Scientia Iranica F (2015) 22(6), 2736–2744

Sharif University of Technology
Scientia Iranica
Transactions F: Nanotechnology
www.scientiairanica.com

Green synthesis of silver nanoparticles using pollen extract of rose flower and their antibacterial activity

M.A. Karimi[a,b,*], M.A. Mozaheb[a,c,d], A. Hatefi-Mehrjardi[a,b], H. Tavallali[a], A.M. Attaran[a] and G. Deilamy-Rad[a]

a. *Department of Chemistry, Payame Noor University, Tehran, P.O. Box 19395-4697, Iran.*
b. *Department of Chemistry & Nanoscience and Nanotechnology Research Laboratory (NNRL), Payame Noor University, Sirjan, P.O. Box 78185-347, Iran.*
c. *Department of Chemistry, Payame Noor University (PNU), Mashhad, Iran.*
d. *Department of Chemistry, Technical and Vocational University of Hazrat Roghayeh, Yazd, Iran.*

Received 18 June 2014; received in revised form 13 June 2015; accepted 10 November 2015

KEYWORDS
Green synthesis;
Silver nanoparticles (AgNPs);
Pollen;
Antibacterial activity;
Surface Plasmon Resonance (SPR).

Abstract. The present study reports a simple, rapid, easy, eco-friendly, and cost efficient method for green synthesis of silver nanoparticles (AgNPs) using pollen extract of rose flower as reducer and stabilizer. AgNPs synthesis was performed at room temperature in solutions by reduction rapidly taking place for 15 min; the synthesized AgNPs were stable for a long time. Moreover, highlights for this method avoid utilizing toxic organic solvents and using nontoxic water as the solvent for pollen extract from rose flower. Microscopy and UV-Vis spectroscopy confirmed the formation of nanoparticles as well as the colloid of silver samples. Surface Plasmon Resonance (SPR) peaks in UV-Vis spectra, observed at 427 nm, indicated the formation of poly dispersive AgNPs using pollen of rose flower. The synthesized AgNPs were mostly spherical in shape with an average size of 12 nm. It shows the significant antibacterial efficacy against *Staphylococcus aureus (S. aureus)*, *Escherichia coli (E. coli)*, *Bacillus cereus (B. cereus)*, and *Enterococcus faecalis (E. faecalis)* by disk diffusion method using Mueller-Hinton Agar. From the results, it is suggested that green synthesized AgNPs could be used effectively in cosmetics, foods, and medicine applications.

1. Introduction

Nanoscience has flourished during the past twenty years. The progress in this area largely depends on the ability to synthesize nanoparticles from various materials in various sizes and shapes.

Silver nanoparticles (AgNPs) have gained much attention for their unique nature and advantages when used in various experiments [1,2]. AgNPs are the most fashionable functionalizing and commercializing nanoparticles due to their exclusive physicochemical properties such as electric, optical, catalytic, and particularly antimicrobial properties [3,4]. AgNPs showed potential antibacterial activity and significantly higher synergistic effect with many antibiotics [5-7]. The interactions of AgNPs with bacteria are dependent on the size and structure of the nanoparticles [8,9]. AgNPs as antibacterial agents are now used extensively in different fields of medicine such as molecular imaging, diagnosis, and treatment of cardiovascular diseases and drug delivery [10].

AgNPs can be synthesized by various methods such as application of reducing chemicals of hydrazine hydrate and sodium citrate, sodium borohydride, formaldehyde, polyethylene glycol, glucose, heating and electrochemical reduction, ultraviolet irradiation,

*. *Corresponding author.*
E-mail addresses: ma_karimi43@yahoo.com; and m_karimi@pnu.ac.ir (M.A. Karimi)

ultrasonic fields, photochemical reduction, etc. [11]. The chemical and physical technologies used for the synthesis of nanoparticles are fairly expensive and their by-products and wastes are toxic and harmful for the environment [11-13]. Scientists propose the synthesis of nanoparticles using a variety of biological systems, such as yeast, fungi, bacteria, fruit and plant extracts as an alternative to the chemical and physical technologies [14,15]. However, using plant parts is reasonable, because they are economic, easily available, and ecofriendly.

Plant extracts are often environmentally and economically friendly materials and have been explored in the synthesis of AgNPs. The reason plant extracts work so well in the synthesis of nanoparticles is that they act as reducing agents as well as capping agents [16]. The use of plant extracts as the production assembly of AgNPs has drawn attention, because it is often environmentally and economically friendly and non-pathogenic, providing a single step method for the biosynthetic processes. The reduction and stabilization of silver ions is possible by combination of biomolecules, such as amino acids, enzymes, polysaccharides, alkaloids,tannins, phenolics, saponins, terpinoids, proteins, and vitamins in the plant extracts.

Several plant extracts have been used for extracellular biosynthesis of AgNPs, such as Cassia roxburghii DC [17], Alternanthera dentate [18], Acorus calamus [19], Tea [20], Pistacia atlantica [21], Centella asiatica [22], commercially available plant powders (Syzygium cumini, Citrus sinensis, Solanum tricobatum, and Centella asiatica) [23], Argyreia nervosa [24], Thevetia peruviana [25], Eclipta prostrate [26], Meliadubia [27], Aloe vera [28], Nelumbo nucifera [29], Vitis vinifera [30], Citrus sinensis [31], and Cocous nucifera [32].

Various colors, shapes, and sizees (diameters of 6 to 200 μm) have been observed for Pollen grains. Pollen grains have very hard porous outer shells (sporoderm), (Figure 1), allowing the pollen to germinate and also provide material removal. Pollen contains compounds like sugars (up to 50 percent), such as glucose, fructose, sucrose, pectin, cellulose, lignin, and starch (up to 18 percent). Pollen is a source of many enzymes and all kinds of essential amino acids for humans (over 40 percent), such as phenyalanine, leucine, valine, arginine, histidine, lysine, and tryptophan [33].

The present study aims to synthesize AgNPs by a green biological route, using pollen extract of rose flower, and characterize the synthesized nanoparticles, utilizing UV-Visible spectroscopy, Transmission Electron Microscope (TEM), X-Ray Diffraction (XRD), and Fourier transform infrared spectroscopy (FT-IR) analyses. The present work has focused on the development of easy synthesis of silver nanoparticles by an environmentally friendly procedure. Besides, the antibacterial activity of synthesized AgNPs against pathogenic bacteria of *Staphylococcus aureus (S. aureus)*, *Escherichia coli (E. coli)*, *Bacillus cereus (B. cereus)*, and *Enterococcus faecalis (E. faecalis)* was investigated.

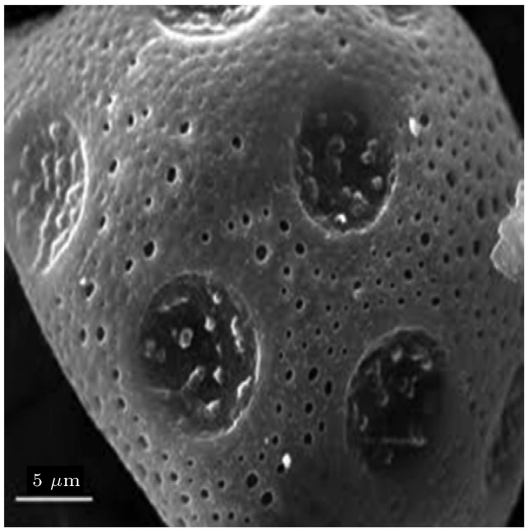


Figure 1. SEM image of pollen grain of rose flower.

2. Experimental

2.1. Apparatus and reagents

The UV-Vis spectra were recorded on a (Cintera 6GBC Australia) UV-Vis spectrophotometer. The Fourier transform infrared spectra were obtained on a (JASCO FT/IR-4200) FTIR spectrometer with the samples as KBr pellets. The morphology of nanoparticles was analyzed using the image obtained with a transmission electron microscope (Phillips Em208 90 kv). All chemicals were of analytical grade and used as received without further purification. Silver nitrate ($AgNO_3$) used for the synthesis of silver nanoparticles was purchased from Merck. The pollen was provided of rose flower stamens available on the campus of Payame Noor University of Sirjan. All of the other solutions were freshly made for the whole experimental procedure. Ultrapure water was used throughout the study, and all glassware was cleaned and rinsed with ultrapure water prior to use.

2.2. Synthesis of silver nanoparticles

We separated the stamen of rose flowers carefully and 0.012 g of them was added to 10 mL of ultrapure water at room temperature. The mixture was allowed to stand for 30 min. Whatman filter paper (No. 40) was used for filtration of material to prepare the aqueous pollen extract. The filtrate was used as reducing agent and stabilizer. Then, 3 mL of the mentioned extracted pollens was added to a vigorously stirred 0.1 mL aqueous solution of $AgNO_3$ (0.1 mol L^{-1}) into a

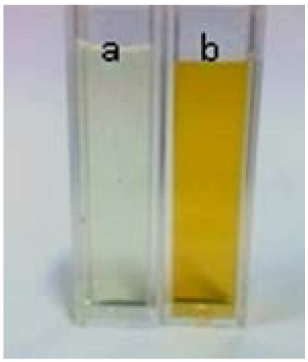


Figure 2. Pollen extract (a) before and (b) after addition of aqueous solution of 1.0×10^{-3} mol L^{-1} $AgNO_3$ at 1 min.

10 mL volumetric flask and ultrapure water was added up to the mark. The obtained solution was stirred well for 15 min at room temperature. Reduction takes place rapidly as indicated by the golden yellow color (Figure 2). The reduction of silver ions was monitored by measuring the absorbance of the reaction mixture in a range of wavelength from 300 to 800 nm by using a UV-Vis spectrophotometer to find the absorbance peak. The effects of reaction conditions, such as the extract concentration, metal ion concentration, pH, reaction time, and reaction temperature, were also studied. The AgNPs was used for antibacterial assay without further purification.

3. Results and discussion

Figure 3 represents the UV-Vis spectra of aqueous component as a function of time variation of pollen solution containing 1.0 mmol L^{-1} $AgNO_3$. There are free electrons in the metal nanoparticles, which result in Surface Plasmon Resonance (SPR) absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light waves. After 15 min, changes in color were observed from colorless to pale with the sharp bands of silver colloids at 427 nm. The intensity of absorption band increases with time period of aqueous component and the following color changes are observed from pale to reddish yellow up to 5 hours, and then, subsequently, only slight variations can be observed. This characteristic color change is due to the excitation of SPR in the metal nanoparticles.

3.1. Optimization of conditions

3.1.1. Effect of metal ion concentration

SPR spectra for AgNPs were obtained at 427 nm with golden yellow color under the natural constant pH and temperature conditions and various metal ion concentration (Figure 3). In the case of AgNPs, the peak absorbance were increased with Ag^+ addition in the concentration ranges of 5.0×10^{-5} to 1.0×10^{-3} mol L^{-1}. The solution color in low concentration of metal ion was yellow, but in higher concentrations turned to black and then precipitated.

3.1.2. Effect of time duration of the formation of AgNPs

AgNPs synthesis was also evaluated at different contact times taking the absorbance with UV-Vis spectroscopy. The nanoparticles formed after 1 minute and increased with time. It was also observed that by increasing the collision time, peaks become sharper (Figure 4).

3.1.3. Effect of pH

The effect of pH was studied under the constant concentration of metal ion at room temperature and various solution pHs (pHs 6 to 10). There is an enhancement in the absorbance with increasing the solution pH and also at the higher pHs; the solution color quickly changes into darker color (Figure 5). The other hand, inset indicated that the difference between the absorption spectrum of solution without adjusting the pH and that with the pH adjusted to 7.5 is negligible. Therefore, in order to provide simplicity

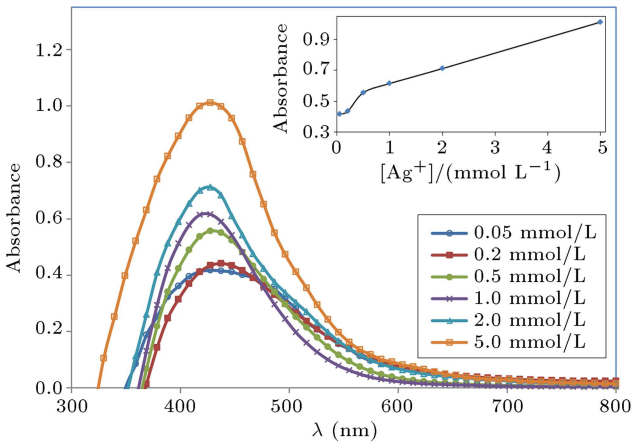


Figure 3. Change in SPR absorption spectra resulted from the different concentrations of silver ions.

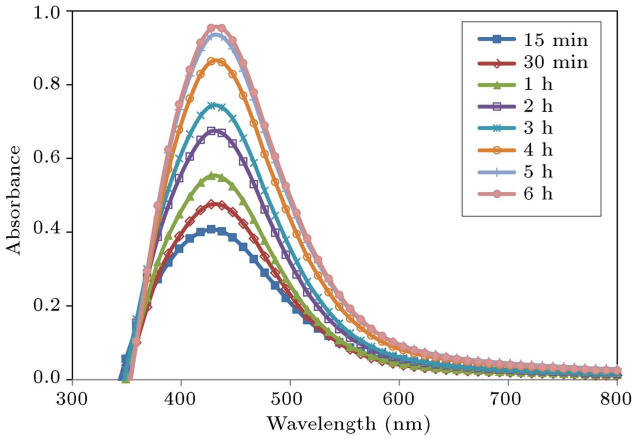


Figure 4. Effect of time duration on the formation of AgNPs at the room temperature. The concentration of $AgNO_3$ is 1.0×10^{-3} mol L^{-1}.

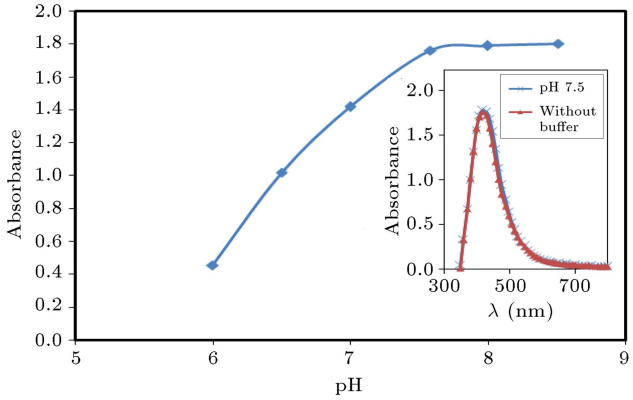


Figure 5. Effect of pH on the formation of AgNPs in solution containing 1.0×10^{-3} mol L^{-1} $AgNO_3$ at the room temperature.

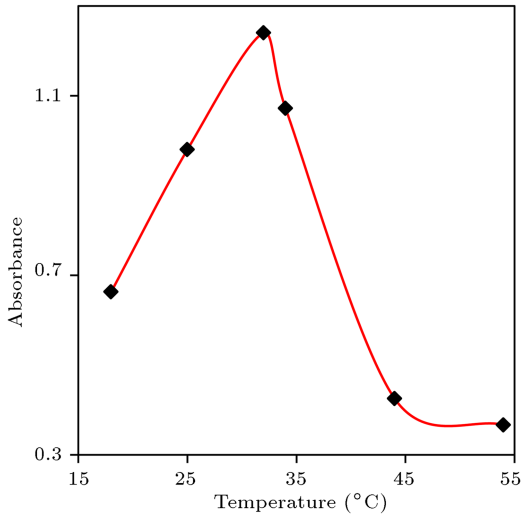


Figure 6. Effect of reaction temperature on AgNPs synthesis. $AgNO_3$ concentration is 1.0×10^{-3} mol L^{-1}.

of the work procedure, the raw materials were used without pH adjustment.

3.1.4. Effect of temperature

Production of AgNPs in a water bath at different temperatures of 16 to 54°C and the SPR spectra were studied. According to Figure 6, the highest absorption was observed at 32°C and a further increase in temperature leads to a decrease in the absorption peak as a consequence of dissolution of the precipitate in solution. Since the absorption of the solution at room temperature (27°C) was acceptable, for the sake of simplicity, this temperature was used.

3.2. Characterization of AgNPs

The size and morphology of the prepared AgNPs form this method were monitored by TEM (Figure 7). The spherical nanoparticles clearly have a narrow particle size distribution with the largest having a diameter of 38 nm and average size of 12 nm.

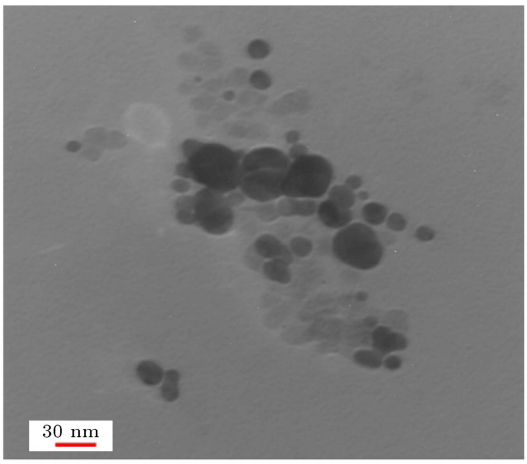


Figure 7. TEM image of synthesized AgNPs.

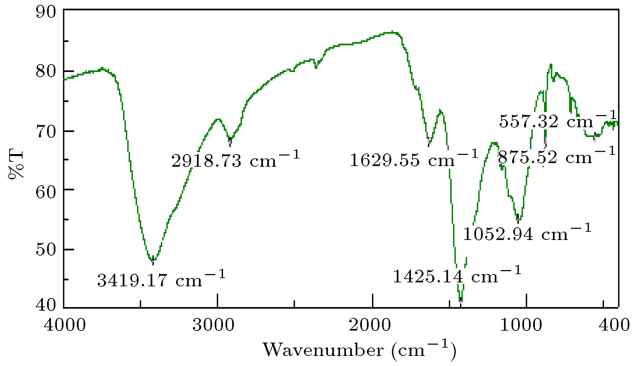


Figure 8. FTIR spectrum of synthesized AgNPs by pollen extract from rose flower.

FTIR measurements were carried out to identify the bio-molecules for capping and efficient stabilization of the AgNPs synthesized by pollen extract. The FTIR spectrum of AgNPs is shown in Figure 8. The band of 3419 cm^{-1} corresponds to O-H stretching H-bonded alcohols and phenols. The peak of 2918 cm^{-1} corresponds to C-H stretch. The assignment at 1629 cm^{-1} corresponds to C=C and C=N bands. The peak at 1052 cm^{-1} corresponds to C-O-H and C-O-C stretching alcohols, carboxylic acids, and ethers. The peaks presented at 1425, 1052, 875, and 557 cm^{-1} correspond to C-C, C-N, and C-O. Therefore, the synthesized nanoparticles were surrounded by sugars and amino acids having functional groups of alcohols and carboxylic acids.

3.3. X-ray diffraction

X-Ray Diffraction (XRD) pattern of dry nanoparticles powder was obtained using X'Pert Pro MPD (PANalytical) X-ray diffractometer with CuKα radiation (λ = 1.54 Å). The experimental powder diffraction (XRD) pattern of the prepared AgNPs is shown in Figure 9. In the XRD pattern of AgNPs, diffraction peaks at 38.13°, 44.21°, 64.45°, and 77.47° are assigned to face-centered cubic (fcc) metallic silver corresponding to

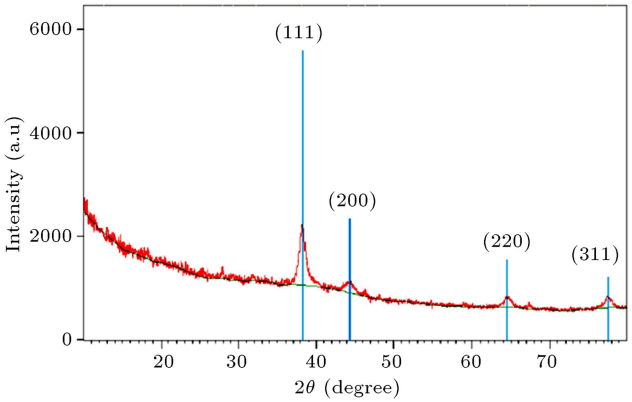


Figure 9. X-ray diffraction pattern of synthesized AgNPs by pollen extract from rose flower.

the (111), (200), (220), and (311) facets of the silver crystals.

3.4. Size and shape of AgNPs

A large number of plants, reported to facilitate AgNPs syntheses, are mentioned in Table 1. Table 1 shows a comparison of the proposed method with some other reported methods for extracellular biosynthesis of AgNPs. It could be seen that the obtained values for average size of AgNPs for the proposed method are as or better than those of some previously reported methods.

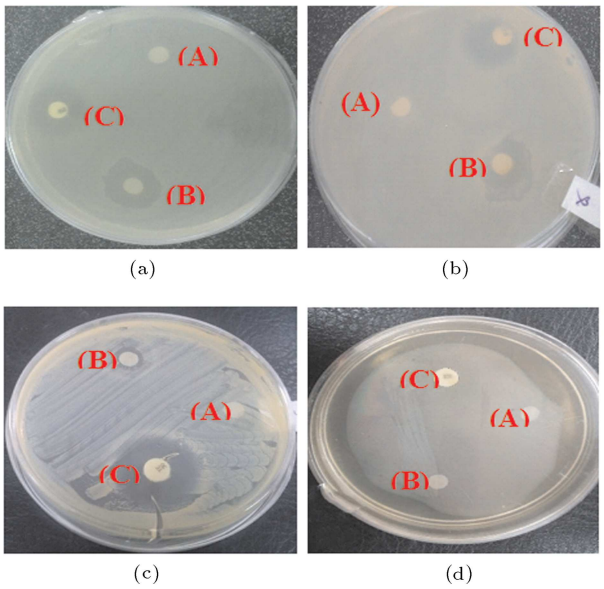
3.5. Evaluation of the antibacterial effect of AgNPs

Antibacterial activity of AgNPs to penetrate the Kirby-Bauer against Gram-positive bacteria, including *S. aureus* (PTCC1431) and Gram-negative bacteria including *E. coli* (PTCC1394), *B. cereus* (PTCC1015), and *E. faecalis* (PTCC1237), was investigated [7,34,35]. Whatman filter paper discs with a diameter of 5 mm were placed in a solution of AgNPs for 24 hours to drive the nanoparticles penetrate. The discs on Mueller-Hinton agar plates, containing bacterial suspensions of the desired concentration before 106 to 107 CFU mL^{-1}, were uniformly distributed on the surface they were placed on. The discs impregnated with tetracycline, as antibiotics, were placed on the plate. The disc drenched in pollen extract was used as blank. Plates containing discs were cultured for 24 hours at 35°C. The average diameter of the area around the discs that stops the growth of bacteria (Zone of inhibition) was measured with a ruler (Figure 10). The diameter of inhibition zone (mm) around each well with AgNPs solution is represented in Table 2. As the results show, the highest antimicrobial activity of synthesized AgNPs by pollen extract were 13.0, 13.0, 11.0, and 0.6 against *E. coli*, *S. aureus*, *B. cereus*, and *E. faecalis*, respectively.

The mechanism of inhibitory action of AgNPs on microorganisms is partially known. AgNPs have

Table 1. Comparison of the sizes and shapes of the synthesized AgNPs by the proposed method with some other reported methods for extracellular biosynthesis.

Plants	Plant's part	Size (nm)	Shape	References
Cassia roxburghii	Flower	5-360	Triangular	[17]
Alternanthera dentate	Leaves	10-50	Spherical	[18]
Acorus calamus	Rhizome	31	Spherical	[19]
Tea	Leaves	20-90	Spherical	[20]
Pistacia atlantica	Seeds	10-50	Spherical	[21]
Centella asiatica	Leaves	30-50	Spherical	[22]
Syzygium cumini	Leaf	53	Triangular	[23]
Citrus sinensis	Leaf	41	Triangular	
Solanum tricobatum	Leaf	52	Triangular	
Centella asiatica	Peel	42	Triangular	
Argyreia nervosa	Seeds	20-50	—	[24]
Thevetia peruviana	Latex	10-30	Spherical	[25]
Eclipta prostrata	Leaves	30-60	Triangles, Pentagons, Hexagons	[26]
Meliadubia	Leaf	35	Spherical	[27]
Aloe vera	Leaves	50-350	Spherical, Triangular	[28]
Nelumbo nucifera	Leaves	25-80	Spherical, Triangular	[29]
Vitis vinifera	Fruit	30-40	—	[30]
Citrus sinensis	Peel	10-35	Spherical	[31]
Cocos nucifera	Inflorescence	22	Spherical	[32]
Rose flower	Pollen	12	Spherical	This work



(a) (b)

(c) (d)

Figure 10. Comparative study of inhibitory zones of (a) *E. coli*, (b) S. aureus, (c) *B. cereus*, and (d) *E. faecalis* bacteria with (A) pollen extract from rose flower (blank), (B) synthesized AgNPs by pollen extract from rose flower, and (C) tetracycline antibiotic on Mueller-Hinton Agar plates after incubation at 35°C for 24 h. Conditions: 30.0 μg/disc of tetracycline; 1.0×10^{-3} mol L^{-1} AgNPs; 1.2 mg mL^{-1} pollen extract.

Table 2. The zone of inhibition of synthesized AgNPs by pollen extract from rose flower against various pathogenic bacteria. Conditions: 30.0 μg/disc of tetracycline; 1.0×10^{-3} mol L^{-1} AgNPs; 1.2 mg mL^{-1} pollen extract.

Plants	Pollen extract	AgNPs	Tetracycline
Escherichia coli	0	13.0	13.0
Staphylococcus aureus	0	13.0	14.0
Bacillus cereus	0	11.0	19.0
Enterococcus faecalis	0	0.6	0.7

positive charge; it will attach to the negative charged microorganisms by the electrostatic attraction in the cell wall membrane [36]. AgNPs are associated with thiol groups of cell wall resulting in the generation of reactive oxygen species and disrupting the cell [37]. AgNPs' binding with bacteria depends on the surface area for the interaction. Smaller particles affect the larger surface area of the bacteria; thus, they have more bactericidal activity than the larger sized nanoparticles [38]. The AgNPs are synthesized in this way to prevent more than 97% of bacteria. Our experimental results showed that the AgNPs, synthesized by pollen extract, have a significant inhibitory effect on the growth of microorganisms; so, they can be used to prevent the growth and propagation of microbes in different environments.

3.6. *Determination of minimum inhibitory (MIC) and Minimum Bactericidal Concentration (MBC)*

The ability to inhibit bacterial growth for synthesized AgNPs by pollen extract was determined by standard dilution micro method, determining the MIC leading to inhibition of bacterial growth. AgNPs, synthesized 2 to 8 times, were diluted with 100 μL of Mueller-Hinton broth solution, and the lowest concentration, causing inhibition of bacterial growth, can be measured by the bacteria concentration of 10^6 to 10^7 CFU mL^{-1}. The MBC is the lowest concentration that causes death in 99.9% of bacteria and AgNPs are measured in bath culture studies. In this study, at first, 10 μL of bacteria was added into diluted AgNPs and then placed in an incubator at 37°C. After 24 hours, each of the solutions was grass cultured in Mueller-Hinton Agar plates, separately. Then, all of them were placed in incubator for 24 hours for their MIC and MBC to be determined (Table 3). Figure 11 also shows the plate of every bacteria, separately.

The inhibitory effect of AgNPs may also depend on the CFU bacteria used in this experiment. As the results show, the highest and lowest resistance was observed for *E. coli* and *E. faecalis*, respectively. Future research is required to evaluate the number of parameters, such as optimum concentration of AgNPs, needed for the inhibitory effect on bacteria and size and shape of the AgNPs to understand the relationship between these parameters and antibacterial activity.

4. Conclusions

This research is new, easy, fast, and compatible with the environment to produce AgNPs. This method does not require the use of complex physical and chemical

Table 3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of AgNPs for various microorganisms. Conditions: 30.0 μg/disc of tetracycline; and 1.2 mg mL^{-1} pollen.

Culture	Strain no.	MIC (μg mL^{-1})	MBC (μg mL^{-1})
Staphylococcus aureus	PTCC1431	21.0	42.0
Escherichia coli	PTCC1394	10.5	21.0
Bacillus cereus	PTCC1015	21.0	42.0
Enterococcus faecalis	PTCC1237	41.0	84.0

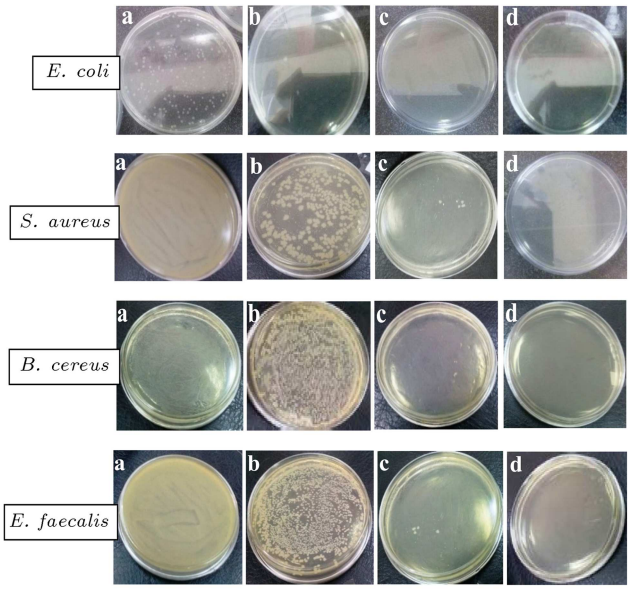


Figure 11. Bacteria grow on the agar plates at different concentrations of AgNPs. The concentrations of AgNPs, used against every bacteria of *E. coli*, *S. aureus*, and *B. cereus*, were (a) 5.2, (b) 10.5, (c) 21.0, and (d) 42.2 μgmL^{-1}; but for *E. faecalis* bacteria, these concentrations were (a) 10.5, (b) 21.0, (c) 42.0, and (d) 84.2 μg mL^{-1}. Conditions: 30.0 μg/disc of tetracycline, 1.2 mg mL^{-1} pollen extract.

processes as well as harmful chemicals of silver ions to reduce their stability. Spherical silver nanoparticles were synthesized with the help of rose flower pollen extract and the resulting nanoparticles were stable in water. The synthesized nanoparticles, having antibacterial properties against both Gram Gram-positive bacteria (*S. aureus*) and Gram-negative bacteria (*E. coli, B. cereus* and *E. faecalis*), were examined and were found to be effective in inhibiting the growth of bacteria used in the medical field applications.

Acknowledgments

The authors would like to express their appreciations to Professor Afsaneh Safavi and Dr. Mahboubeh Mirhosseini for their valuable discussion and useful suggestions. This research was supported by the Nanostructured coating Institute of Payame Noor University of Yazd.

References

1. Geddes, J.R., Carney, S.M., Davies, C., Furukawa, T.A., Kupfer, D.J., Frank, E. and Goodwin, G.M. "Relapse prevention with antidepressant drug treatment in depressive disorders: A systematic review", *The Lancet*, **361**, pp. 653-661 (2003).
2. Ghosh, P.K., Saxena, T.K., Gupta, R., Yadav, R.P. and Davidson, S. "Microbial lipases: Production and applications", *Sci. Prog.*, **79**, pp. 119-157 (1996).
3. Tricoli, A. and Pratsinis, S.E. "Dispersed nanoelectrod devices", *Nat. Nanotechnol.*, **5**, pp. 54-60 (2010).
4. Chen, M., Yang, Z., Wu, H., Pan, X., Xie, X. and Wu, C. "Antimicrobial activity and the mechanism of silver nanoparticle thermos sensitive gel", *Int. J. Nanomed.*, **6**, pp. 2873-2877 (2011).
5. Devi, L.S. and Joshi, S.R. "Antimicrobial and synergistic effect of silver nanoparticles H using soil fungi of high altitudes of eastern Himalaya", *Mycobiology*, **40**, pp. 27-34 (2012).
6. Mahdieh, M., Zolanvari, A. and Azimee, A.S. "Green biosynthesis of silver nanoparticles by Spirulina platensis", *Sci. Iran.*, **19**, pp. 926-929 (2012).
7. Faghri Zonooz, N. and Salouti, M. "Extracellular biosynthesis of silver nanoparticles using cell filtrate of Streptomyces sp ERI-3", *Sci. Iran.*, **18**, pp. 1631-1635 (2011).
8. Panacek, A., Kvitek, L., Prucek, R., Kolar, M., Vecerova, R., Pizurova, N., Sharma, V.K., Tatjana, N. and Zboril, Z. "Silver colloid nanoparticles: Synthesis, characterization, and their antibacterial activity", *J. Phys. Chem. B*, **110**, pp. 16248-16253 (2006).
9. Pal, S., Tak, Y.K. and Song, J.M. "Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram-negative bacterium Escherichia coli", *Appl. Environ. Microbial*, **73**, pp. 1712-1720 (2007).
10. Dar, M.A., Ingle, A. and Rai, M. "Enhanced antimicrobial activity of silver nanoparticles synthesized by Cryphonectria sp. evaluated singly and in combination with antibiotics", *Nanomedicine*, **9**, pp. 105-110 (2013).
11. Mallick, K., Witcombb, M.J. and Scurrella, M.S. "Self-assembly of silver nanoparticles in a polymer solvent: Formation of a nanochain through nanoscale soldering", *Mater, Chem. Phys.*, **90**, pp. 221-224 (2005).
12. Yu, D.G. "Formation of colloidal silver nanoparticles stabilized by Na^+ _poly (γ-glutamic acid)-silver nitrate complex via chemical reduction process", *J. Colloids Surf. B*, **59**, pp. 171-178 (2007).
13. Smetana, A.B., Klabunde, K.J. and Sorensea, C.M. "Synthesis of spherical silver nanoparticles by digestive ripening stabilization with various agents, and their 3-D and 2-D superlattice formation", *J. Colloid Interface Sci.*, **284**, pp. 521-526 (2005).
14. Senapati, S., Ahmad, A., Khan, M.I., Sastry, M. and Kumar, R. "Extracellular biosynthesis of bimetallic Au-Ag alloy nanoparticles", *Small*, **1**, pp. 517-520 (2005).
15. Shahverdi, A.R., Minaeian, S., Shahverdi, H.R., Jamalifar, H. and Nohi. A.A. "Rapid synthesis of silver nanoparticles using culture supernatants of enterobacteria: A novel biological approach", *Process Biochem.*, **42**, pp. 919-923 (2007).
16. Hebbalalu, D., Lalley, J., Nadagouda, M.N. and Varma, R.S. "Greener techniques for the synthesis

of silver nanoparticles using plant extracts, enzymes, bacteria, biodegradable polymers, and microwaves", *ACS Sustainable Chem. Eng.*, **1**, pp. 703-712 (2013).

17. Moteriya, P. and Chanda, S. "Biosynthesis of silver nanoparticles using flower extract of Cassia roxburghii DC and its synergistic antibacterial ecacy", *Sci. Iran.*, **21**, pp. 2499-2507 (2014).
18. Nakkala, J.R., Mata, R., Kumar Gupta, A. and Rani Sadras, S. "Biological activities of green silver nanoparticles synthesized with Acorous calamus rhizome extract", *Eur. J. Med. Chem.*, **85**, pp. 784-794 (2014).
19. Nakkala, J.R., Mata, R. and Rani Sadras, S. "Green synthesis and characterization of silver nanoparticles using Boerhaavia diffusa plant extract and their antibacterial activity", *Indus. Crop. Prod.*, **52**, pp. 562-566 (2014).
20. Nabikhan, A., Kandasamy, K., Raj, A. and Alikunhi, N.M. "Synthesis of antimicrobial silver nanoparticles by callus and leaf extracts from saltmarsh plant, Sesuvium portulacastrum L.", *Colloids Surf. B: Biointerfaces*, **79**, pp. 488-493 (2010).
21. Sadeghi, B., Rostami, A. and Momeni, S.S. "Facile green synthesis of silver nanoparticles using seed aqueous extract of Pistacia atlantica and its antibacterial activity", *Spectrochim. Actat A*, **134**, pp. 326-332 (2015).
22. Rout, A., Jena, P.K., Parida, U.K. and Bindhani, B.K. "Green synthesis of silver nanoparticles using leaves extract of Centella asiatica L. For studies against human pathogens", *Int. J. Pharm. Biol. Sci.*, **4**, pp. 661-674 (2013).
23. Logeswari, P., Silambarasan, S. and Abraham, J. "Ecofriendly synthesis of silver nanoparticles from commercially available plant powders and their antibacterial properties", *Sci. Iran.*, **20**, pp. 1049-1054 (2013).
24. Thombre, R., Parekh, F. and Patil, N. "Green synthesis of silver nanoparticles using seed extract of Argyreia nervosa", *Int. J. Pharm. Biol. Sci.*, **5**, pp. 114-119 (2014).
25. Rupiasih, N.N., Aher, A., Gosavi, S. and Vidyasagar, P.B. "Green synthesis of silver nanoparticles using latex extract of Thevetia peruviana: A novel approach towards poisonous plant utilization", *J. Phys. Conf. Ser.*, **423**, pp. 1-8 (2013).
26. Rajakumar, G. and Abdul Rahuman, A. "Larvicidal activity of synthesized silver nanoparticles using Eclipta prostrata leaf extract against filariasis and malaria vectors", *Acta Trop.*, **118**, pp. 196-203 (2011).
27. Kathiravan, V., Ravi, S. and Kumar, S.A. "Synthesis of silver nanoparticles from Meliadubia leaf extract and their in vitro anticancer activity", *Spectrochim. Acta A*, **130**, pp. 116-121 (2014).
28. Chandran, S.P., Chaudhary, M., Pasricha, R., Ahmad, A. and Sastry, M. "Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract", *Biotechnol. Prog.*, **22**, pp. 577-583 (2006).
29. Santhoshkumar, T., Rahuman, A.A., Rajakumar, G., Marimuthu, S., Bagavan, A. and Jayaseelan, C. "Synthesis of silver nanoparticles using Nelumbo nucifera leaf extract and its larvicidal activity against malaria and filariasis vectors", *Parasitol. Res.*, **108**, pp. 693-702 (2011).
30. Gnanajobitha, G., Paulkumar, K., Vanaja, M., Rajeshkumar, S., Malarkodi, C., Annadurai, G. and Kannan, C. "Fruit-mediated synthesis of silver nanoparticles using Vitis vinifera and evaluation of their antimicrobial efficacy", *J. Nanostruct. Chem.*, **3**, pp. 1-6 (2013).
31. Kaviya, S., Santhanalakshmi, J., Viswanathan, B., Muthumary, J. and Srinivasan, K. "Biosynthesis of silver nanoparticles using Citrus sinensis peel extract and its antibacterial activity", *Spectrochem. Acta A*, **79**, pp. 594-598 (2011).
32. Mariselvam, R., Ranjitsingh, A.J.A., Usha Raja Nanthini, A., Kalirajan, K., Padmalatha, C. and Mosae Selvakumar, P. "Green synthesis of silver nanoparticles from the extract of the inflorescence of Cocous nucifera (Family: Arecaceae) for enhanced antibacterial activity", *Spectrochim. Acta A*, **129**, pp. 537-541 (2014).
33. Krell, R. "Value-added products from beekeeping", *FAO Agricultural Services Bulletin No. 124*, Food and agriculture Organization of the United Nations, Rome (1996).
34. Raffi, M., Hussain, F., Bhatti, T.M., Akhter, J.I., Hameed, A. and Hasan, M.M. "Antibacterial characterization of silver nanoparticles against E. Coli ATCC-15224", *J., Mater. Sci, Technol.*, **24**, pp. 192-196 (2008).
35. Kokkoris, M., Trapalis, C.C., Kossionides, S., Vlastou, R., Nsouli, B., Grotzschel, R., Spartalis, S., Kordas, G. and Paradellis, T. "RBS and HIRBS studies of nanostructured $AgSiO_2$ sol-gel thin coatings", *Nucl. Instrum, Methods Phys. Res. Sect, B*, **188**, pp. 67-72 (2002).
36. Dibrov, P., Dzioba, J., Gosink, K.K. and Hase, C.C. "Chemiosmotic mechanism of antimicrobial activity of Ag(+) in Vibrio cholera", *Antimicrob. Agents Chemother.*, **246**, pp. 2668-2670 (2002).
37. Lara, H.H., Ayala-Nuaez, N.V., Ixtepan-Turrent, L. and Rodriguez-Padilla, C. "Bactericidal effect of silver nanoparticles against multidrug resistant bacteria", *World J. Microbiol. Biotechnol.*, **26**, pp. 615-621 (2010).
38. Shrivastava, S., Tanmay, B.E., Roy, A., Singh, G., Rao, P.R. and Dash, D. "Characterization of enhanced antibacterial affects of novel silver nanoparticles", *Nanotechnology*, **18**, pp. 1-9 (2007).

Biographies

Mohammad Ali Karimi is Professor of Analytical Chemistry in the Department of Chemistry and the Nanoscience and Nanotechnology Research Laboratory

(NNRL) and Associate Member of the Payame Noor University. He received his PhD degree in Analytical Chemistry from Shiraz University, Iran. His research interests include: chemiluminescence, chemometrics, synthesis and application of magnetite nanoparticles, metal oxide nanoparticles, carbon nanotubes, and graphene in analytical chemistry.

Malihe Alsadat Mozaheb obtained her BS degree in Pure Chemistry from the Islamic Azad University of Yazd, and her MS degree in Analytical Chemistry from Islamic Azad University of Yazd, Iran. She is PhD student in Analytical Chemistry at the Payame Noor University. Her research interests include: synthesis and applications of silver and gold nanoparticles.

Abdolhamid Hatefi-Mehrjardi is Assistant Professor of Analytical Chemistry in the Department of Chemistry and the Nanoscience and Nanotechnology Research Laboratory (NNRL) and Associate Member of the Payame Noor University of Sirjan. He received his PhD degree in Analytical Chemistry from Isfahan University, Iran. His research interests include: self-assembled monolayers, synthesis and application of magnetite nanoparticles, metal oxide nanoparticles, carbon nanotubes, and graphene in analytical and electroanalytical chemistry.

Hossein Tavallali is Professor of Analytical Chemistry at the Payame Noor University of Shiraz, Iran. He graduated in 1998 from Shahid Beheshti University. His main research interests cover atomic spectroscopy, chemical sensors, separation and measurement, kinetic analysis, and complexometry.

Abdol Mohammad Attaran is Assistant Professor of Analytical Chemistry in the Department of Chemistry at the Payame Noor University, Iran. He received his PhD degree in Analytical Chemistry from Shahid Chamran University, Iran. His main research interests cover electrochemical sensors, separation, and measurement.

Gohar Deilamy-Rad is working as an Assistant Professor in the Department of Chemistry at the Payame Noor University of Shiraz, Iran. Her research is concerning with the development of supramolecular chemistry, studies of metal complexes in solution, chemosensors for anion and cation detection, and sensors for biologically activations.