

RESEARCH PAPER

Biologically Green Synthesis of High-quality Silver Nanoparticles Using *Scrophularia striata* Boiss Plant Extract and Verifying Their Antibacterial Activities

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ABSTRACT

In the present work, we demonstrate a facile way to study the biosynthesis of silver nanoparticles (Ag-NPs) with strong bactericidal properties using an aqueous extract of *Scrophularia striata* Boiss. The bio-reduction of Ag⁺ ions resulted in FCC cubic structures of Ag-NPs with spherical shapes of about 16 nm. As a main aim of the present work, the antibacterial activity of the bio-synthesized Ag-NPs against Gram-negative (*Escherichia coli* and *Salmonella typhi* ATCC) and Gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) bacteria was evaluated by disk diffusion method and results were compared with those of ciprofloxacin antibiotic. Interestingly the antibacterial activity of as-prepared Ag-NPs against all pathogenic bacteria was considerably higher than those obtained for *Ciprofloxacin*, and also better than the recent reports on the bactericidal activity of bio-synthesized Ag-NPs. As a simple, cost-effective and biocompatible method, the present work proposes a facile way toward bio-synthesis of large-scale Ag-NPs with an excellent antibacterial activity which can be suitable for future biological applications.

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INTRODUCTION

Nanomaterials often exhibit properties and behaviors drastically different from their corresponding bulk materials of the same chemical composition. Metallic nanoparticles (NPs) have generated novel materials that exhibit unique optical, catalysis, electrochemistry or biological properties making them attractive for a number of application areas [1–4]. Among all metallic NPs, silver NPs exhibit unique optical and electrical properties at the nanoscale, along with a most valuable enhanced-antibacterial activity. They have received considerable attention owing to their remarkable applications in nanomedicine, food container, anti-bacterial intentions, and

material engineering [5]. The Ag-NPs can be prepared through biological methods using microorganisms such as fungus, [6], bacteria [7], enzymes [8], and plant extracts [9–11]. Komal and Kashyap have recently prepared Ag NPs employing plant extracts of *Actinidia deliciosa* with applications in antimicrobial and detecting Hg²⁺ heavy metal ions [12]. Rawat's group also reported a green synthesis of silver NPs by Mulberry leaves extract as a reducing agent as well as a stabilizing agent [13]. In the green synthesis of NPs, the bioreduction of silver nitrate by reducing agents in plant extracts led to the formation of Ag-NPs. Researchers reported that reducing agent in plant extracts such as carbohydrates, reducing sugars, alkaloid, terpenoids, tannin, saponins, phenolics,

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and flavones compounds are responsible for the reduction of silver nitrate to Ag-NPs [14,15].

As a traditional medicinal plant of the *Scrophulariaceae* family, *Scrophularia striata* (*S. striata*) Boiss is used as a home remedy in Iran and is known by the local name of *Tashnedari* among the people of Ilam province in Iran. The *S. striata* have been and still used by natives as a useful remedy for different diseases such as infectious diseases, allergies, wound healing, rheumatism, and chronic inflammatory disorders. Several studies on its biological activities using aqueous extracts of the *S. striata* showed that *S. striata* have promising antioxidant, antibacterial, anti-tumor, and anti-inflammatory characteristics [16–18].

While there has been a high level of attention devoted to Ag-NPs, there is still an undeniable need in finding new routes with several improved characteristics like; simplicity, utilizing environment/ user-friendly precursors, cost-effectiveness, the scaling-up potent, with the final products exhibiting promising applications. For this purpose, the present manuscript presents a success in providing a facile way to bio-synthesize Ag-NPs using an aqueous extract of *S. striata* with a capability of up-scaling. Then, their potential application as an antibacterial agent was examined against several Gram-positive and -negative pathogenic bacteria. Results showed an excellent antibacterial activity even better than conventional antibiotics, nominating the present Ag-NPs as a suitable case for future biological intentions.

MATERIAL AND METHODS

Preparation of plant extracts

The *S. striata* plants were collected from Ilam province in Iran from August to September 2016. Aerial parts of *S. striata* were washed with sterile distilled water, dried, and then reduced to a fine powder. After that, a fine dried powder of samples (10 g) was added to 100 mL of deionized water. Then, it was transferred to a 100 °C water bath and remained at that temperature for 15 min. Finally, the solution was allowed to cool down to room temperature and the extracts were filtered using Whatman No. 2 filter paper.

Biosynthesis of silver NPs

For the green synthesis of Ag-NPs, 10 mL of extract was added to varying concentration of

AgNO₃ (Merck, 99.0-100.5 %) precursor solutions and the reaction mixture was stirred at 3000 rpm for about 30 min at the room temperature. Bio-reduction of Ag⁺ ions was monitored by the color change of the solution from colorless to brown along with results of UV-Vis measurements. After 24 h incubation at room temperature, the reaction solution was centrifuged at 10000 rpm for 15 min and the obtained precipitations were then washed with deionized water, followed by 3 times centrifuging. Finally, the obtained powders were placed in an oven at 60 °C for 24 h and were used for further experiments.

Characterization of synthesized silver NPs

The optical property of Ag-NPs was determined by Cary 300 Bio UV-vis spectrophotometer (VARIAN) in the wavelength range of 275–675 nm. The morphological features of the bio-synthesized NPs were observed by field emission scanning electron microscopy (FE-SEM; Hitachi S-4200) under an acceleration voltage of 15 kV. Transmission electron microscopy (TEM) image was recorded using a Philips CM30 electron microscope. The chemical composition of the silver NPs was studied by an energy dispersive X-ray spectrometer (EDX) instrument (Oxford INCA II energy solid state detector). The FT-IR spectroscopy (Bruker Vertex 70 spectrometer with 0.5 cm⁻¹ resolution) was used to obtain information about the possible interactions during NPs' preparation. Information about phase variety and grain size of the bio-synthesized Ag-NPs was determined by X-ray diffraction pattern (Philips X'pert diffractometer by Cu-Kα radiation at 40 kV and 30 mA). The size of the crystallite was estimated by using the well-known Scherrer equation as follows;

$$D = \frac{0.9 \times \lambda}{\beta_D \times \cos \theta} \quad (1)$$

where D is the approximate crystal size, λ is the wavelength of X-ray radiation used (1.54 Å), θ is the Bragg's angle correspond to the most intense peak, and β_D is the full width at half maximum of the peak in radians.

Antibacterial activity study

The antibacterial activity of the bio-synthesized silver NPs was evaluated by disk diffusion method, against Gram-negative (*Escherichia coli* and *Salmonella typhi* ATCC) and Gram-positive

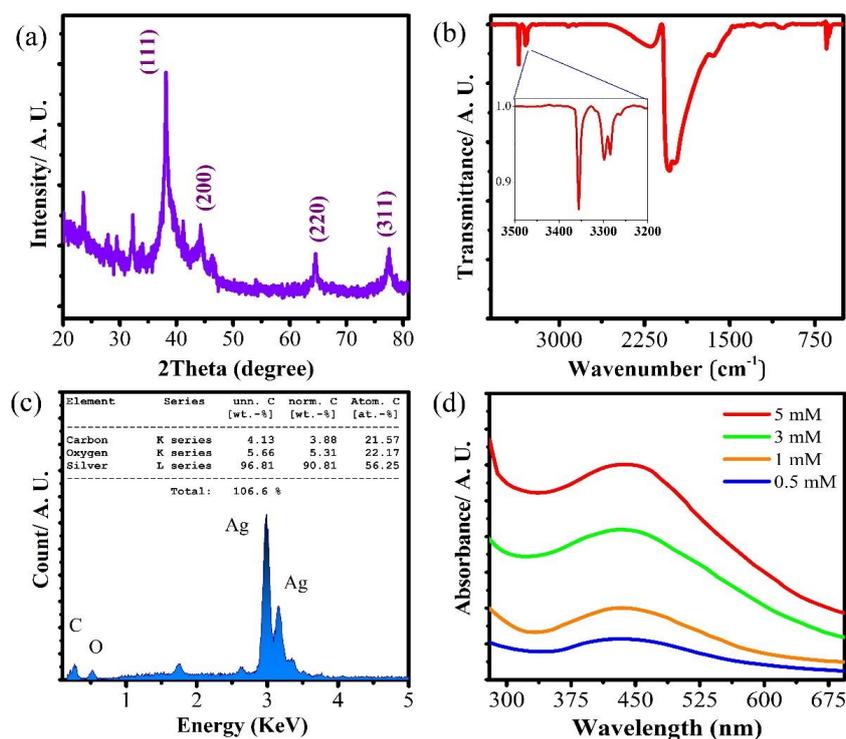


Fig. 1. Typical (a) XRD diffraction pattern, (b) FTIR spectrum, (c) EDX analysis, and (d) UV-Vis spectra of a typical Ag-NPs synthesized by plant extract.

(*Staphylococcus aureus* and *Bacillus cereus*) bacteria. Briefly, the Muller–Hinton Agar (MHA-Merck) powder was utilized as a culture medium for bacterial growth. A single colony of each strain with a turbidity of 0.5 McFarland was swabbed uniformly on the individual plates using sterile cotton swabs. Using a micropipette, 100 μ L of the Ag-NPs solution was poured into the plate, and after incubation at 37 $^{\circ}$ C for 24 h the antibacterial activity was measured based on the inhibition zone (clear zone). The diameter of the inhibition zones in the plate was measured carefully, and the mean values were recorded. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were also determined using the microdilution technique.

RESULTS AND DISCUSSION

Physical and chemical characteristics

The XRD pattern of the purified silver NPs (Fig. 1a), clearly determines the FCC structure of metallic silver. The observed diffraction peaks at 2 theta values of 38.18 $^{\circ}$, 44.2 $^{\circ}$, 64.5 $^{\circ}$, and 77.5 $^{\circ}$ can be well-indexed to (111), (200), (220), and (311) miller indices-related planes (standard metallic silver XRD pattern JCPDS data 04-0783),

respectively [19]. Using the Scherrer formula, the average crystallite size of the Ag NPs was estimated to be about 15 nm. The obtained broad peaks further indicate that the present bio-synthesized samples are in the nanoscale range. Several unassigned peaks were also found in XRD pattern which can be attributed to the crystallization of bio-organic phases at the surface of NPs [20].

To identify the effective presence of reducing agent groups, we utilized the FT-IR spectroscopy. As seen in Fig. 1b, and noted in Table 1, the observed bonds indicate the key roles of amine, alcohol, and aldehyde groups in authority to reduce Ag⁺ ions to Ag⁰. EDX analysis of a typical synthesized silver NPs shows excellent purity of Ag-NPs (Fig. 1c). However, observation of C, and O species in the obtained spectrum, further determines the presence of different functional groups in active phytoconstituents of the plant extract, at which is in accordance with present FT-IR outcomes and other reports [21].

Finally, the successful formation of the Ag-NPs was monitored by employing UV-Vis spectroscopy at different concentrations of the metal ion (Fig. 1d). As can be seen, a very broad peak located around 435 nm is a characteristic peak of Ag-NPs

Table 1. The characteristic bonds revealed in the FT-IR spectrum of a typical Ag-NPs synthesized plant extract

Wavenumber (cm ⁻¹)	Corresponding bond	Reference
3280-3370	O-H hydroxyl groups (in alcohol or polyphenol)	[22]
	N-H amines (in aromatic amines)	[23]
2200	C≡N stretching (presence of nitriles)	[23]
	C≡C stretching vibrations	[24]
The very intense and broad peak at 1700- 2150	C=O functional groups of aldehydes, ketones and carboxylic acids	[10]
	C=C stretching vibrations	[19]
The intense and broad shoulder at 1651	C=O carbonyl groups	[21]
	C-C aromatic vibrations	[25]
	C-N stretch polyphenols	[26]
1041	C-O stretching mode of esters	[9]
660	C-H of Alkynes	[26]

[27], which undergoes a slight red-shift to 445 nm with the increase in metal ion concentration. This absorption peak is attributed to the collective oscillation of electrons known as localized surface plasmon resonance (LSPR) [28], resulting in the yellow-brown color of silver NPs in different media. On the other hand, based on the Si and Mandal [29] report, synthesis of metallic NPs is occurred through initial reduction of metal ions to metals and their nucleation, followed by an Ostwald ripening growth step. Therefore, the higher concentration of silver ions would probably lead to larger NPs.

Morphology and particle size analysis on bio-synthesized Ag-NPs were achieved by FE-SEM and TEM measurements. The typical top view FE-SEM images in Fig. 2a, b reveals smooth and spherical morphology of the Ag-NPs with the mean size of about 19.7 nm. As suggested elsewhere, the shape of Ag-NPs is significantly influenced by their optical property and spherical NPs usually show a similar absorption property [30]. On the other hand, the TEM image of that sample (Fig. 2c) confirms the formation of relatively monodisperse and nanometer size particles with average size value of 16 ± 1.4 nm.

Antibacterial activity

The antibacterial activity of the bio-synthesized Ag-NPs against various pathogenic bacteria (*B. cereus*, *S. aureus*, *S. typhi* ATCC., and *E. coli*) was performed by disk diffusion method, which was compared with those examined for aqueous plant extract and ciprofloxacin antibiotic. Results have been presented in Fig. 3.

It was indicated that the maximum antibacterial activity was observed against *B. cereus* followed

by *S. aureus*, *S. typhi* ATCC, and *E. coli* with a zone inhibition diameters of 33, 30, 28, and 25 mm, respectively (Table 2). The results indicated that MIC and MBC test have the same trend so that *E. coli* with lower zone inhibition diameters have higher MIC and MBC values (Table 3). Interestingly, the plant extract did not show any antibacterial activity against different pathogenic bacteria. In all experiments, ciprofloxacin antibiotic was used as a control for better evaluating of the antibacterial activity of present bio-synthesized silver NPs. There were significant differences ($p < 0.05$) between plant extract, ciprofloxacin, and Ag-NPs in all bacteria so that the antibacterial activity of bio-synthesized Ag-NPs against both Gram-positive and Gram-negative bacteria was remarkably greater than that of ciprofloxacin antibiotic and those reported by others (see Table 2).

In general, the as-prepared Ag NPs were found to be more effective on Gram-positive bacteria than Gram-negative ones, which can be attributed to the structural and compositional differences of the cell wall. The mechanism of antibacterial activity of Ag-NPs is still not well known. However, regarding the high degree of scientific attention, several mechanisms have been proposed for the bactericidal effects of Ag-NPs.

1. On one hand, the electrostatic interaction between the positive surface charge of the Ag-NPs and negatively charged cell membrane of bacteria, and on the other hand, the small size of Ag-NPs with high surface energy, cause effective adhesion of Ag-NPs onto the cell membrane. This adhesion leads to subsequent damages on cell wall and membrane such as disruption of the cell wall, alteration of membrane structure and permeability, the formation of pit and leakage of

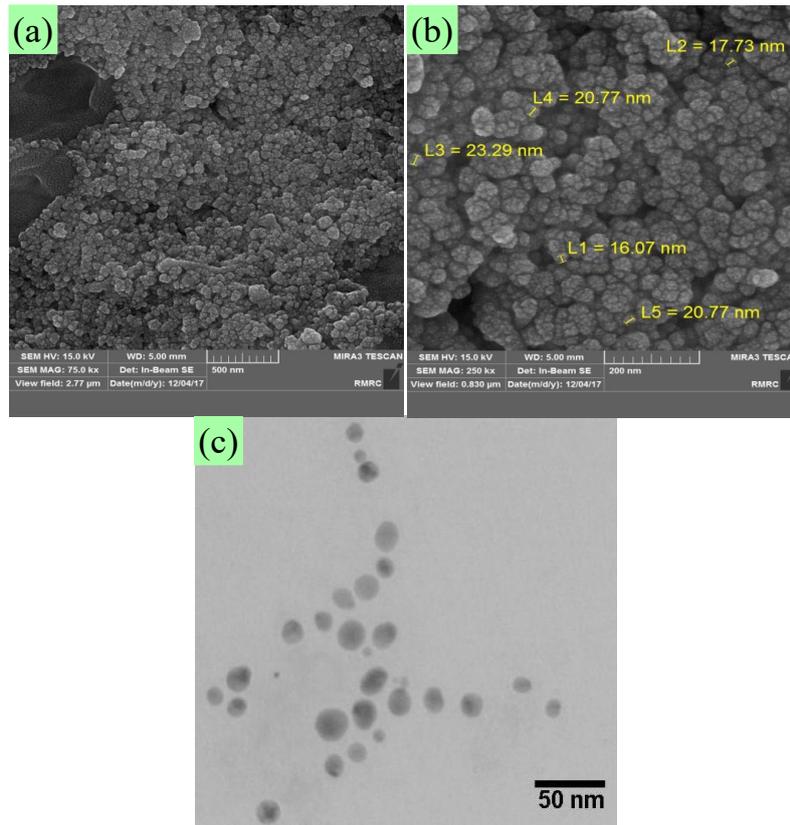


Fig. 2. (a, b) FE-SEM images of the bio-synthesized Ag-NPs at different magnifications. (c) TEM image of the same sample of Ag-NPs.

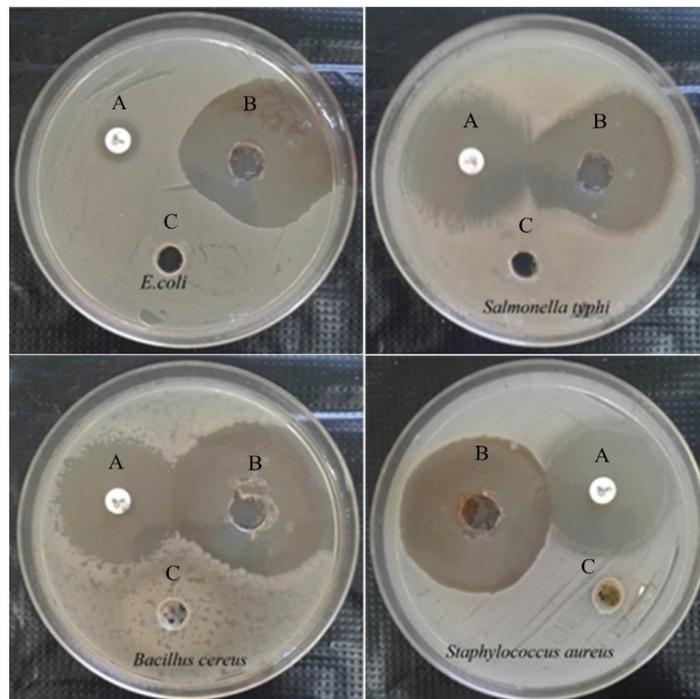


Fig. 3. Antibacterial activity of ciprofloxacin (CIP) antibiotic (A), bio-synthesized silver NPs (B), and plant extract (C) against various pathogenic bacteria.

Table 2. Comparison of antibacterial activity of other bio-synthesized silver NPs with the results of the present study. Numbers indicate the zone of inhibition (mm). In each row, different letters mean significant differences $p < 0.05$. NI: No inhibition

Plant source	Zone of inhibition (mm)				References
	<i>S. aureus</i>	<i>B. cereus</i>	<i>E. coli</i>	<i>S. typhi</i>	
Plant extract	NI c	NI c	NI c	NI c	Present study
Ciprofloxacin (CIP, 5 μg)	28 \pm 0.5 b	22 \pm 0.7 b	6 \pm 0.3 b	27 \pm 0.6 b	
<i>S. striata</i> Boiss shoot extract	30 \pm 0.3 a	33 \pm 0.5 a	25 \pm 0.5 a	28 \pm 0.7 a	
<i>Garcinia indica</i> fruit extract	15	-	14	-	
<i>S. striata</i> Boiss flower extract	17	-	16	-	
<i>Lippia citriodora</i> leaf extract	15	-	21	20	
<i>Delphinium denudatum</i> root extract	10	10	8	-	
<i>Ficus sycomorus</i> leaf extract	9	-	14	7	
<i>Ficus sycomorus</i> latex extract	15	-	13	9	

Table 3. MIC and MBC results for Bacteria

	MIC ($\mu\text{g.ml}^{-1}$)	MBC ($\mu\text{g.ml}^{-1}$)
<i>E. coli</i>	312	625
<i>S. typhi</i> (ATCC 6539)	156	312
<i>S. aureus</i>	156	312
<i>B. cereus</i>	156	312

cellular content, and also disturbance in transport activity [34].

2. After absorption of Ag-NPs to the bacterial membrane, the Ag-NPs can transport across the cell membrane and penetrate into the bacterial cell. Inside the bacterial cell, the Ag-NPs can affect the cell's macromolecules such as DNA, Proteins, and lipids, which results in negative effects on vital cellular functions, including protein synthesis [35], sugar metabolism [36], and cell division [37].

3. Formation of reactive oxygen species (ROS) and free radicals by the Ag-NPs. The effects of an increase in ROS level in the bacterial cells can be denaturation of protein, hyper-oxidation of lipids and DNA [38]. Therefore, high levels of ROS in a bacterial cell can lead to cell death.

4. Ag-NPs can effect on the signal transduction pathway in the bacterial cell. These signal transduction pathways influence different cellular activities including recombination, DNA replication, cell cycle, and bacterial metabolism. Ag-NPs with modulation of these signaling pathways would inhibit bacterial growth [34].

Although, the general antibacterial activity of bio-synthesized NPs depends on different parameters such as plant species, plant organ type, precursors and methods used, and concentration of nanomaterials in antibacterial activity assay, however, the results obtained in the present work are remarkably better than the best results reported by other groups. This shows

great potential for our cost-effective and scalable bio-synthesized Ag-NPs using *S. striata* Boiss plant extract, for future biological applications.

CONCLUSIONS

A novel, facile and green approach was suggested for bio-synthesis of high-quality Ag-NPs using a very safe, biocompatible and accessible *S. striata* Boiss plant extract. The synthesized Ag-NPs were relatively spherical in shape exhibiting FCC cubic structure with the average size of ~ 16 nm and plasmonic-related absorption peak at about 435 nm. The presence of different functional groups in active phytoconstituents of the plant extract has been shown by FT-IR spectroscopy. The antibacterial activity of the as-prepared Ag-NPs was also investigated using the disk diffusion method. Results revealed a strong antimicrobial activity against different Gram-positive and -negative microorganisms (zone of inhibition of 25, 28, 30, and 33 mm for *E. coli*, *S. typhi* ATCC, *B. cereus*, and *S. aureus*, respectively). It demonstrated better bactericidal property against Gram-positive bacteria compared to Gram-negative bacteria. On the other hand, the bactericidal activities of NPs were better than those of *Ciprofloxacin* antibiotic and even than other reports. Therefore, the present scalable bio-synthesized Ag-NPs using *S. striata* plants can be considered as excellent candidates for a wide range of biological intentions.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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