



Green Synthesis and Investigation of Antibacterial Activity of Silver Nanoparticles Using *Eryngium bungei* Boiss Plant Extract

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Abstract

Metal nanoparticles synthesis using plant extracts and the study of their medicinal effects is a relatively new topic. Many chemical techniques have been proposed for the synthesis of silver nanoparticles (AgNPs), but green synthesis has a special place due to its clean and non-toxicity. *Eryngium bungei* Boiss plant was selected for this study due to its reducing properties and medicinal properties. The effect of various factors including concentration of silver nitrate, contact time and pH were investigated to reach optimum conditions. Silver nanoparticles were synthesized by the reaction between extract and silver nitrate after 20 min at room temperature and pH 12. The obtained nanoparticles were analyzed by XRD, UV–Vis, FESEM, EDX analysis to determine the crystals, morphology, identification of constituents, identification of unknown substances and particle distribution, respectively. The absorption of silver nanoparticles at 438 nm was measured using spectrophotometer machine. Particle size, morphology and antibacterial activity against standard strains of *Escherichia coli* (*E. coli*; ATCC® 25922TM), *Klebsiella pneumoniae* (*K. pneumoniae*; PTCC® 700603TM) and *Staphylococcus aureus* (*S. aureus*; PTCC® 16538TM) were investigated and the results showed that the synthesized silver nanoparticles have antibacterial properties. Due to the antibacterial results, biosynthesized silver nanoparticles enhanced antibacterial efficiency against *S. aureus*, *K. pneumoniae* and *E. coli* bacteria with MIC value of 27.34, 27.34, 6.83 µg/ml and MBC value of 54.68, 109.3 and 13.67 µg/ml, respectively. The results of this study showed that the synthesis of silver nanoparticles can be performed using the extract of *Eryngium bungei* Boiss and the synthesized nanoparticles by this extract have effective antibacterial effects.

Keywords Green synthesis · Silver nanoparticles · *Eryngium bungei* Boiss · Antibacterial activity

Introduction

In the recent years, nanotechnology is growing rapidly and has far-reaching effects in various fields such as agriculture, medicine, pharmacology, environmental and industry [1–4]. In this regard, the use of nanoparticles whose size is in the range of 1 to 100 nm, due to their unique electrical, optical, mechanical and magnetic properties has received more attention in various areas such as environmental health, chemical industry, energy science, optics, electronics, biomedical and optical-electricity sciences [5–10]. The main methods

of nanoparticle synthesis include physical, chemical and biological methods which physical and chemical methods have been less used due to high costs, high energy consumption, and use of toxic reducing agents and even production of toxic waste. Biological or green synthesis methods are more important due to their environmental compatibility and cost-effectiveness [11, 12]. Plant extracts, fungi and bacteria are the three main methods for synthesizing nanoparticles biologically [13–17]. It has been proven that there is an inverse relationship between particle size and surface to volume ratio of nanoparticles, so the surface energy of the nanoparticles and the catalytic reaction increase intermittently, causing the biological effect of the nanoparticles to increase proportionally [18].

In the green synthesis method, metal ions can be converted into nanoparticles using plant extracts. Plant extracts contain chemicals such as enzymes, retinoic acid, cyclic peptides, terpenoids, tannins, proteins, amino acids, polysaccharides, ascorbic acid, polyphenols and more. Polyphenols

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in plant extracts have antioxidant properties that play an important role in providing reducing and covering agents in the green synthesis of nanoparticles and also improve the performance of the nanoparticles [19, 20]. Also, due to the prevention of cell culture and extensive storage processes, the use of plants in green synthesis has received more attention than microorganisms [21]. Many studies have been conducted on the green synthesis using plant extracts and the use of metal nanoparticles such as Ag, Au, Zn, Fe and Cu which can be attributed to Suman's research on the green synthesis of gold nanoparticles using *Morinda citrifolia* extracts. In the mentioned study, gold nanoparticles were absorbed at 540 nm and the anti-diabetic effect of nanoparticles was investigated [22]. Also, the green synthesis of silver nanoparticles was performed using the extract of *Annona squamosa* plant by Vivek which the absorption of silver nanoparticles at 444 nm was measured using a UV spectrometer machine and the effect of these nanoparticles on MCF-7 human breast cancer cells has been investigated [23]. Various factors can affect the rate of synthesis and properties of nanoparticles, including plant extract concentration, metal concentration, pH, temperature and contact time [24].

Nowadays, noble colloidal metal nanoparticles such as silver, platinum and gold have received more attention due to their size, shape and tendency to accumulate. Among these, silver nanoparticles are more used due to their antimicrobial properties [25]. Silver can react with skin moisture and wound fluid and ionize, although its antibacterial properties depend on the amount of silver and the amount of silver released. Due to its high reactivity, silver ions can cause changes in the cell wall structure of bacteria and nuclear membranes and eventually lead to cell death [26]. In various studies, the antibacterial effects of silver nanoparticles have been studied, including the research of Nagsh et al. which had the antibacterial effects of silver nanoparticles have been investigated using *Eucalyptus* plant extract. The effect of silver nanoparticles on *Escherichia coli* was examined at concentrations of 25, 50, 100, 200, 300, 400 and 500 mg/l and it was concluded that the most suitable concentration of silver nanoparticles for the growth inhibitory effect of *Escherichia coli* has been 25 mg/l [27]. Parthiban et al. also investigated the effect of silver nanoparticles on control of *Aedes aegypti* mosquito larvae population and found that a concentration of 20 µg/l of nanoparticles synthesized by *Annona reticulata* leaf extract could kill 100% of this larval population [28].

In the present study, the synthesis of silver nanoparticles was performed in a bio-friendly way without the use of harmful chemicals by methanolic extract of *Eryngium bungei* Boiss plant. This plant belongs to the Apiaceae family, which grows in most of the foothills of South Khorasan Province (Iran). This plant is rich in tannins, alkaloids, saponins and flavonoids. Various extracts of the genus *Eryngium*

can have anti-inflammatory, anti-scorpion, anti-glycemic and anti-seizure effects [29]. *Eryngium bungei* Boiss has many properties including enhancing sexual power, digestion, treating flank, chest pain, treating inflammation, controlling blood sugar and crushing kidney stones. The leaves of this plant are also used to treat colds, asthma, cough, sinusitis and rheumatism. Therefore, this study was performed to investigate the antibacterial effect of silver nanoparticles synthesized by the extract of *Eryngium bungei* Boiss plant against Gram-positive and negative bacteria.

Materials and Methods

Materials

In the present study, silver nitrate for synthesis of nanoparticles and methanol (Merck Co. German) with 99.9% purity for preparation of plant extract was used. Also sodium hydroxide solution was used for pH adjustment. For antibacterial experiments, Mueller Hinton Broth and Mueller Hinton Agar (Merck Co. German) culture mediums were used.

Methanolic Plant Extract

In order to synthesize silver nanoparticles, *Eryngium bungei* Boiss plant was collected in spring from around the Birjand city (the capital of South Khorasan province, Iran) and transferred to laboratory. After rinsing with double distilled water, place at room temperature to dry. 20 g of dried *Eryngium bungei* Boiss was contacted with 400 ml of methanol for three days to prepare the extract. Then the obtained solution was passed from the Whatman paper No. 42 and a rotary apparatus was used to concentrate methanol and separate the extract.

Biosynthesis of Silver Nanoparticles

For synthesis of silver nanoparticles, 10 ml of extract with a concentration of 10 g/l was mixed with 10 ml of 15 mM silver nitrate (AgNO_3) solution and the sample was placed at room temperature for 20 min on a shaker at 200 rpm (Fig. 1). The color of mentioned solution changed to dark brown, which was a sign of the formation of silver nanoparticles ($\text{Ag}^+ \rightarrow \text{Ag}^0$) [30]. To optimize the concentration of silver nitrate, concentrations of 5, 10, 20 and 30 mM with 10 ml of *Eryngium bungei* Boiss plant extract were placed at constant temperature and pH for 20 min, respectively. For evaluation of the optimum pH, 10 ml of silver nitrate solution with optimized concentration with 10 ml of *Eryngium bungei* Boiss plant extract was brought to the pH of 10, 12 and 14 using a solution of sodium hydroxide (2 N) and was tested for



Fig. 1 Synthesis of silver nanoparticles (Ag-NPs) by *Eryngium bungei* Boiss extract

20 min at a constant temperature. Optimally, 10 ml of silver nitrate solution with optimized concentration with 10 ml of *Eryngium bungei* Boiss plant extract was placed on the shaker at 5, 20, 60 and 120 min at a constant temperature and pH. Finally, the absorption spectrum was taken using a two-beam spectrophotometer machine for all samples in the range of 200 to 500 nm. To obtain the nanoparticle precipitate, the optimized solution was placed in a centrifuge at 20,000 rpm for 20 min. The precipitate was washed twice with distilled water and methanol to wash the nanoparticles thoroughly four times, and the supernatant was discarded. The precipitate was placed in an oven at 60 °C for 24 h.

Antibacterial Activity

In this research, antibacterial activity of synthesized silver nanoparticles against gram-positive bacteria such as *Staphylococcus aureus* (*S. aureus*; PTCC@16538™) and Gram-negative bacteria such as *Klebsiella pneumoniae* (*K. pneumoniae*; PTCC@700603™) and *Escherichia coli* (*E. coli*; ATCC@25922™) were determined. To prepare the 0.5 McFarland standards: some bacterial colonies were first removed after 24 h by sterile looping near the flame and then dissolved in Muller Hinton Broth medium and incubated at 37 °C for 2 h. 96-well micro plate was used to determine the minimum inhibitory concentration (MIC) by dilution in liquid medium (Micro Broth dilution). All wells of the microplate were filled with 100 µl of Mueller–Hinton Broth including diverse concentrations of silver nanoparticles (200 µl) at ambient temperature. Then, 100 µl of microbial suspension with a concentration of 10⁶ CFU/ml was added to each well. These biological experiments were performed in triplicate. MIC assay was determined visually as the

lowest concentration without visible growth. To obtain the minimum bactericidal concentration of biosynthesized silver nanoparticles, we discharge 10 µl of the well that reported as MIC along with three higher wells (higher concentration) on the blood agar medium. At that concentration where no growth of bacteria was observed, we declare it as the minimum bactericidal concentration (MBC).

Results and Discussion

Determination of Nanoparticle Characterization

The following methods were used to determine the properties of synthesized silver nanoparticles:

UV–Vis Analysis

Results of the UV–Vis spectra analysis of green synthesized silver nanoparticles (colored solutions) are shown in Figs. 2, 3 and 4. Characteristic peaks originating from surface plasmon resonance of AgNPs solutions confirmed the preparation of silver nanoparticles (about 420 nm). Different parameters like pH (10, 12 and 14), AgNO₃ concentration (5, 10, 20, and 30 mM) and temperature (5, 20, 60, and 120 min) have significant effect on shape and size of products. So, the morphology and grain size of samples were optimized and observed by UV–Vis spectrum. The UV–Vis absorption spectra of the silver nanoparticles with various concentrations of silver nitrate solution of 5, 10, 20, and 30 mM are recorded and shown in Fig. 2. When the concentration of Ag solution increased (5 mM to 10 mM), the intensity of the SPR bands increased and the absorption band shifted

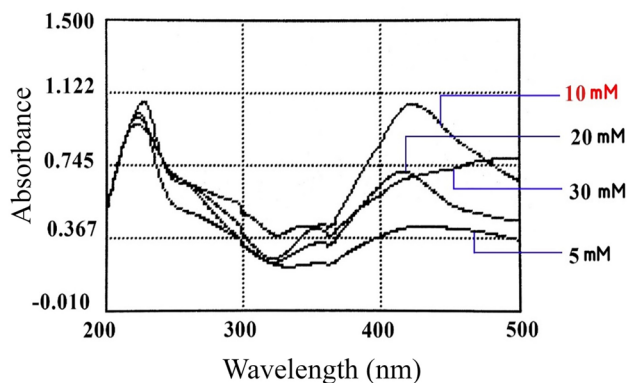


Fig. 2 UV–Vis absorption spectra of AgNPs: optimization of AgNO_3 concentration

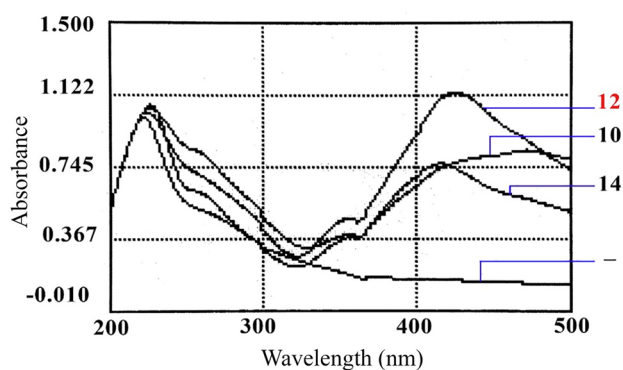


Fig. 3 UV–Vis absorption spectra of AgNPs: optimization of pH

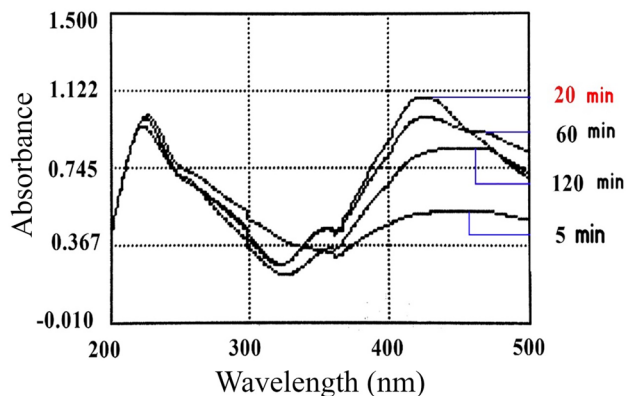


Fig. 4 UV–Vis absorption spectra of AgNPs: optimization of time (min)

to shorter wavelengths (blue shift). This displacement can be due to the small size of biosynthesized silver nanoparticles. In contrast, when molar concentration of silver solution increased to 30 mM, the intensity of SPR band decreased. This decline could be owing to aggregation of synthesized

nanoparticles [31, 32]. Past studies have shown that SPR bands intensity and reaction solution color were pH-dependent. Figure 3 illustrates the influence of pH on the absorption spectra of green synthesized silver nanoparticles. The SPR band intensity improved with increasing of pH from 10 to 12. Due to the UV–Vis results, the optimum pH reaction condition was pH 12. At this pH, low aggregation and small size were features of green synthesized silver nanoparticles. As the pH increased (to pH 14), the intensity of the adsorption band decreased, which could be due to the accumulation of particles. The reaction among silver ion and *Eryngium bungei* Boiss extract as capping, reducing and stabilizing agents was followed at various times (5, 20, 60 and 120 min). Figure 4 illustrates the UV–Vis spectra of synthesized silver nanoparticles after different reaction times. The UV–Vis absorption spectrum recorded (after 60 and 120 min) illustrated a sharp decrease in the SPR absorption which indicating the silver nanoparticles synthesis reaction tended towards aggregation. So, best time for the synthesis of silver nanoparticles being 20 min. As the results showed, the size and aggregation of the synthesized nanoparticles were reduced (from 5 to 20 min).

X-ray Diffraction (XRD)

XRD is a method of studying the structure of crystalline materials. X-ray diffraction (Philips X'pert PRO, The Netherlands) was used for fuzzy analysis and to study the grain size and nanomaterials. This technique can be performed by processing and analyzing the reflected X-ray from the sample surface. The diffraction peaks related to the (111), (200), (220) and (311) crystallographic planes that are relevant to 2θ values of 38.3° , 44.7° , 64.4° and 77.2° , respectively (Fig. 5). This illustrates that the biosynthesized AgNPs are faced centered cubic in nature. Three extra weak peaks at $2\theta = 28.18^\circ$, 32.24° and 46.57° were recognized to AgCl impurity as depicted at the diagram. These impurities were related to the presence of chlorine ions in the solution, which reacted with silver nitrate, leading to the formation of silver chloride precipitate. Our result was in agreement with previous studies [33].

Energy Dispersive X-ray spectroscopy (EDS)

The elemental analysis (EDS) recorded from the synthesized silver nanoparticles in presence of *Eryngium bungei* Boiss are shown in Fig. 6. The energy dispersive X-ray spectroscopy analysis illustrates a strong silver signal along (3 keV) with weak oxygen and carbon peaks. O and C are most likely associated with the organic compounds from the *Eryngium bungei* Boiss extract absorbed on the surface of silver nanoparticles.

Fig. 5 XRD pattern of biosynthesized silver nanoparticles

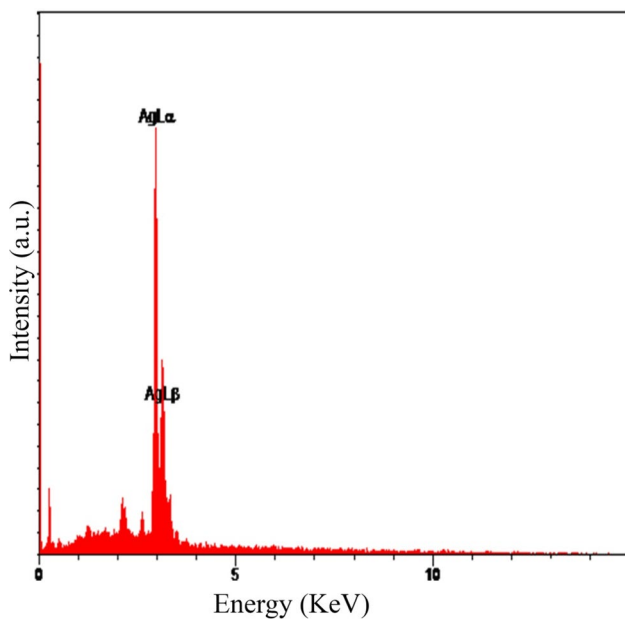
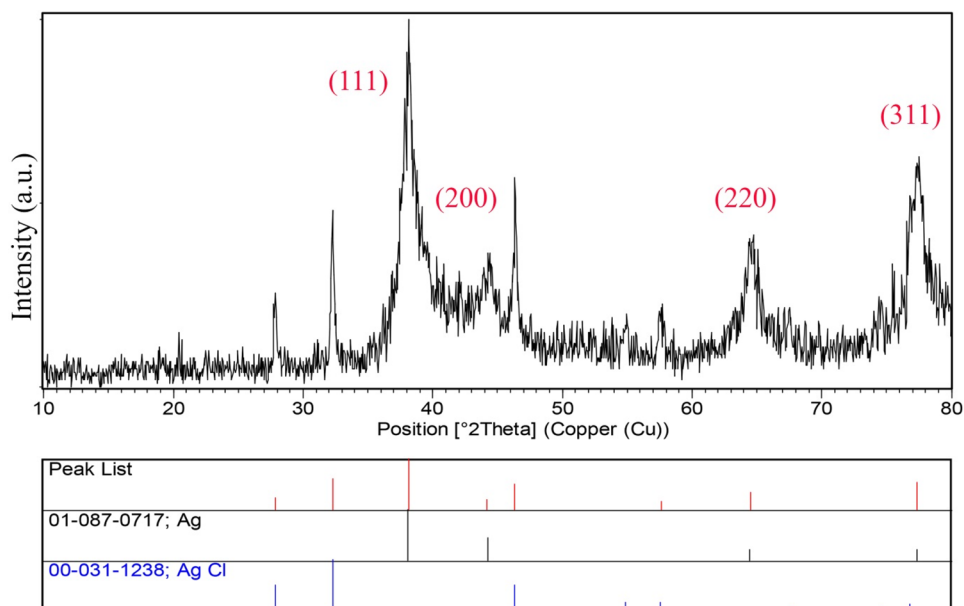


Fig. 6 EDS spectrum of AgNPs from *Eryngium bungei* Boiss extract

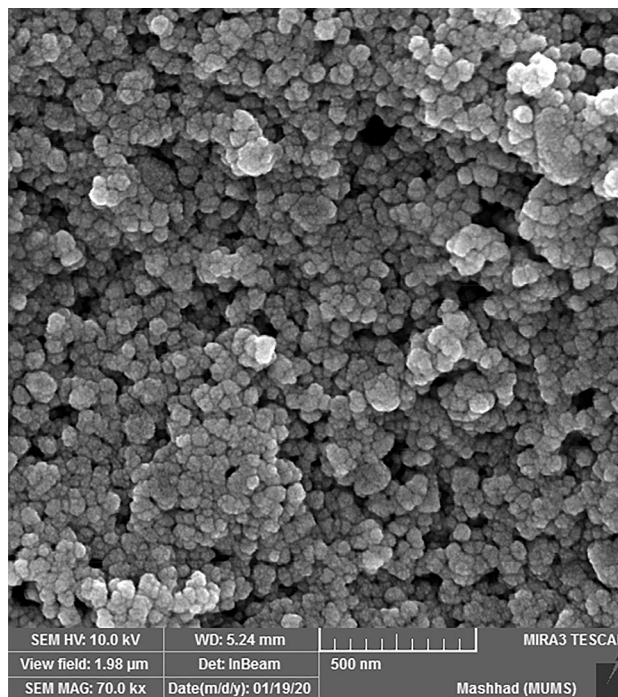


Fig. 7 Surface morphology of synthesized silver nanoparticle from the aerial extracts of *Eryngium bungei* Boiss

Field Emission Scanning Electron Microscopy (FESEM)

The grain size and morphology of the green synthesized silver nanoparticles in presence of natural extract was performed by FESEM technique (operated at 10.0 kV; JSM 6380 A Jeol, Japan). The FESEM results of the biosynthesized silver nanoparticles are shown in Fig. 7. The image obtained by the SEM demonstrated oval-like and spherical-like shape of silver nanoparticles from aerial extracts of *Eryngium bungei* Boiss being applied as stabilizing, capping and reducing agents. In the present study, the particles size

of the as-obtained silver nanoparticles was found to be in the range of 60–80 nm.

Antibacterial Activity

In present study three series of ATCC strains including Gram positive and Gram negative bacteria were selected to

Table 1 Results of MIC and MBC of bacteria for silver nanoparticles synthesized from *Eryngium bungei* Boiss extract

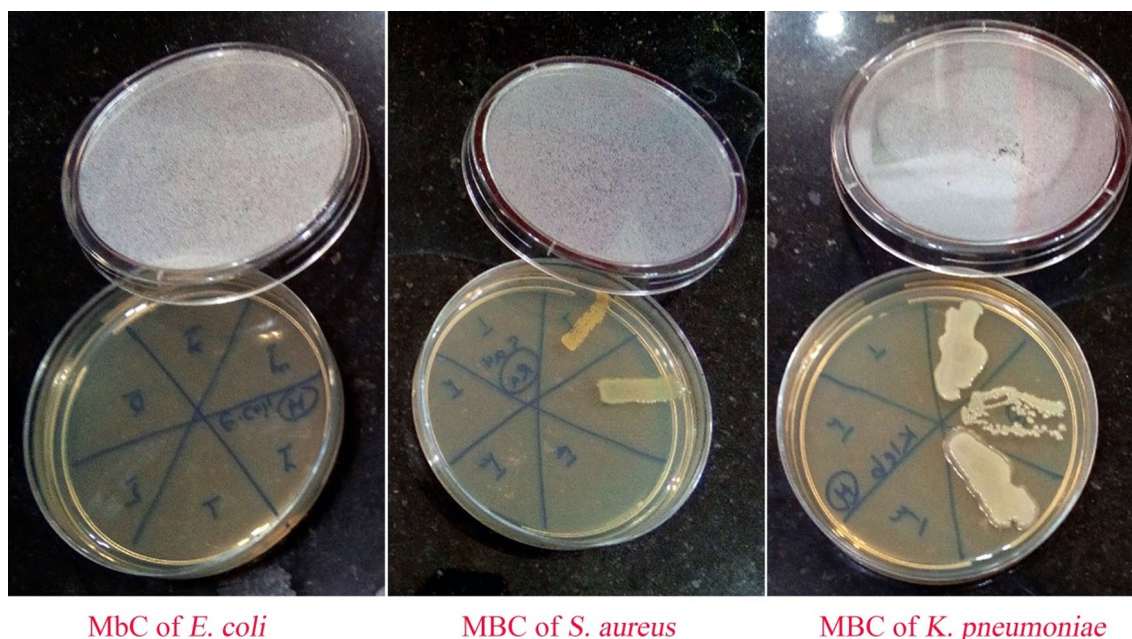
Bacteria	MIC ($\frac{\mu\text{g}}{\text{ml}}$)	MBC ($\frac{\mu\text{g}}{\text{ml}}$)
<i>Escherichia coli</i>	6.83	13.67
<i>Klebsiella pneumoniae</i>	27.34	109.3
<i>Staphylococcus aureus</i>	27.34	54.68

examining their susceptibility against synthesized AgNPs (Table 1). The biosynthesized silver nanoparticles from *Eryngium bungei* Boiss extract were analyzed for antibacterial activity against *S. aureus*, *K. pneumoniae* and *E. coli* strains. In continue of work, MIC three bacterial for ATCC strains with broth dilution method was determined. The antibacterial activity of AgNPs has demonstrated that as the concentration of AgNPs was increased the MIC for strains of bacteria decreased parallel. The study showed that high antibacterial activity was revealed against ATCC strains at very low concentration of AgNPs (in $\mu\text{g}/\text{ml}$). The antibacterial effects of the extract which tested separately no significant inhibition of this extract and consequently the bacterial growth has occurred in all wells. The MIC and MBC values in this research are listed in Table 1. The greater antibacterial activity could be due to the amount of silver. The results of antibacterial activity showed that higher antibacterial activity was revealed against *E. coli* with MIC value of 6.83 $\mu\text{g}/\text{ml}$, while intermediated antibacterial activity was observed against *S. aureus*, *K. pneumoniae* with MIC values of 27.34

and 27.34 $\mu\text{g}/\text{ml}$, respectively. In addition, minimum bactericidal concentration values for *S. aureus*, *K. pneumoniae* and *E. coli* were 54.68, 109.3 and 13.67 $\mu\text{g}/\text{ml}$, respectively (Fig. 8). In our opinion, the possible mechanism for killing bacteria can be as follows: In the first stage, silver ions enter the bacterial cell (via cell wall destruction). In the next step, the DNA is converted to a concentrated form which in turn prevents DNA replication. This process finally leads to cell death.

Conclusion

In this research, a eco-friendly and inexpensive method for green synthesis of silver nanoparticles using *Eryngium bungei* Boiss extract as reducing and capping agents were carried out. The nature of biosynthesized silver nanoparticles was characterized with various techniques including FESEM, XRD, EDS and UV/Vis. FESEM image showed the formation of silver nanoparticles with a mean diameter of 60–80 nm. Biosynthesized AgNPs illustrated high antibacterial activity against *E. coli*, *S. aureus* and *K. pneumoniae* microorganisms with MIC values of 6.83, 27.34 and 27.34 $\mu\text{g}/\text{ml}$, respectively.

**Fig. 8** Photographs of MBC tests of various bacteria

Acknowledgements In this investigation, the entire procedures were conducted according to the Helsinki Declaration and ethical standards of the institutional research committee. The ethics code was taken from Birjand University of Medical Sciences (Grant No. IR.BUMS.REC.1399.205).

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