diffraction pattern strongly indicated a high purity of biosynthesized AgNPs. This pristine method is facile, cost effective, clean and green, and therefore is applicable for a variety of purposes. Moreover, it is easy to scale up the production of AgNPs to industrial scale using this method.

This green chemistry approach towards the synthesis of AgNPs has many advantages such as ease with which the process can be scaled up, economic viability, etc. Application of such nanoparticles in medicine and other applications makes this method useful for the large scale synthesis of other inorganic nanomaterials. Toxicity studies of AgNPs on pathogen open a door for a new range of antifungal and antibacterial agents. So in the present study, we demonstrated that AgNPs have significant antifungal activity. It was determined that the growth inhibitory effect of AgNPs strongly depends on the concentration of AgNPs and with the increase concentration of AgNPs in the medium the inhibitory effect on fungal growth increased. So AgNPs can be used as excellent new antifungal agent.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All of them have been also involved in the drafting and revision of the manuscript. All authors read and approved the final manuscript.

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