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Antibacterial activity of biosynthesized silver nanoparticles from fruit extracts of Bunium persicum Boiss

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ABSTRACT: Biological production of silver nanoparticles, due to its high antimicrobial properties and environmental compatibility, can be used as an alternative to antibiotics. The aim of this study was to optimize the green synthesis of silver nanoparticles using fruit extract of *Bunium persicum Boiss* and investigate the antimicrobial effect of nanoparticles against *Escherichia coli* and *Staphylococcus aureus*. In this study, AgNPs were synthesized by B. persicum fruit extract, as a green reducing agent. The color change of the solution is the first indication of the formation of silver nanoparticles. The quality of nanoparticles produced by SEM and UV-Vis spectroscopy was investigated and the average size of nanoparticles was also measured by dynamic light scattering. Antimicrobial effect of synthesized nanoparticles against *E. coli* and *S. aureus* was evaluated by disk diffusion and broth microdilution method. The results of this study showed maximum absorbance at 340 nm. SEM showed that AgNPs are spherical and the mean average size of them which is indicated with DLS was 21.3 nm. Minimum inhibitory concentration (MIC) of AgNPs was determined for *E. coli* 0.78 μg/ml and for *S. aureus* 1.56 μg/ml. The synthesized silver nanoparticles possess an effective antimicrobial activity against the selected microorganisms.

Keywords: *Antimicrobial effect; Bunium persicum; fruit extract; Green synthesis; Silver nanoparticles*

INTRODUCTION

Nanoparticles have an important role in the pharmaceutical and biotechnology industries. Because of the small size and hence the high surface-to-volume ratio, nanoparticles find a high level of contact with the environment and microorganisms, which can increase their biological and chemical activity (Vijayakumar, *et al.*, 2013). This causes the antimicrobial effects of metal nanoparticles to be much higher than those of metals themselves (Prabhu and Poulose, 2012, Nazeruddin, *et al.*, 2014). Biological methods of synthesis have paved the way for the "greener synthesis" of nanoparticles and these have proven to be better methods due to slower kinetics, they offer better manipulation and control over crystal growth and their stabilization. This has motivated an upsurge in research on the synthesis routes that allow better control of shape and size for various nano technological applications (Singh, *et*

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al., 2010). The use of environmentally benign materials like plant extracts (Jain, *et al.*, 2009, Banerjee, *et al.*, 2014), bacteria (Saifuddin, *et al.*, 2009) and fungi (Bhainsa and D'Souza, 2006) for the synthesis of silver nanoparticles offer numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications. Many research papers reported the synthesis of silver nanoparticles using plant extracts such as *Acalypha indica* leaf (Krishnaraj, *et al.*, 2010); *Chenopodium album* leaf (Dwivedi and Gopal, 2010); Rosa rugosa (Dubey, *et al.*, 2010); *Ocimum sanctum* stems and roots (Ahmad, *et al.*, 2010); *Eucalyptus oleosa* leaf (Vidhu, *et al.*, 2011) and Olea europaea leaves (Awwad, *et al.*, 2012).

In the present research, fruit extract of Bunium persicum have been used for silver nanoparticles (AgNPs) biosynthesis as a reducing agent of Ag^+ ions to Ag0 nanoparticles from silver nitrate. The antibacterial effect of Biosynthesized silver nanoparticles was evaluated against two pathogenic bacteria, including Escherichia coli (Gram negative) and Staphylococcus aureus (Gram positive) using the agar disk diffusion and broth microdilution method.

MATERIALS AND METHODS

Materials

The fruit extract of Bunium persicum were purchased from the market. Methanol (CH3OH, 99.9%), AgNO3 (99.98%), nutrient agar and Mueller-Hinton agar (MHA) were purchased from Merck (Germany). The standard strains of E. coli (ATCC 25922) and S. aureus (ATCC 25923) prepared from microbial collection of Pasteur institute of Iran.

Extract preparation

Bunium persicum fruits weighing 5g were into powder and were boiled into 100 ml sterile distilled water for 15 min. The aqueous extracts were filtered through Whatman paper No. 1 pore size 125mm and then by 25 mm and was stored at in dark bottles at 4 °C until used.

Synthesis of silver nanoparticles

Aqueous solution of 10 mM AgNO3 was prepared for the synthesis of silver nanoparticles. 0.5 mL of the AgNO3 aqueous solution was drop-wise added to 7 mL of the resulted aqueous extract solution. The volume was adjusted to 10 mL by de-ionized water and heated with stirrer in dark condition at 150 rpm and 60°C for 2 hours. The pH of the solution was adjusted to 9. The observed color change of reaction mixture from yellow to dark brown indicates the formation of silver nanoparticles. The formation of AgNPs was furthermore confirmed by spectrophotometric analysis. Then, the obtained solution was centrifuged at 10,000 rpm for 10 min and subsequently dispersed in sterile distilled water to get rid of any uncoordinated materials. Finally, The biosynthesized AgNPs were dried at room temperature and stored properly for future use.

Characterization of silver nanoparticles

The synthesized silver nanoparticles were characterized using FTIR analysis, Scanning Electron Microscopy, Dynamic Light Scattering (DLS) technique and Ultraviolet-Visible spectroscopy. UV–Vis spectral analysis was done by using a Double Beam spectrophotometer (SHIMADZU Prestige- 21, Japan) in a wavelength range between 200 and 800 nm. The reduction of silver ions (Ag+) to silver Nanoparticles was monitored through measuring the UV–Vis spectrum of the reaction medium after diluting a small amount of the sample into deionized water, using deionized water as a reference. FTIR analysis was used to detect the possible biomolecules responsible for the reduction of the silver ions into silver nanoparticles. A Nicolet IR100 instrument was used for FTIR spectroscopies. The SEM observations were carried out using KYKY (EM3200) Scanning Electron Microscopy for morphology identification. Dynamic light scattering measurements were detected by a Malvern zeta size analyzer instrument (Malvern 3600 Instruments Ltd., Malvern, UK).

Antibacterial activity

In vitro antibacterial activity of the prepared nanoparticles was evaluated using the Kirby-Bauer technique, which conformed to the recommended standards of the Clinical and Laboratory Standards Institute (CLSI). One species each of a Gram positive (Staphylococcus aureus) and Gram negative bacteria (Escherichia coli) were used for the antibacterial assay. The

sterile paper discs (6 mm) impregnated with 20 µL of biosynthesized AgNPs solution in normal saline (25, 50 and 100 µg/ml) and were left to dry at 30 °C for 24 h in an incubator. Several isolated colonies of bacteria were selected from a culture of 12–18 h on nutrient agar and dissolved in sterile saline which equal to suspension was adjusted to match the tube 0.5 McFarland standard (1.5×10^8 CFU/ml). The surface of MHA was completely cultured using a cotton swab which steeped in prepared suspension of bacterium. Finally, dried impregnated discs were placed on inoculated medium and incubated in 37 °C for 18–24 h. The diameter of zone inhibition was measured in millimeter, and was recorded as mean ± SD of the triplicate experiment. Gentamicin (10 µg) was used as positive standard for comparison purposes.

The minimal inhibitory concentration (MIC) of silver nanoparticles was examined using the standard broth microdilution method and was determined in BHI broth using serial two-fold dilutions of AgNPs in concentrations ranging from 100 µg/ml to 0.19 µg/ml with adjusted bacterial concentration at 1×10^6 CFU/ml. The positive control used in this study contained BHI broth medium with tested bacterial concentrations and negative control contained only inoculated broth and the time and temperature of incubation being 24 h and 37ºC respectively. The MIC was noted by the visual turbidity of the tubes both before and after incubation. After the MIC determination of the

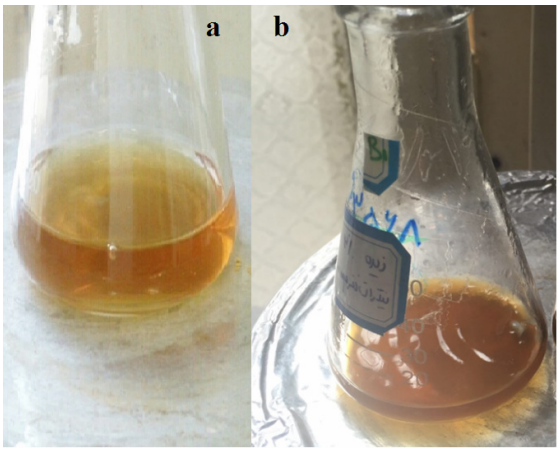

Fig. 1. a) Photograph of *Bunium persicum* fruit extract. b) AgNO3 with *B. persicum* fruit extract after 30 min.

AgNPs, aliquots of 20 µl from all microplate which showed no visible bacterial growth were seeded in BHI agar plates, which are not supplemented with AgNPs, were incubated for 24 h at 37ºC. The MBC was observed for presence or absence of bacterial growth in agar plates both before and after incubation. The MBC endpoint is defined as the lowest concentration of antimicrobial agent that kills 99.9% of the initial bacterial population. All of the experiments were triplicated.

RESULTS AND DISCUSSION

Green synthesis of silver nanoparticles is shown in

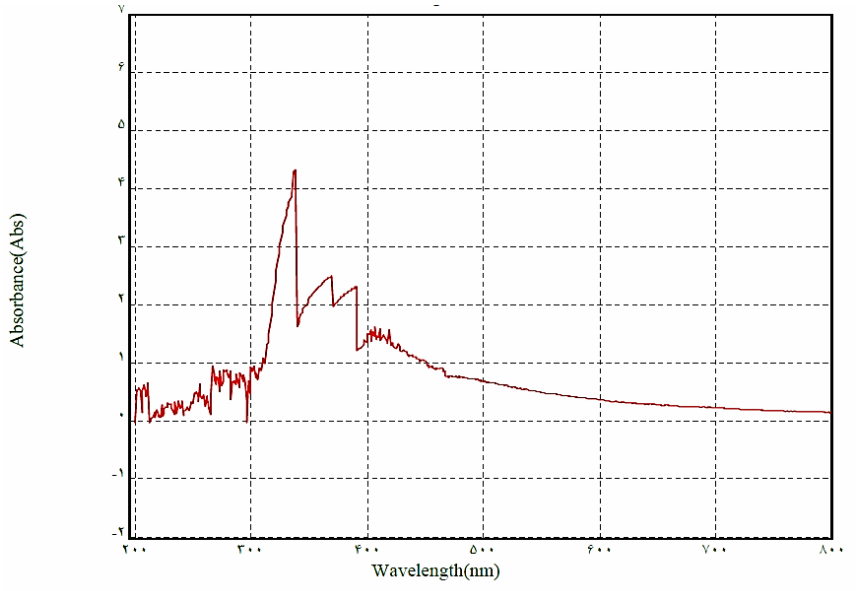

Fig. 2. UV–Vis absorption spectrum of silver nanoparticles.

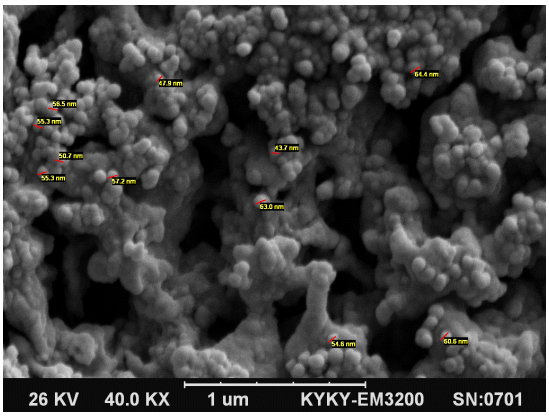

Fig. 3. SEM micrograph of the silver nanoparticles synthesized by B. persicum extract.

Fig. 1. The color change of solution was recorded through visual observation. The color of the reaction mixture changed from yellow to dark brown which indicated that the silver nanoparticles were yielded (Fig. 1).

UV-Vis spectral analysis was done and nanoparticle solution showed maximum absorbance at 340 nm (Fig. 2). Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts was indicated absorbance peak at 420 nm (Krishnaraj, *et al.*, 2010) and Synthesis of Pomegranate Peel Extract Mediated Silver Nanoparticles showed absorbance maximum at 370 nm (Shanmugavadivu, *et al.*, 2014).

The SEM image reveals the formation of cluster of spherical silver nanoparticles synthesized by B. persicum fruit extract with high density. The images of Scanning Electron Microscopy showed that shape of synthesized AgNPs is spherical. (Fig. 3).

Results of the FT-IR study of biosynthesized AgNPs showed sharp absorption peaks located 3388, 2922, 1598, 1404 and 1257 cm^{-1} (Fig. 4). The band at 3388 cm^{-1} was assigned to the stretching vibration of OH groups of alcohol. The peak at 2922 cm^{-1} related to the stretching vibration of aliphatic C-H groups available in extract, as well as peaks in 1598 cm^{-1}, 1404 cm^{-1} and 1257 cm^{-1} related to the stretching vibration of alkenes and bending vibration of C-H (Fig. 4). The synthesized nanoparticles were characterized by FT-IR to identify functional groups and organic compounds in the surface of particles. Peaks obtained were consistent with results of Jayapria & Lalitha in 2013.

The mean average sizes of nanoparticles were 21.3 nm as shown in DLS histogram (Fig. 5). Poinern *et al.* in 2013 synthesized spherical AgNPs with 10-100 nm size using leaf extract of Eucalyptus macrocarpa.

Antibacterial activity of synthesized silver nanoparticles was studied against E. coli and S. aureus by disk diffusion method. The results of the study presented in Table 1. It was observed that microbial growth of E. coli was independent on AgNPs concentration (25, 50, 100 µg/ml). The zone of inhibition For E. coli ranged from 12±0.1 to 17±0.5 mm and for S. aureus 10±0.5 to 13±0.3 mm. Ahmed *et al.* in 2016 studied the antibacterial effect of nanoparticles synthesized by the plant Azadirachta indica leaf extract on Staphylococcus aureus and Escherichia coli and 9 mm reported as inhibition zone diameter in both bacteria (Ahmed, *et al.*, 2016).

The minimum inhibitory concentration (MIC) was determined as the lowest concentration of silver

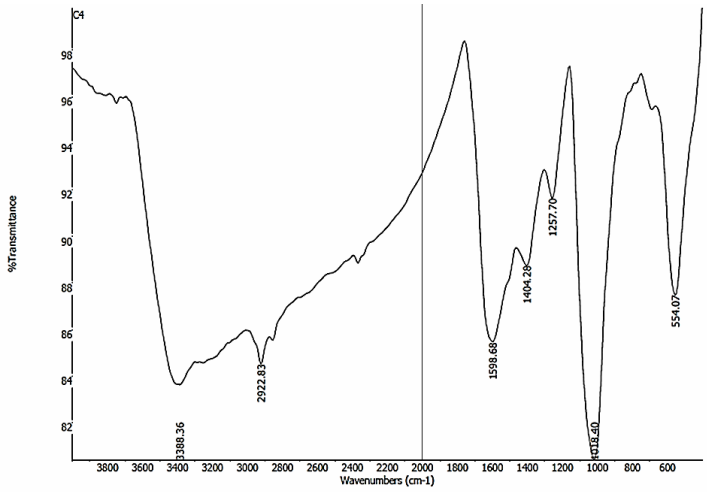

Fig. 4. FT-IR of dried silver nanoparticles synthesized by B. persicum fruit extract.

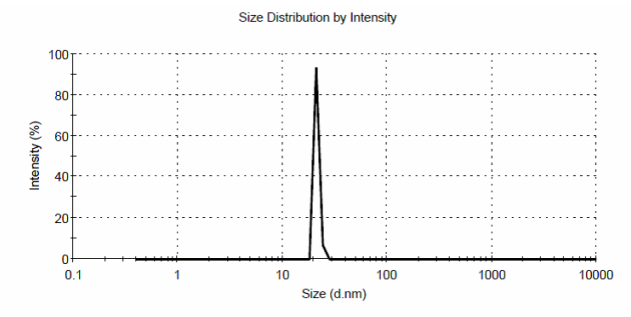

Fig. 5. Histogram of dynamic light scattering (DLS).

nanoparticles that inhibited the visible growth of E. coli and S. aureus. It was found that the MIC for E. coli was 0.78 µg/ml and for S. aureus was 1.56 µg/ml. MBC is defined as the lowest concentration of antimicrobial agent that will prevent the growth of microorganism after culture. The MBCs of silver nanoparticles were found to be 1.56 and 3.12 µg/ml, respectively in E. coli and S. aureus. According to the results, E. coli was showed more susceptible to silver nanoparticles compared to S. aureus which may be due to the variation in cell wall composition. The cell wall of Gram positive bacteria composed of a thick peptidoglycan layer, consisting of linear polysaccharide chains cross linked by short peptides, thus forming more rigid structure leading to difficult penetration of the silver nanoparticles (Shrivastava, *et al.*, 2007).

It is well known that few medicinal plants exhibit anti-oxidant property. Thus they can act as biological source of reducing agent. On this belief choice of plants for this purpose were those carrying medicinal and aromatic properties. B. persicum plant has traces of antioxi-dants and polyphenols. The main compounds of B. persicum fruit oil was included γ-terpinene, cuminaldehyde, ρ-cymene, and γ-terpinen-7-al respectively (Rustaie, *et al.*, 2016). This Phytochemical compounds within the plant result in the effective reduction of silver salt to AgNPs. Phenolic compounds possess hydroxyl and carboxyl groups which are able to bind metals (Nazeruddin, *et al.*, 2014).

CONCLUSIONS

The present green synthetic method for the synthesis of silver nanoparticles from silver nitrate using B. persicum fruit extract is an easy, economical, rapid and eco-friendly way to synthesize metallic nanoparticles and is a better alternative to chemical synthesis. This method produced spherical nanoparticles, with average particle size of 21.3 nm. The results showed that synthesized silver nanoparticles possess significant antimicrobial activity against the selected pathogenic microorganisms.

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Table 1. Average of inhibition zones, MIC and MBC silver nanoparticles synthesized (AgNPs) by B. persicum fruit extract.

	Inhibition zone (mm)				MIC (mg/ml)	MBC (mg/ml)
	Gentamicin (10 µg)	AgNPs (25 µg/ml)	AgNPs (50 µg/ml)	AgNPs (100 µg/ml)		
E. coli	18±0	12±0.1	14±0.2	17±0.5	0.78	1.56
S. aureus	15±0.1	10±0.5	11±0	13±0.3	1.56	3.12

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