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Green Synthesis, Characterization and Antibacterial Activity of Silver Nanoparticles Using Fruit Aqueous and Methanolic Extracts of *Berberis vulgaris* and *Ziziphus vulgaris*

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In the present study biosynthesis of silver nanoparticles (SNPs) by *Berberis vulgaris* and *Ziziphus zizyphus* fruit extracts was examined and antibacterial effect of the produced nanoparticles against some bacterial pathogenic strains was investigated. For this reason, the fruit aqueous and methanolic extracts of barberry and jujube were prepared and subjected to the silver nitrate solution at the final concentration of 1 mM. After nanoparticles production, all the color changed reaction mixtures were analyzed through visible spectrophotometer, X-ray diffraction analysis (XRD) and transition electron microscope (TEM). Finally the antibacterial effect of the produced nanoparticles was investigated by agar well diffusion method. Results showed that after nanoparticles production, the color of the plant extracts was converted to dark brown. Visible spectra of all the color changed extracts had maximum absorption peaks around 420-440 nm wavelength. Furthermore, presence of the SNPs was confirmed by XRD. TEM analysis revealed that the obtained SNPs were spherical in their shapes and their average sizes were around 5-50 nm. Antibacterial assays revealed that the produced nanoparticles in comparison with the control and the pure fruit extracts had more effective antibacterial activity. Moreover, statistical analysis has demonstrated that the antibacterial effects of these nanoparticles against all of the tested bacterial strains have showed similarity.

Key words: *Berberis vulgaris* fruit extract, *Ziziphus zizyphus* fruit extract, Silver nanoaprticles, biosynthesis.

Nanotechnology is one of the most attractive research area that deals with the tiny materials that their sizes are usually less than 100 nm. In this area, the nanoparticles exhibit different properies because of their higher surface area in contrast to their bulk materials. Among different metal nanoparticles, silver one in contrast to it's bulk material has found various atributes in the medicinal field because it has better antibacterial (Rai *et al*., 2009), anti fungal (Wiley *et al*., 2006), anti viral (Nadworny *et al*., 2008), anti-angiogenesis (Rogers *et al*., 2008), anti-inflammatory (Panacek *et al*., 2009) and anti platelet (Gurunathan *et al*., 2009) activities. Although there are some different chemical and physical methods for nanoparticles production (Goia & Matijevi cacute; 1998) there is still a need for environmental friendly, compatible with the human body and clean strategy for nanoparticles production. In the chemical method of nanoparticles production, the use of the toxic chemical reagents, releasing of the toxic byproducts and sometimes remaining of the toxic reagents on the surface of the synthesized nanoparticles are it's deficiencies for medical applications. Moreover, difficulties in the

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purification, size control and repression of the aggregation of the nanoparticles are the other reasons for the need of an alternative way. So recently green approach of nanoparticles production is emerged (Begum *et al*., 2009). In this approach, by the use of some plant extracts or microorganisms, nanoparticles are produced. This strategy is simple, fast and does not use high energy and toxic ingredients. Furthermore, plant extracts and microorganisms have some different proteins that are act as capping agents and consequently the nanoparticles production process can be scaled up. Among different organisms that are used for nanoparticles production, the use of the plant extracts has some advantages over the use of microorganisms such as they do not need culture media or other difficulties that are present in the use of microorganisms for nanoparticles production such as using the aseptic condition. Moreover, the use of plant extracts other than microorganisms for nanoparticles production is more acceptable (Veerasamy *et al*., 2011). So in the present study the use of the two medicinal important herbs for silver nanoparticles (SNPs) production was examined.

Ziziphus vulgaris known as Jujube, is a native tree of the subtropical and warm-temperate regions such as Australia, Asia, Middle East, Mediterranean, South Europe, North Africa and tropical America (Yossef *et al*., 2011). This plant is a member of *Rhamnaceae* family in the *Rosales* order. *Ziziphus* genus consists of 100 different species that one of them is named *vulgaris* (Abalaka *et al*., 2010). Different parts of this species are used for treatment of fever, bronchitis, pharyngitis, diabetes, liver dysfunctions and different bacterial infections. The extract of this species has different types of proteins, flavonoids, triterpenoids, alkaloids, saponins, lipids, free sugar and mucilage (Adzu *et al*., 2003). *Berberis vulgaris* is a member of *berberidaceae* family in the order of *Ranunculales*. This plant is known as barberry, is growing in Asia and Europe. Different parts of this plant such as root, leaf, fruit and bark are used for treatment of gastrointestinal disorders such as colitis and diarrhea and liver dysfunction. It has different types of alkaloids that one of them is named berberine. Some important properties of this component are anti-tumor, antimicrobial and anti-inflammatory effects. Moreover other component of this herb are berbamine, palmatine, glucoside, stigmasterol, stigmasterol, terpenoids lupeol and oleanolic acid (Hermenean *et al*., 2012).

In this article, the ability of SNPs production by the fruit aqueous and methanolic extracts of *Berberis vulgaris* and *Ziziphus vulgaris* was examined. After that the antibacterial activity of the obtained naoparticles and plant extarcts were tested against some bacterial pathogenic strains.

MATERIALS AND METHODS

Preparation of fruit extracts

Fruit aqueous extracts

The fresh fruits of *Berberis vulgaris* and *Ziziphus vulgaris* were obtained and the seeds and membranes of *Ziziphus vulgaris* were brought out. Then the fruits were washed twice with distilled water and 50g of them were boiled in 200mL of sterile distilled water for 10 min. The obtained extracts were filtered through *Whatman* filter paper (Sigma Aldrich, USA), freeze dried and kept in dark condition at 4°C until the experiments were started (Nanda & Saravanan; 2009).

Fruit methanolic extracts

The air-dried and powdered fruits of *Berberis vulgaris* and *Ziziphus vulgaris* samples were extracted by the method described previously by Kahkonen *et al*. Briefly, 100 g of each of the samples was extracted with methanol using a Soxhlet extractor at 60° C for 6 h. The obtained extracts were filtered through *Whatman* filter paper, freeze dried and kept in the dark at 4° C until the experiments were started (Kahkonen *et al*., 1999).

Silver nannoparticles synthesis

In order to production of the SNPs , 0.5g of each of the freeze dried extract was suspended in the 50mL of sterile distilled water and each of them was challenged with the 50µL of 1M silver nitrate solution at the final concentration of 1mM. All the mixtures were incubated at room temperature for 10 min. Production of the SNPs was observed by the formation of the brown-yellow color due to the surface plasmon resonance (SPR) of the SNPs (Pourali *et al*., 2013).

Characterization of the produced SNPs

Visible spectra analysis

Synthesis of the SNPs was detected by NanoDrop spectrophotometer (Thermo scientific, USA). The absorption spectra of the fruit aqueous and methanolic extracts were obtained by using spectrophotometer that was operated in 350–700nm wavelength. The aqueous and methanolic extracts without the silver nitrate ions were used as blank (Pourali *et al*., 2014).

Transmission Electron Microscopic (TEM) analysis

TEM images were obtained on a Ziess Leo 910 transmission electron microscope. For this aim, 10 µL of each sample was placed on the carbon coated copper grid and excess of the sample was removed by a blotting paper. The grid was dried under an infrared lamp. The accelerating voltage was 40–120 kV and images were taken by 0.4 nm resolution and a Gatan SC1000 camera (Pourali *et al*., 2014).

X-ray diffraction (XRD) analysis

In order to determine the biosynthesis of the SNPs, all the extracts containing SNPs were freeze-dried and analyzed by Philips Automatic X-ray Diffractometer. The diffracted intensities were recorded from 30° to 80° 2¸ angles (Pourali *et al*., 2014).

Antibacterial activity test

Antibacterial activity of the produced SNPs was examined by well diffusion method against some bacterial pathogenic strains. The pathogenic bacteria were as follow: *Staphylococcus aureus* (PTCC 1113), *Escherichia coli* (PTCC 1330), *Pseudomonas aeruginosa* (PTCC 1310) and *Bacillus cereus* (PTCC 1015). For the antibacterial test, the bacterial single colony was transferred to a tube of sterile normal saline and it‘s turbidity was compared to0.5 McFarland standard. After that, each of the obtained suspension was streaked by the sterile cotton swab over the entire surface of Muller Hinton agar (HiMedia, India) plate and five wells of 6 mm in diameter were made in the medium. On each plate, wells were filled with 50µL of fruit aqueous extract, fruit methanolic extract, fruit aqueous extract containing SNPs, fruit methanolic extract containing SNPs and 1 mM silver nitrate solution (since silver nitrate has the antimicrobial property). Finally, plates were incubated at 37°C for 24 hours and the growth inhibition zones were determined. All the experiments were done in triplicate and analysis of the obtained data was performed by one-way ANOVA in *SPSS* (Pourali *et al*., 2013).

RESULTS

Silver nannoparticles synthesis

Formation of the brown-yellow color was observed and confirmed the bioproduction of the SNPs. Figure 1 has showed changing in the color of the aqueous extract of *Ziziphus vulgaris* after formation of the SNPs.

Characterization of the produced SNPs

Visible spectra analysis

The obtained spectra for the both of the aqueous and methanolic fruit extracts of *Ziziphus vulgaris* and *Berberis vulgaris* revealed the formation of the SNPs. Both of the aqueous and methanolic fruit extracts of *Ziziphus vulgaris* and

Table 1. Antibacterial activity test of the aqueous and methanolic fruit extracts of *Ziziphus vulgaris* and *Berberis vulgaris* and the produced SNPs by each of them in contrast to the 1 mM silver nitrate solution

Type of the extract	Inhibition zones (mm) of the extracts against the tested bacterial strains* (Mean ± S.E.M)			
	E.coli	*P.aeruginosa*	*B. cereus*	*S. aureus*
Aqueous extract of *Z. vulgaris*	7.1 ± 0.33	7.33 ± 0.33	7.00 ± 0.00	7.33 ± 0.33
SNPs produced by aqueous extract of *Z. vulgaris*	7.33 ± 0.33	11.66 ± 0.88	9.33 ± 0.33	14.00 ± 0.57
Aqueous extract of *B. vulgaris*	7.00 ± 0.00	7.00 ± 00	7.66 ± 0.33	7.66 ± 0.33
SNPs produced by aqueous extract of *B. vulgaris*	8.33 ± 0.33	11.33 ± 1.20	10.00 ± 0.57	12.33 ± 0.88
Methanolic extract of *Z. vulgaris*	7.33 ± 0.33	7.33 ± 0.33	7.66 ± 0.33	8.00 ± 0.57
SNPs produced by methanolic extract of *Z. vulgaris*	11.00 ± 1.00	10.33 ± 0.66	12.33 ± 0.66	18.33 ± 1.20
Methanolic extract of *B. vulgaris*	7.00 ± 0.00	7.66 ± 0.33	7.66 ± 0.33	7.66 ± 0.33
SNPs produced by methanolic extract of *B. vulgaris*	9.33 ± 0.33	13.33 ± 0.66	13.33 ± 0.33	18.66 ± 1.85
Silver nitrate (1mM)	8.66 ± 0.33	8.00 ± 0.00	8.66 ± 0.33	8.33 ± 0.33

* Tests were done in triplicate

Berberis vulgaris had maximum absorption peaks around 410–450 nanometer due to the surface plasmon resonance (SPR) of the SNPs . Figure 2 shows visible absorption spectrum of the produced SNPs obtained from the aqueous extract of *Ziziphus vulgaris*.

Transmission Electron Microscopic (TEM) analysis

TEM analysis showed that the produced SNPs had spherical structures and their sizes were

Fig. 1. Color changing of the aqueous extract of *Ziziphus vulgaris* after formation of the SNPs. A: blank and B: extract containing SNPs.

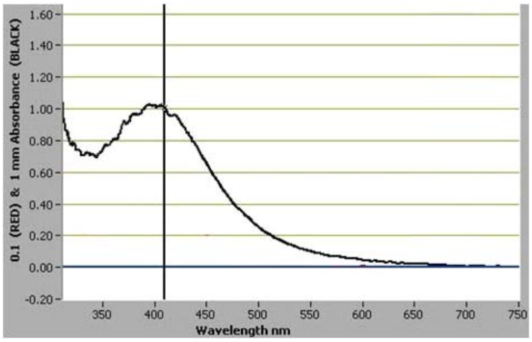

Fig. 2. Visible absorption spectrum results for the produced SNPs obtained from the aqueous extract of *Ziziphus vulgaris*

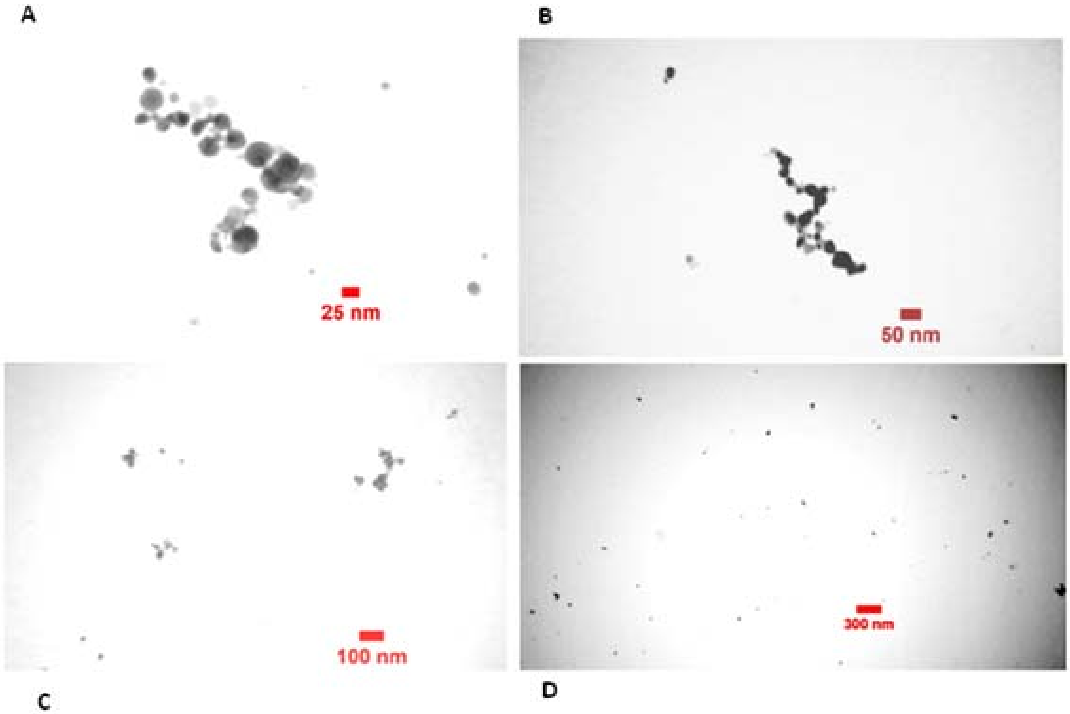

Fig. 3. TEM images of nanoparticles that were produced by both of the aqueous and methanolic fruit extracts of *Ziziphus vulgaris* and *Berberis vulgaris*. A: nanoparticles obtained from the methanolic extract of *Berberis vulgaris*. B: nanoparticles obtained from the methanolic extract of *Ziziphus vulgaris*. C: nanoparticles obtained from the aqueous extract of *Berberis vulgaris* and D: nanoparticles obtained from the aqueous extract of *Ziziphus vulgaris*

around 5–50 nm. Figure 3 shows the TEM images that were obtained from the both of the aqueous and methanolic fruit extracts of *Ziziphus vulgaris* and *Berberis vulgaris*.

X-ray diffraction (XRD) analysis

Results from the X-ray showed the presence of the sharp Bragg peaks at 2¸ values of 38.126°, 44.313°, 64.464°, and 77.424° confirming presence of the elemental silver in the both of the aqueous and methanolic fruit extracts. Figure 4 indicated the XRD result that was obtained from the aqueous fruit extract of *Ziziphus vulgaris*.

Antibacterial activity test

Antibacterial activity tests indicated that both of the aqueous and methanolic fruit extracts and the produced nanoparticles of each of them had antibacterial activity against all of the tested bacteria but antibacterial activity of the extracts containing nanoparticles were higher than the pure herb fruit extracts. Analysis of the obtained data showed that the SNPs produced by the aqueous plant extracts had the same antibacterial activity against all of the bacterial strains but the antibacterial activity of the SNPs produced by the methanolic extracts had greater antibacterial activity against *Bacillus cereus* and *Staphylococcus aureus* in contrast to *Escherichia coli* and *Pseudomonas aeruginosa*. Table 1 shows data that were obtained from antibacterial activity test.

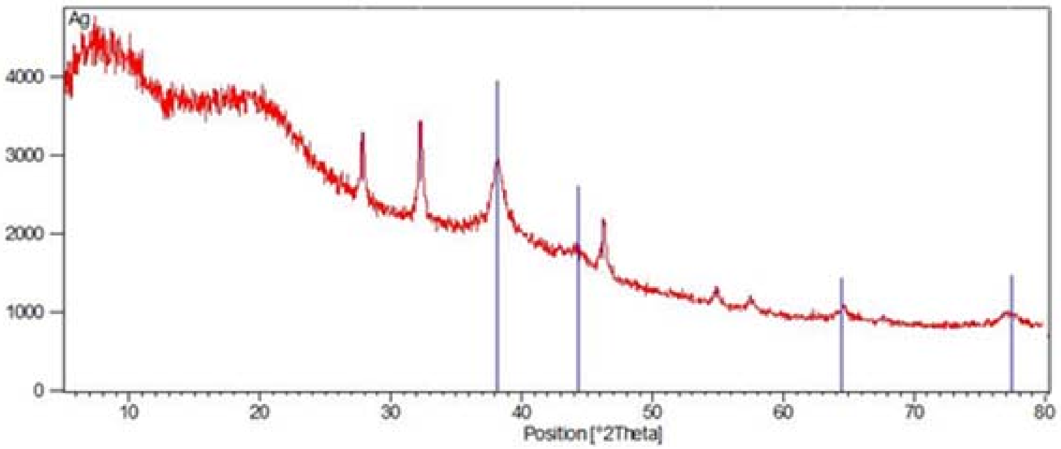

Fig. 4. The XRD result that was obtained from the produced nanoparticles by the aqueous fruit extract of *Ziziphus vulgaris*.

DISCUSSION

Although nanoparticles can be synthesized by various chemical and physical methods, the green synthesis approach of the nanoparticles production is nontoxic, environmental friendly, reliable and does not release any harmful by-products in the nature (Cao, 2004). There are different available organisms that were reported for green synthesis of nanoparticles and among them plant extracts have been used mainly to produce nanoparticles (Luangpipat *et al*., 2011). The use of the plant extracts for nanoparticles production is safe, eco-friendly, non toxic, simple, rapid and the most important is they are more acceptable by the society in contrast to the use of the bacteria or fungi for nanoparticles production. According to the importance of the nanoparticles production by plant extracts, in the present research the production of SNPs was done by using the fruit aqueous and methanolic extracts of two important Iranian medicinal plants: *Berberis vulgaris* and *Ziziphus vulgaris*.

There is reported that some components of the plants extracts such as alkaloids, proteins, enzymes, amino acids, alcoholic compounds, flavonoids, quinols, terpenoids, polyphenols, chlorophyll pigments and polysaccharides are responsible for the reduction and stabilization of the silver ions to SNPs (Kesharwani *et al*., 2009). It is also reported that *Berberis vulgaris* has several alkaloid component like berberine, berbamine and palmatine (Ivanovska & Philipov; 1996). Moreover, other molecules such as oleanolic acid, terpenoids lupeol, stigmasterol, stigmasterol glucoside (Saied & Begum; 2004), polyphenols (Imanshahidi & Hosseinzadeh; 2008) and some other active components that are responsible for the reduction of the silver ions to SNPs were identified in the extract of this plant. Among these active molecules berberine is the most important alkaloid that has many medicinal properties (Imanshahidi *et al*., 2008). Moreover, different parts of the *Ziziphus vulgaris* have various types of active components such as cyclopeptide, alkaloids, flavonoids, terpenoids and glycosides (Ali *et al*., 2006). The fruits of Z. vulgaris have mucilage, vitamin C, proteins, sugar and ziziphique acid that may be responsible for the nanoparticles production (Salehi; 2010).

In the first part of this research, the fruit aqueous and methanolic extracts of *Berberis vulgaris* and *Ziziphus vulgaris* were obtained and after adding the silver ions to each of them the SNPs production was detected by changing in the color of the extracts from yellow to brown-yellow. Production of the SNPs was confirmed by visible spectrophotometer, TEM and XRD analysis. The extracts containing SNPs had maximum absorption peaks around 410–450 nanometer due to the surface plasmon resonance (SPR) of the SNPs. The resulted nanoparticles were spherical in their shapes and the size of them were around 5-50 nanometer. After that, the antibacterial tests were done agains four bacterial pathogenic strains: *E.coli*, *P.aeruginosa*, *B. cereus* and *S. aureus.* The extracts alone as it was previousley reported by Manshahidi et al., had antibacterial activities (Imanshahidi *et al*., 2008).

Antibacterial activity tests for the extracts containing SNPs revealed that the produced SNPs had the same effects against all of the four bacterial strains tested. Several studies on antibacterial activity of SNPs were carried out and some of them reported that the SNPs had better antibacterial activity against Gram positive bacteria and some of them reported that they have better antibacterial activity against Gram negative ones (Kim *et al*., 2007) but according to Ruparelia *et al*., the susceptibility to the SNPs is depends on the bacterial species (Ruparelia *et al*., 2008).

Further studies are needed for better understanding the exact mechanism of the production and antibacterial activities of the produced SNPs by these two medicinal important plant extracts in the future.

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