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Original Research

Green synthesis of silver nanoparticles using *Salvadora persica* L. and its antibacterial activity

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Abstract: The silver nanoparticles (AgNPs) shows special physicochemical properties, therefore they use many applications such as catalysis, health, electronic and optical. In this study, AgNPs was synthesized using aqueous extract of *Salvadora persica* bark. The synthesized AgNPs were characterized by UV-Vis spectroscopy, Powder X-ray diffraction (PXRD) and Transmission electron microscopy (TEM) methods. The optimal synthesis condition to prepare nanoparticles was determined as silver nitrate 3 mM, 5 ml of aqueous extract in the room temperature for 1 h. The TEM image of AgNPs showed the formation of spherical, non-uniform nanoparticles of mean size of 50 nm. The antibacterial activity of synthesized AgNPs was evaluated using disk diffusion, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) methods on *Escherichia coli* and *Staphylococcus aureus* bacteria. The MIC values of AgNPs were 100 and 400 µg/mL on *E. coli* and *S. aureus*, respectively. Also the MBC of AgNPs was 200 µg/mL for *E. coli* and there was no result observed for *S. aureus* bacteria. The results showed that synthesized nanoparticles have favorable antibacterial properties.

Key words: AgNPs, *Salvadora persica,* MIC, *Escherichia coli*, *Staphylococcus aureus*, PXRD, TEM.

Introduction

The metal nanoparticles have been attracted many researchers and scientists due to their widely uses in industry and medicine (1, 2). In this way, silver nanoparticles (AgNPs) has been more considered because of their unique features. The studies have shown the AgNPs have catalysis (3, 4), photonics (5), photo-catalysis (6), antibacterial (7-10), antifungal (11-13) and anti-inflammatory (14-15) properties. In addition, recent studies of the AgNPs have been done in drug delivery (16-17), biosensors (18-19) and treatment of cancer (20-22). Therefore, finding an inexpensive, available and nontoxic method for producing AgNPs is one of the challenge in nanoscience, in this case green synthesis method has been more considered by researchers.

Herbs continue to be an extremely important resource due to their complex molecular compositions (23). The herbs produce ingredients through secondary metabolism and numerous of these derivatives (e.g., phenolic compounds, alkaloids, tannins, terpenoids, glycosides, coumarins, flavonoids and isoflavonoids) have various properties (24-30). *Salvadora persica* L. is a shrub belonging to *Salvadoraceae* and it grows in Asia and South Africa (31). In traditional medicine, it has used as abrasive, astringent, antiseptic, detergents and inhibitors of enzymes (32). The studies showed seeds, leaves and bark of the plant were containing sterols, fatty acids, tocopherols and phenolic compounds that these compounds have strong antioxidant properties (33). Flavonoids, saponins, tannins, sulfur, benzyl isothiocyanate, chloride, fluoride, calcium and silicon exist in the root, stem and leave of the *S. persica* (31). Khan *et al.* (2015) evaluated root extract of *S. persica* for bioreduction of graphene oxide and reported that *S. persica* can act as a bioreductor (34) and also they synthesized palladium nanoparticles using *S. persica* (35). Tahir *et al.* (2015) investigated the extract of stem of *S. persica* as a reducing and coating agent for synthesis silver nanoparticles (36). The aim of this study was to synthesis of AgNPs using *S. persica* bark aqueous extract and examination of its antibacterial activity.

Materials and Methods

Preparation of *S. persica* L. extract

The *Salvadora persica* bark was collected from Khash, Sistan and Baluchestan province, Iran. The *S. persica* bark was prepared by adding 50 mL distilled water to 5 g of plant powder (10:1 ratio). Mixture was shaken at 150 rpm for 6 h. The solution was filtered by Whatman No.1 and resulting clear brown solution was stored at 4 °C for future assays.

Preparation of silver nanoparticles

For the synthesis of AgNPs, 45 ml of 3 mM silver nitrate solution (Merck) was added to 5 mL of aqueous extract of *S. persica*. The mixture was shaking at room temperature for 1 h. The silver nanoparticles formation confirmed by creation brown colloid solution (Figure 1).

Antibacterial activity studies

Disk diffusion method

The Antibacterial activity of AgNPs was evaluated using disk diffusion assay against *Escherichia coli*

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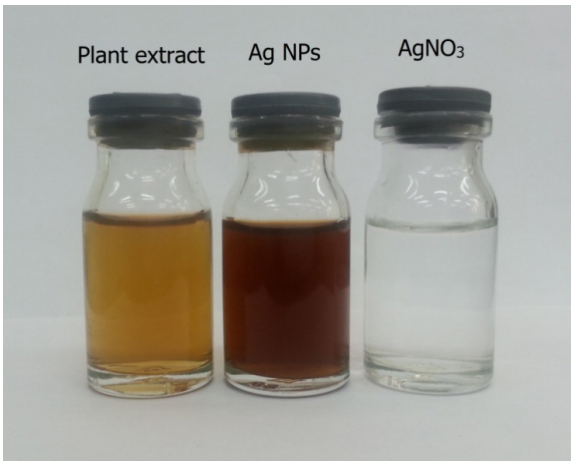

Figure 1. The change of solution color from light brown to brown after 1 h of the reaction, indicating the formation of AgNPs.

(ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) according to standard methods (37). The suspension of bacteria was prepared according to standard of 0.5 McFarland equivalents to 1.5×10^8 colony-forming units/mL. The bacteria cultivated on Nutrient agar plates. In each plate placed sterilized paper disks were impregnated with 10 µL AgNPs and aqueous plant extract. Also Gentamicin and Streptomycin antibiotic disks (Padtan Teb Co) used as positive control. All plates were incubated at 37 °C for 24 h. The inhibition zones diameters were measured (Table 1). The experiments carried out in triplicate.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Minimum inhibitory concentration (MIC) of samples (AgNPs and extract) were determined by the microdilution assay in 96 multi-well microtiter plates, according to the standard procedure of the Clinical and Laboratory Standards Institute (37). Streptomycin and Gentamicin were used as positive control; distilled water and aqueous extract were used as blank. The samples were diluted with 100 µL of Mueller-Hinton broth (Merck) and then added 100 µL tested bacteria in each well and microplates were inoculated at 37 °C for 24 h. The MICs were determined as the lowest concentration that inhibited the visible growth of the bacteria. To determinant of minimum bactericidal concentration (MBC) of samples, 100 µL of solution from each well of microbroth assay was sub-cultured on MH agar plates and incubated at 37 °C for 24 h. The lowest concentration of synthesized AgNPs that resulted in no bacterial growth was considered as MBC.

Characterization of AgNPs

The synthesized AgNPs solution (1:4 diluted) was characterized by measuring the UV-vis spectrum. The UV–vis spectrum was recorded on double beam spectrophotometer (Cecil, model CE 7250, England) from 300 to 900 nm. The double distilled water containing the extract was used as a blank. The reflection planes of AgNPs was observed in PXRD diffraction pattern (PANalytical, model X'pert, Cu Kα radiations (k = 1.5418 Å, Netherlands). The size and morphology of synthesized AgNPs were determined using TEM (Zeiss, model EM 900, Germany).

Results and Discussion

In the study, aqueous extract of *S. persica* bark was used to synthesize AgNPs. The formation of AgNPs was investigated by the observation of the change in the color of the solution. The appearance of brown color in solution confirmed the AgNPs formation (Figure 1). The change of color of extract after adding silver nitrate solution, from light brown to brown is observed within 60 min. In case metal nanoparticles, creation color in reaction solution is due to excitation of surface plasmon vibrations (38). The color change is important evidence for synthesis of AgNPs (39). As a result of reactions of *S. persica* bark extract that involve reducing agents such as tannins, flavonoids, alkaloids, enzymes and other reducing factors with solution salts at room temperature, the silver ions reduced and their electrical charge became zero and finally the AgNPs were produced (40).

UV-vis spectroscopy

The UV-vis spectroscopy is an important method to determining the formation and stability of metal nanoparticles in aqueous solution (41). The UV-vis spectrum of synthesized silver nanoparticles displays a broad peak in 450 nm, which shows the formation of silver nanoparticles (Figure 2) (42, 43). Appearance of this peak, assigned to a surface plasmon, is well-documented for various metal nanoparticles with size ranging from 2 to 100 nm (44).

Powder X-ray diffraction

The XRD was used to confirm the crystalline nature and purity of the synthesized AgNPs (45). The XRD pattern of AgNPs was showed four major peaks appeared with 2θ values of 38/1°, 44/3°, 64/4°and 77/4° that can be indexed to (111), (200), (220) and (311), respectively (Figure 3) (38, 39). This pattern shows crystalline nature and face-centered cubic (FCC) structure for AgNPs (46, 47).

Transmission electron microscopy

The morphology and size of the synthesized AgNPs were investigated by TEM analysis. A representative transmission electron micrograph recorded from the synthesized AgNPs deposited on carbon coated copper

Table 1. Zone of inhibition (mm) of AgNPs against bacteria tested.

Bacteria	Inhibition zones (mm)			
	Test	control (-)	control (+)	
	Ag NPs	water	GM	S
E. coli	14	NA	23	22
S. aureus	12	NA	23	21

NA: not appearing; GM: Gentamicin; S: Streptomycin.

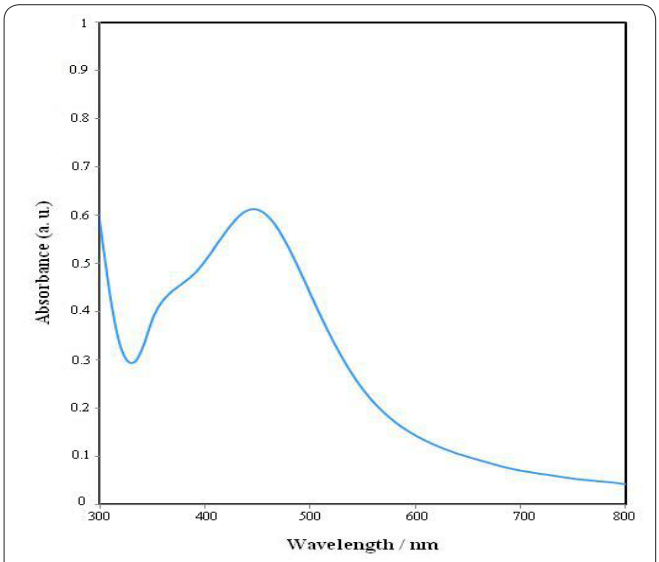

Figure 2. The UV-vis absorption peak of synthesized AgNPs in *S. persica* extract. The absorption spectrum of AgNPs displayed a strong peak at 450 nm.

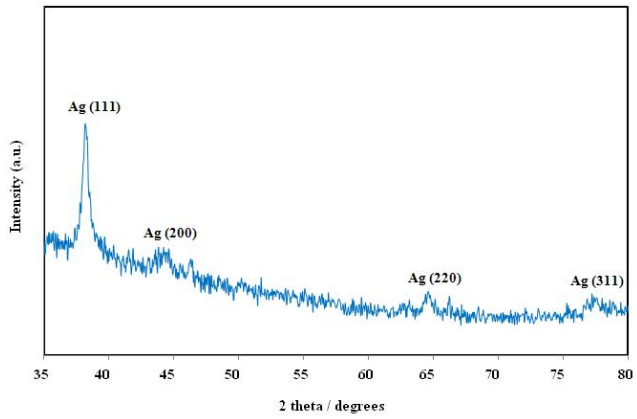

Figure 3. The powder X-ray diffraction pattern of synthesized AgNPs by *S. persica* (a.u. = arbitrary units).

grids is shown in Figure 4. Results showed that morphology of AgNPs was spherical and formed AgNPs were in a size range of 50 nm. Tahir *et al.* (2015) synthesized of silver nanoparticles using aqueous extract of *S. persica* stem that these nanoparticles have been FCC structure and size of 1-6 nm (36). Different size of AgNPs in various parts of the plant can be related to variety of compounds such as tannins, flavonoids, alkaloids, enzymes and other reducing factors in each part of the plant. In addition, shape and size of nanoparticles can be influence via reaction conditions such as light, pH, reaction time, reaction temperature, reducing agent and stabilizer.

Antibacterial activity of synthesized AgNPs

Antimicrobial activities of synthesized AgNPs and controls on bacteria are shown in Figure 5. The inhibition zones of AgNPs on *E. coli* and *S. aureus* were 14 and 12 mm, respectively (Table 1).

The results of MIC of synthesized AgNPs against *S. aureus* and *E. coli* bacteria showed 100 and 400 µg/mL for *E. coli* and *S. aureus*, respectively (Figures 6 and 7). The results of MBC of AgNPs showed in Table 2. The results showed that MBC of AgNPs against *E. coli* was 200 µg/mL. In this test, there was no result observed for *S. aureus* bacteria. The synthesized AgNPs not

Table 2. Minimum inhibition concentrations (MIC) and Minimum bactericidal concentration (MBC) of AgNPs against bacteria tested.

	E. coli		*S. aureus*	
	MIC (µg/mL)	**MBC (µg/mL)**	**MIC (µg/mL)**	**MBC (µg/mL)**
AgNPs	100	200	400	-
Extract	-*	-	-	-

*means no antibacterial effect.

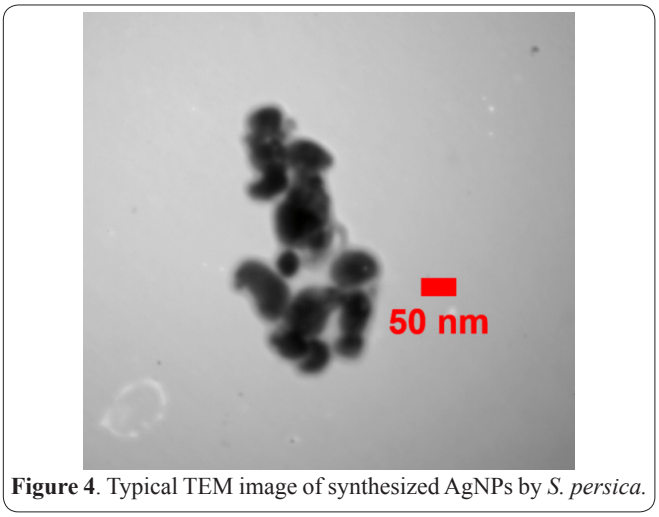

Figure 4. Typical TEM image of synthesized AgNPs by *S. persica*.

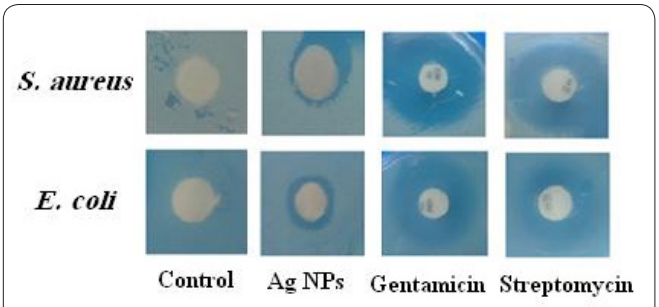

Figure 5. Antibacterial activities of synthesized AgNPs and controls on *E. coli* and *S. aureus*.

only inhibited the growth of *E. coli* but also killed them. Abubacker and Sathya (2015) investigated the synthesis of AgNPs from *S. persica* and their antibacterial activity on dental pathogen. They reported that the antibacterial effect of AgNPs from *S. persica* revealed a highly effective against oral pathogens followed by *Azadirachta indica* and *Ficus bengalensis* when compared with control ($AgNO_3$) at 1 mM concentration, ethyl alcohol and standard antimicrobial discs (48).

The antibacterial activity of AgNPs is attributed to cell wall structure in gram-positive and gram-negative bacteria (46). Silver has a strong tendency to interact with phosphor and sulfur atom that these exist in bacteria cell wall. The silver disrupts respiration process in bacterial by interact with thiol and phosphor groups in bacteria cell membrane, that this cause inhibitory or death bacteria (49).

The cell wall of gram-positive bacteria has a rigid layer of liner polysaccharide, that there is no in gram-negative bacterium; this causes difficult permeation into gram-positive bacteria layer. Therefore, inhibitory activity of AgNPs is stronger in gram-positive bacteria than gram-negative (50).

The plant synthesis of metal and oxide metal nano-

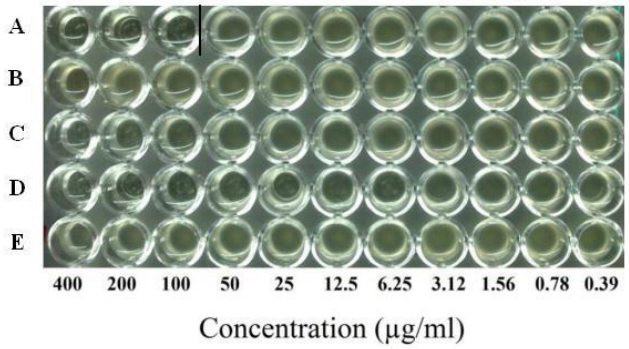

Figure 6. Minimum inhibitory concentration of silver nanoparticles on *E. coli*. A: synthesized AgNPs; B: Extract; C: Streptomycin; D: gentamycin; E: Distilled water.

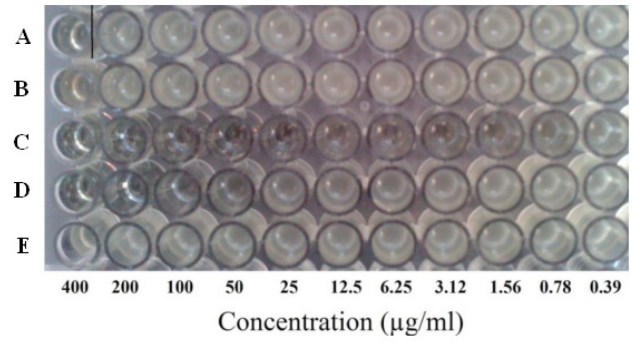

Figure 7. Minimum inhibitory concentration of silver nanoparticles on *S. aureus*. A: synthesized AgNPs; B: Extract; C: Streptomycin; D: gentamycin; E: Distilled water.

particles is an emerging field in nanosience and nanothnology. Among nature materials, plants are best choice for green synthesis of nanoparticles. Different parts of plants such as roots, stems, leaves and fruit can use for synthesis of nanoparticles. Several physical and chemical methods such as laser ablation, electron irradiation, chemical reduction, photochemical methods have been used for synthesizing silver nanoparticles. Most of these methods are expensive, time consuming, toxic and difficult (39). Green synthesis is an appropriate choice for reduce of these disadvantages and risks. The important parameters in assay of NPs synthesis are shape and particle size and for control these parameters, reaction conditions should be optimized (51). Results of our study showed that AgNPs were appropriate effect on Gram negative bacteria. We suggest that it will be need to more studies on the animal models before use the synthesized AgNPs as antimicrobial agents.

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