J Med Bacteriol. Vol. 10, No. 1, 2 (2021): pp. 30-47 jmb.tums.ac.ir

Journal of Medical Bacteriology

Green Synthesis of Silver Nanoparticles Using an *Ephedra sinica* Herb Extract with Antibacterial Properties

Bahman Fazeli-Nasab [1*], *Mahmood Solouki* [2], *Ali Sobhanizadeh* [3]

[1] *Research Department of Agronomy and Plant Breeding, Agricultural Research Institute, University of Zabol, Zabol, Iran.*

[2] *Department of Breeding and Biotechnology, Faculty of Agriculture, University of Zabol, Zabol, Iran.*

[3] *MSc. of Plant Medicinal, Faculty of Agriculture, University of Zabol, Zabol, Iran.*

ARTICLE INFO

Article type:
Research Article

Article history:
Received: 20 Apr 2020
Revised: 13 May 2020
Accepted: 27 May 2021
Published: 30 Jun 2021

Keywords: *Antimicrobial, Biomedicine, Ephedrine, FTIR, Nanoscience, XRD*

ABSTRACT

Background: The exceptional properties of the silver nanoparticles play an important role in nanoscience and nanotechnology, particularly in nanomedicine and also offer several applications in the biomedicine field. The development of antibacterials which are clinically useful against bacteria and drug resistant microorganisms, it is one of the main approaches of silver nanoparticles. However, it is essential to improve environmentally friendly methods for their synthesisin this respect, the principal aim of this research is focused on to propose a simplified and efficient green synthesis of silver nanoparticles with proven antibacterial properties.

Methods: The green synthesis route is based on the use of the *Ephedra sinica* as reducing agent of the silver ions in aqueous solution at room temperature. Complementary, the antibacterial activity of the silver nanoparticles against *E. coli*, *S. aureus*, *S. dysenteriae*, *B. cereus* and *L. monocytogenes* was confirmed. Green synthesized silver nanoparticles have been characterized by UV-Vis spectroscopy, XRD, TEM and FTIR.

Results: The silver nanoparticles revealed Gaussian distributions with the average diameter of 10 nm and results showed that the lowest MIC and MBC of *Ephedra sinica* herb extract were 25 and 50 mg/mL, respectively and also the lowest MIC and MBC of the antibacterial activity of the silver nanoparticles produced by *Ephedra sinica* herb extract were 6.25 and 12.5 mg/mL, respectively.

Conclusion: The observed results suggested that using *Ephedra sinica*, it is possible to performed silver nanoparticles with controlled characteristics and with significant inhibitory activity against the *E. coli*, *S. aureus*, *S. dysenteriae*, *B. cereus* and *L. monocytogenes*.

- ***Please cite this paper as:*** Fazeli-Nasab B, Solouki M, Sobhanizadeh A. Green Synthesis of Silver Nanoparticles Using an *Ephedra sinica* Herb Extract with Antibacterial Properties. *J Med Bacteriol.* 2021; **10** (1, 2): pp.30-47.

*Corresponding Authors: Bahman Fazeli-Nasab, Research Department of Agronomy and Plant Breeding, Agricultural Research Institute, University of Zabol, Zabol, Iran

Tel: +98-5432240696, *Email: Bfazeli@uoz.ac.ir*

Introduction

Ephedra sinica Stapf (Fam. *Ephedraceae*) is taxonomically classified as gymnosperms that is one of the most important plants in traditional medicine and medicinally important as the botanical origin of crude drugs. It's used as a diuretic, antipyretic, diaphoretic and as bioresources that contain pharmacologically active chemicals (1, 2). It has an official monograph in some pharmacopoeias in the world, where it is standardized against the major alkaloids, ephedrine and pseudoephedrine (3). The final satges of ephedrine and pseudoephedrine biosynthesis in genus members of *Ephedra* involve *N*-methylation of norephedrine and norpseudoephedrine, respectively (4). Because of ephedrine and pseudoephedrine, *Ephedra sinica* has been used to treat cold, cough, asthma, edema, and urine negative embolism from ancient times (5). Therefore, with all the above mentioned actions, the main emphasis is conventionally given to its alkaloidal content, despite the fact that these substances represent only about 0.7–0.8% of the whole plant (6, 7). Recent advances emphasize that It's proven the clinical effects of these secondary metabolism on the respiratory, central nervous, and cardiovascular systems (8). However, many species of *Ephedra* have also been shown to contain significant amounts of oligomeric flavonoids (9).

Many health benefits of foods and medicinal plants have been attributed to proanthocyanidins (10-12), and some of their pharmacological activity, such as hypotensive and vasorelaxant effects (13, 14), enhancement of the airflow obstruction, airway hyperresponsiveness and the airway microenvironment in asthma (15), and the inhibition of inflammation and remodeling in murine models of chronic inflammatory disease like asthma (16), are responsible for the aforementioned activities of *Ephedra sinica*, especially its respiratory and cardiovascular effects. In some researches related that *Ephedra* spp. Also display other pharmacological activitys that are not attributed to alkaloids, including antioxidant (17), antiviral (18), anti-inflammatory (19, 20), antimicrobial (21, 22), immunosuppressive (23, 24), anti-invasive, antiangiogenic, antitumor (25), and cytotoxic (26) properties. The dimeric proanthocyanidins of *Ephedra sinica* exhibit cytotoxic activity against the cancer cell lines SGC-7901, HepG2, and HeLa (26). In addition, the administration of proanthocyanidin oligomers of *Ephedra sinica* induce decreasing in the uremic toxin parameters of rats (27).

One of the most produced nano-matrials is silver nanoparticles (five hundred tons each year) and it seems to be increasing every year (28) and is estimated to increase in future. Including its profound role in field of high sensitivity bio molecular detection, catalysis, biosensors and medicine; it is been acknowledged to have strong inhibitory and bactericidal effects along with the anti-fungal, anti-inflammatory and anti-angiogenesis activities (29, 30).

There are several metods for the silver nanoparticles synthesis, for example, ion sputtering, chemical reduction, sol gel, etc. (31); unfortunately many of the nanoparticle syntheses methods involve the use of hazardous chemicals or high energy requirements, which are rather difficult and including wasteful purifications (32). Chemical approaches are the most popular methods for the production of nanoparticles. Since, it is impossible to eliminate harmful materials from the silver nanoparticles synthesis methods, and also the accessibility of noble metal

nanoparticles such as gold, silver and platinum nanoparticles, it seems to be felt more than ever the need to develop new methods with a view to adapting to the environment and the absence of harmful substances (33). Biological methods of nanoparticles synthesis using microorganism (34-36), enzyme (37), and plant extracts have suggested as a possible alternatives for chemical and physical methods. Using plant extracts in nanoparticls synthesis has the advantage of eliminating the elaborate maintaining processes of cell cultures (38). Also, it is suitable for larg scale synthesis of nanoparticles.

Greener syntheses of nanoparticles also provides advancement over other methods as they are simple, one step, cost-effective, environment friendly and relatively reproducible and often results in more stable materials (39). Microorganisms have utilized to nanoparticles producing, however, the rate of synthesis is slow (32) and it is still unexplored the potential of higher plants as a source of nanoparticles.

Silver nanoparticles has long been recognized as having an inhibitory effect toward many bacterial strains and microorganisms commonly present in medical and industrial processes (40). The most widely used and known applications of silver and silver nanoparticles are in the medical industry. Some of creams and topical oinments have silver in their ingredients to prevention of woud infection (41). Also, silver-impregnated polymers widely can be find in the surgical components like a dental implant.(42). In addition, silver-containing consumer products such as colloidal silver gel and silver-embedded fabrics are now used in sporting equipment (33).

Some plants extracts have reprted as a source of nanosilver particles synthesis with high antibacterial activities, for example, marigold flower (43, 44), *Spirogyra varians* (45), *Solanum tricobatum* (46), *Melia dubia* (32), *Erythrina* indica (47), beet root (48), mangosteen (30), *Ocimum tenuiflorum* (49), olive (50), *Acalypha indica* (51) and of *Sesuvium portulacastrum* also reported in literature with nanoparticle size ranging from 5 to 20 nm (52).

In this work, the green synthesis of AgNP's by *Ephedra sinica* herb extract and the bactericidal effect against *Escherichia coli*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Bacillus cereus* and *Listeria monocytogenes* were confirmed.

Materials and Methods

Plant Materials and Preparation of Herb extract for Silver Nanoparticles

The herb of *Ephedra sinica* were collected in 2017 from dahane-sanklider village, sabzvar region of Khorasan-Razavi province of Iran and identified by the Department of Plant Biology, Unievrsity of Zabol.

The *Ephedra sinica* herbs were washed several times with deionised water and dried at room temperature. 10g of the herbs was homogenized in 100 ml of double distilled water and stirred and kept on a rotator shaker at 190–220 rpm for 24h at room temperature in an airtight container till further use with the help of electric grinder, glass dish and filtered (53). After centrifugation at 10,000 rpm for 15 mins, the supernatant was collected and stored at 4°C.

Preparation of aqueous silver nitrate and silver nanoparticles synthesis

Silver nitrate solution was purchased from Merck Company with Catalogue Number 101510 and with the mediation of the Parsa store, Iran and also 1 mM silver nitrate solution was prepared and stored in amber coloured bottle.

The experiment of optimization and synthesis of silver nanoparticles was carried out on different concentrations of *Ephedra sinica* herb extract (3, 5, 10 and 20 milliliters) and different concentrations of silver nitrate (0.5, 1, 3 and 5 mM) combined to obtain the best mode for synthesizing silver nanoparticles. Typically, the synthesis of silver nanoparticles was done by mixing *Ephedra sinica* herb broth and 1mM of

aqueous silver nitrate solution (AgNO3) for reduction of Ag+ ions in the ratio 1: 10 and exposed to sunlight with stirred and kept on a rotator shaker at 100 rpm (about for a period of 48 hours) until the color of the solution colour changes were checked periodically and Finally, The colour change of herb extract from brown to reddish-brown demonstrated that the silver nanoparticles were synthesized (Figure 1). The Experiment of optimization and synthesis of silver nanoparticles was carried out on different concentrations of *Ephedra sinica* herb extract (3, 5, 10 and 20 milliliters) and different concentrations of silver nitrate (0.5, 1, 3 and 5 mM) combined to obtain the best mode for synthesizing silver nanoparticles. (Figures 2 and 3). The contents were centrifuged at 10,000rpm for 15 minutes and then the supernatant was discarded and the pellet was washed with diionised water, then dried in an oven at 60 ° C for one hour, and silver nanoparticles were prepared (54). At all stages of the test, due to the sensitivity of the silver nanoparticles to light, all containers were covered with aluminum foil (55).

Characterization of AgNPs.

There are several ways for characterizing nanoparticles (56). Checking of solution colour changing is the most convenient method to confirm nanoparticles synthesis. It were utilized UV-absorption spectra of synthesized AgNPs (UV-visible spectrometer; Shimadzu UV- 2700) and also Fourier Transform Infrared Spectroscopy (FTIR; Nicolet 800, Nicolet, Madison, USA) spectrums for determination of optical properties of AgNPs. The size and shape of the synthesized AgNPs were determined by transmission electron microscopy (TEM)(Zeiss EM 900, Jena, Germany, −80 kv). Prior to analysis, AgNPs were sonicated for 5 minutes, and a drop of appropriately diluted sample was placed onto a carbon-coated copper grid. The liquid fraction was allowed to evaporate at room temperature. FTIR spectral measurements were carried out to identify the potential biomolecules in *Ephedra sinica* herb extract which is responsible for reducing and capping the bioreduced silver nanoparticles. Xray diffraction (XRD; D8 ADVANCE, Bruker) operating at a voltage 35 kV and a current of 30 mA with Ka1 Cu radiation was used for the phase identification and characterization of crystalline metallic AgNPs.

MIC and MBC of Plant Extracts and biosynthesized AgNPs

The antibacterial activity of *Ephedra sinica* herb extract and the biosynthesized AgNPs were assessed against *Escherichia coli* (*E. coli*)(ATCC 25922), *Staphylococcus aureus* (*S. aureus*) (ATCC 25923), *Shigella dysenteriae* (*S. dysenteriae*) (ATCC 12021), *Bacillus cereus* (*B. Cereus*) (ATCC 19123) and *Listeria monocytogenes* (*L. monocytogenes*) (ATCC 19118).

Broth dilution method was used to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) according to the Yu et al. (57). All of the tests were performed in Mueller Hinton Broth supplemented with Tween 80 at a final concentration of 0.5% (v/v). Briefly, serial doubling dilutions of biosynthesized AgNPs and aqueous herb extract of *Ephedra sinica* were prepared in a 96-well microtiter plate ranged from 3.1 to 100 ppm.

They were added, 10 µL of indicator solution (prepared by dissolving a 10-mg extract in 2 mL of DMSO) and 10 µL of Mueller Hinton Broth in each well. Finally, 10 µL of bacterial suspension (10^6 CFU/mL) was added to each well to achieve a concentration of 10^4 CFU/mL. The plates were wrapped loosely by cling film to ensure that the bacteria did not get dehydrated. The plates were prepared in triplicates, and then they were placed in an incubator at 37 °C for 18-24 hours. The color change was then assessed visually.

The MIC is the lowest concentration of extract which the coluor has changed and the microorganism dose not demonstrate visible

growth. It was calculated the average of three repeats as the MIC value of each extract. The microorganism growth was indicated by turbidity.

After MIC determination of the *Ephedra sinica* herb extract and the biosynthesized AgNPs, the MBC was considered the lowest concentration of the *Ephedra sinica* herb extract and the biosynthesized AgNPs which prevented growth and kills >99.9% of the initial bacterial population that it was observed no visible bacteria growth in the initial bacteria population on the Muller Hinton plates. All tubes were incubated for a total of 72 h, and progressive changes in visual turbidity were noted after 48 and 72 h (58).

Result

UV-Vis spectroscopy analysis of the effect of concentration of Ephedra sinica herb extract and the biosynthesized AgNPs

In the study of the combined effect of *Ephedra sinica* herb extract levels (3, 5, 10 and 20 milliliters) and silver nitrate levels (0.5, 1, 3 and 5 mM) for the green synthesis of silver nanoparticles. The absorption bands peak of silver nitrate levels at 0.5, 1, 3 and 5 mM with a volume of 10 ml of the *Ephedra sinica* herb extract at environment temperature and for 1 hour was 442, 442, 435 and 458 nm in their electron spectrum (Figure 2), and also the absorption bands peak of the *Ephedra sinica* herb extract levels at 3, 5, 10 and 20 milliliters with a volume of 1 mM of the silver nitrate at environment temperature and for 1 hour was 433, 441, 469 and 480nm (Figure 3) in their electron spectrum.

In the present study, the investigation of the spectra showed that with increase in the concentration of silver nitrate, peak intensity increased, so it can be said that increasing the concentration of silver nitrate, more Ag + is converted to Ag0 And hence the concentration of synthesized silver nanoparticles increases (Figure 2). It was also observed that with increasing $AgNo_3$ concentration, the color of the reddish brown solution was observed (Figure 1). This indicates an increase in the concentration of silver nanoparticles and also the growth of particle size in the solution (59, 60).

It has also been reported that increasing the concentration of silver nitrate, will increase the percentage of silver nanoparticles, particle diameter and maximum absorption (61). As seen in the figure 2, no significant change in peak intensity is observed between concentrations of 3 and 5 mM from silver nitrate that this indicat the completion of the reduction process (62). According to observations of this research, the most suitable concentration of silver nitrate for the synthesis of silver nanoparticles is 0.5 mM (with 0.741 absorption rate) and 1 mM (with 1.489 absorption rate) but, in some stages of this research after changing the environmental conditions, the synthesis of silver nanoparticles at a concentration of 0.5 mM was difficult (as observed), so the concentration of 1 mM was determined for the subsequent stages of the test.

By resuscitation of silver ions and the production of silver nanoparticles, the color of the herb extract from brown to reddish brown change that this is was matched with others research (63) and also indicated the silver nanoparticles were synthesized from the leaves extract. To prove the presence of silver nanoparticles in samples, The UV-visible spectrum was obtained from them.

One of the coolest features of metallic nanoparticles is their optical properties, which varies fits with the shape and size of the nanoparticles. In metal nanoparticles, surface plasmon resonance is responsible for their unique optical properties which is affected by factors such as the size of the nanoparticles, the shape of the nanoparticles, their distance from each other and the refractive index of the perimeter environment (64). Considering that silver nanoparticles absorb light from 400 to 500 nanometers (63), Figure 2 shows that the characteristic of absorption bands of surface plasmons occurred from 435 to 458 nm for silver

nanoparticles and as shown in this figure, concentration of 1 mM silver nitrate has a better absorption than others concentrations of silver nitrate and then the nanoparticles synthesis more. Therefore, the peak in this range, which is determined using an ultraviolet spectrometer device, is a step to prove the synthesized silver nanoparticles. The displacement of peaks and changes in their intensity and changes in observed colors are factors that are as dependent on nanoparticles. In other words, the optical properties of silver nanoparticles are strongly dependent on the diameter of the nanoparticles. UV-visible spectroscopy can be used as a simple and reliable method to monitor the stability of nanoparticle solutions (65) but when the nanoparticles become unstable, the peak courier is greatly reduced due to the evacuation of stable nanoparticles (44).

Spectroscopic studies in the effect of aqueous extract on the synthesis of silver nanoparticles with a concentration of 1 mM silver nitrate, environment temperature and 1 hour duration, showed that the intensity of absorption increased by increasing the concentration of the extract (Figure 3). This is due to the increase in the number of silver nanoparticles and their agglomeration, and variations in absorption values indicate particle size variations (66) and also the color of the solution was changed from brown to reddish brown with increasing extract volume (Figure 1) represents the synthesis of silver nanoparticles due to the stimulation of surface plasmon resonance in silver nanoparticles. By increasing the volume of the extract, the maximum wavelength rises from 433 to 480 nm, which is a superficial factor indicating the production of silver spherical nanoparticles with uniform distribution (50). Silver nanoparticles synthesized with different concentrations of plant extracts by analyzing the UV-Vis spectrum and viewing the surface plasmon resonance. The average wavelength was 433-480 nm which was similar to the previous research (66, 67). Changes in maximum wavelengths indicate a change in particle size due to changes in the ratio of the concentration of extract to silver ion. In this research, the intensity of absorption and the maximum wavelength decreased by reducing the concentration of the extract; However, as shown in Figure 2, with the increase in the volume of the extract to 10 ml, the absorption rate of the electron spectra increases but in higher volumes (20 ml) absorption was sharply reduced. On the other hand, because the extract of the plant contains organic matter, these materials act as stabilizing and reducing agents (68-70). Therefore, when the volume of the extract increases in steady state, it concentrates more organic matter around the nanoparticles, so the heavier particles are deposited and released from the nano mode, which makes the characteristic peak of these nanoparticles not present in the desired range (71). In general, the results showed that the best combination for the synthesis of silver nanoparticles is 10 ml of *Ephedra sinica* herb extract and 1 mM of silver nitrate.

Characterization of silver nanoparticles by X-Ray Diffraction (XRD)

XRD analysis showed distinct diffraction peaks which can be indexed the angle vales of 33.31 A°, 40.21 A°, 61.3 A° and 74.91 A° that are respectively in accordance with the levels 267.14, 116.65, 86.28 and 94.48 crystalline planes of nano silver. This analysis revealed the orthorhombic crystals of silver nanoparticles (72). The high peaks in the analysis indicated the active silver composition with the indexing (Figure 6).

X-ray diffraction (XRD) is a non-degrading, multi-application method that provides comprehensive information on the chemical composition and crystalline structure of natural and industrial materials. Each crystal has a pattern due to its unique x-ray diffraction, which detects metal element from other metal compounds of the same metal (72). In the present research, the peaks observed in the XRD diagram (Figure 4) confirm the crystalline structure of the

silver nanoparticles. In addition, additional peaks appearing in this spectrum are related to silver chloride and indicating that the final deposition of the reaction in addition to the silver nanoparticles only contains silver chloride. All of these peaks in this diagram can represent a solid center cubic structure for silver nanoparticles, which has been confirmed in various studies (73, 74).

TEM analysis

TEM analysis revealed that the synthesized nanoparticles were stable in solution at room temperature. In order to compare the size, morphology and uniformity of the distribution of nanoparticles produced, the image of the Transmitted Electron Microscope (TEM) showed that the shape of AgNPs was spherical and the average size of them at optimum conditions was 10 nm (Figure 5).

It is related that silver nanoparticles size is depende on plant extract so that The TEM analyses showed the particle size between 25-50nm (72) in Morinda Pubescens and 15 nm (56) Melissa officinalis.

ICP analysis

Silver concentrations and conversions were determined using Inductively Coupled Plasma spectrometry (ICP). The conversion of silver ion into silver nanoparticles got by the formula Q= ((c0-cf)/c0) *100. c0 and cf are respectively the initial and final concentrations of the metal ion (mg/l) and Q is the percentage of conversion of metal ion into a metal nanoparticle (75, 76). The results of this research showed that ICP is 99.6 that represents the linear relationship was obtained between the silver concentration determined by ICP and adsorption (33), which also confirms the results of the UV-visible spectrophotometer (Table 1).

FTIR analysis

FTIR analysis is utilizable for characterizing the surface chemistry of nanoparticles (63). The FTIR spectral analysis of silver nanoparticles showed some absorption bands (peak) at 518, 824, 1033, 1143, 1196, 1384, 1446, 1518, 1609, 1730, 2360, 3324 and 3736 cm-1 (Figure 6). The peaks of 1518 cm-1 and 824 cm-1 in the *Ephedra* sinica herb extract are related to the tensile vibration of C-H (flexural alkins) and O-H, which are commonly found in the building of amino acids (77). Existence of peaks at 1033, 1143, 1196, 1384, 1446 and 11515 cm-1 in the *Ephedra sinica* herb extract also relates to the tensile vibration of C-O and C-N, respectively that there are in the formation of phenolic groups and aliphatic and aromatic amine acids (50, 77, 78). The presence of these peaks confirmed that the nanoparticles were covered by plant secondary metabolites such as terpenoids, flavonoids, glycosides, phenols, tannins, with functional groups such a s ketone, aldehyde, carboxylic acid, and others (63).

The peaks of 1609 cm-1 and 1730 cm-1 is related to the tensile forces C=O and C=C, which are commonly found in proteins, indicating the presence of proteins as a reducing and stabilizing agent for the synthesis of silver nanoparticles (46, 79, 80). The peaks of 3324 cm-1 and 3736 cm-1 is related to the tensile vibration of N-H and O-H, which is commonly found in the structure of alcohols and phenolic materials (40, 78).

Phenolic and flavonoid compounds reduce the factors that reduce Ag+ to Ag0, and also amino acids and proteins stabilize the synthesis of green nanoparticles from AgNPs (70) These metabolites prevent clotting and pairing of the nanoparticles. As reported (Singhal et al., 2011), the secondary structure of proteins has not been affected during the reaction with Ag+ ions or after binding to Ag nanoparticles. These results indicate that the present of functional groups of biological molecules like hydroxyl, amine and carbonyl present in the *Ephedra sinica* herb extract which play an important role in reducing

the Ag + ions and the stability of nanoparticles (63). Therefore, FTIR study showed that the multifunctional effects of *Ephedra sinica* herb extract simultaneously decreased Ag+ and stabilized Ag nanoparticles.

Antibacterial activity of AgNPs and aqueous herb extract of Ephedra sinica

Antibacterial activity potentials of Minimum Inhibitory Concentration (MIC) and minimum bactericidal concentration (MBC) of aqueous herb extract of *Ephedra sinica* and silver nanoparticles synthesized from *Ephedra sinica* herb extract against *Listeria monocytogenes*, *Staphylococcus aureus*, *Shigella disentry*, *Escherichia coli* and *Bacillus cereus* were evaluated and then the results showed that the AgNPs green synthesized (Table 2, 3) and aqueous herb extract of *Ephedra sinica* (Table 4, 5) have inhibiting activity on all five bacteria in tested concentrations.

The MIC of aqueous herb extract of *Ephedra sinica* on *Listeria monocytogenes*, *Staphylococcus aureus*, *Shigella disenetria* and *Escherichia coli* was 25 µg / ml and on *Bacillus cereus* was 50 µg / ml (Table 2, 3). However, the MIC of silver nanoparticles synthesized from *Ephedra* extract on *Staphylococcus aureus* and *Shigella disenchyterum* was 6.26 µg / ml, but on *Listeria monocytogenes*, *Escherichia coli* and *Bacillus cereus* was 12.12 µg / ml (Table 4, 5).

The MBC of the aqueous herb extract of *Ephedra sinica* on *Listeria monocytogenes*, *Staphylococcus aureus*, *Shigella dilentaria* and *Escherichia coli* was 50 µg / ml and on *Bacillus cereus* was 100 µg / ml (Table 2, 3). However, at least the trapping concentration of silver nanoparticles synthesized from *Ephedra* extract on *Staphylococcus aureus* strains and *Shigella disenetria* was 12.5 µg / ml and on *E. coli*, *Bacillus cereus* strains and *Listeria monocytogenes* was 25 µg / ml (Table 4, 5).

Discussion

The results of the current study showed that the lowest MIC and MBC of the aqueous herb extract of *Ephedra sinica* was 25 and 50 mg/mL respectively but the lowest MIC and MBC of silver nanoparticle synthesized by the herb extract of *Ephedra sinica* was 6.25 and 12.5 mg/mL. It's related (81) the lowest MIC of silver nanoparticle synthesized by *P. ovata* seed extract was e 12.5 ppm aginest *Staphylococcus aureus*. It's related (51) that the particle size of silver nanoparticles synthesized by *Acalypha indica* leaves extract was 20-30 nm and the MIC of silver nanoparticles synthesized against *Escherichia coli* and *Vibrio cholera* 10 µg/mL. In another research (82) the average particle size of silver nanoparticles synthesized by *Allium cepa* stem exract was 67 nm particles it's had an antimicrobial activity against *E. coli* and *Salmonella typhimurium*. Soo-Hwan et al. (83) reported that the MIC of Ag-NPs against *S. aureus* and *E. coli* was 100 µg/mL. It's related (56) that the average particles sizeby *Melissa officinalis* extract was 12 nm and then they observed that using Melissa officinalis, it is possible to performed silver nanoparticles with controlled characteristics and with significant inhibitory activity against the *Staphylococcus aureus* and *Escherichia coli*.

One of the important characteristics of extracts and essential oils of the plant and their phenolic components is their hydrophobicity(84, 85), which disrupts the cytoplasmic membrane, disrupts the proton energy and electrical current, coagulates the cell contents, and also the penetration of these materials into the cell membrane lipids bacteria and mitochondria and then disrupts their buildings and increases their permeability (86, 87). This reason causes the leakage of ions and other cellular contents. Although the release of a limited amount of these substances is tolerable to the bacterium, it has a biological effect, and the expulsion of large amounts of cellular contents or the release of vital ions and molecules will cause cell death (87).

It has been reported that the AgNP's have the capacity to hold fast to the bacterial cell membrane and penetrate into the cytoplasm (88, 89). This fact produce structural changes in the cell, and consequently the elimination of this organism. In this sense, can be suppose the cell membrane of *Listeria monocytogenes, Escherichia coli* and *Bacillus cereus* exhibits a major resistance to the AgNP's in low concentration but *Staphylococcus aureus* and *Shigella dysenteriae* can not be resistant to the AgNP's in low concentration.

The effect of silver nanoparticles on living organism cells depends on the diameter, particle size, and shape of the nanoparticles of synthesized, and because the size of the silver nanoparticles is usually low, the surface is more exposed to the outer space and has a greater effect on the membrane of the cells(90, 91). The mechanism of inhibiting silver nanoparticles depends on the performance of silver ions in a colloidal solution, in which the nanosilver metal particles, over time, emit silver ions. In the succession reaction, these ions converted the bands of SH into microorganisms into SA-g bands, which resulted in the destruction of microorganisms (91, 92). Also, silver nanoparticles can disrupt the normal function of the cell membrane, including its selective permeability, as well as the process of cellular respiration, and even after entering the bacterial cell, they can interfere with the function of sulfur-containing proteins and phosphorus-containing molecules such as DNA, efficiency eliminate them(92-94).

According to the mentioned studies, silver nanoparticles have a powerful antimicrobial effect on the antibiotic resistant bacterias such as *Escherichia coli, Staphylococcus aureus, Shigella dysenteriae, Bacillus cereus* and *Listeria monocytogenes*. Though one of the biggest advantages of green synthesis of silver nanoparticle method is that a large quantity of nanoparticles can be synthesized in a short span of time (32). During this type of syntheses; chemicals used are toxic and led to non-ecofriendly by-products. This may be the reason which leads to the biosyntheses of nanoparticles via green route that does not employ toxic chemicals and hence proving to become a growing wanton want to develop environment friendly processes. Thus, the advancement of green syntheses of nanoparticles is progressing as a key branch of nanotechnology; where the use of biological entities like microorganisms, plant extract or plant biomass for the production of nanoparticles could be an alternative to chemical and physical methods in an ecofriendly manner (32).

Antimicrobial and antibiotic resistances are an increasingly serious threat to human health and then it is necessary to overcome it with the help of nature (63). Therefore, there is an increase in the investigation of plants as a source of human infectious diseases management (72, 95). Though there are two potential problems with the use of any silver antibiotic usage. First, it does appear that silver resistance can occur in at least some bacterial strains. It is extremely important to avoid this circumstance since it appears that, at least in the near future, silver ions may soon be the only remaining effective agent for clinical use. There appears to be a tendency to develop other silver compounds that produce low levels of silver ions in the wound environment or have characteristics permitting long term application with fewer dressing changes as a cost saving measure. Both of these characteristics would appear to favor the development of additional silver resistant strains (41).

Conclusion

The proposed green route for the synthesis of silver nanoparticles from *Ephedra sinica* herb extract provide an efficient and functional methodology to obtain well dispersed and antimicrobial silver nanoparticles. Also, this methodology is simple, economic and environmental friendly. Based on the results observed by FTIR spectroscopy, is possible to affirm that the organic compounds such as

metabolites and phenols presents in the *Ephedra sinica*, promote the reduction of Ag+ ion to Ag0. The intensity of AgNP's antibacterial activity can be associated with the distribution of particle size, in the other words, extensive distribution of particle size have a promoting effect on the antibacterial activities and has a major interaction between the cell membrane of the *Listeria monocytogenes, Staphylococcus aureus, Shigella dysenteriae, Escherichia coli* and *Bacillus cereus*. consequently, AgNP's obtained by green methodologies have an eliminating potential against antibiotic resistance bacterias.

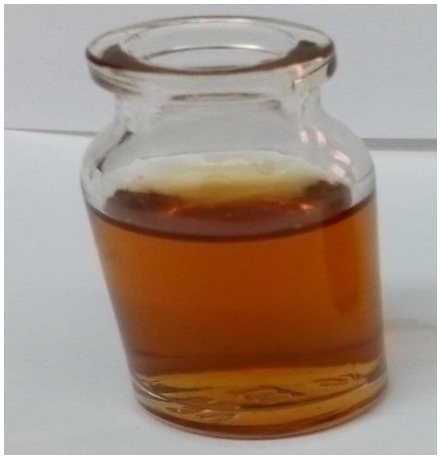

Figure 1. Silver nanoparticles synthesized in the *Ephedra sinica* herb extract**.**

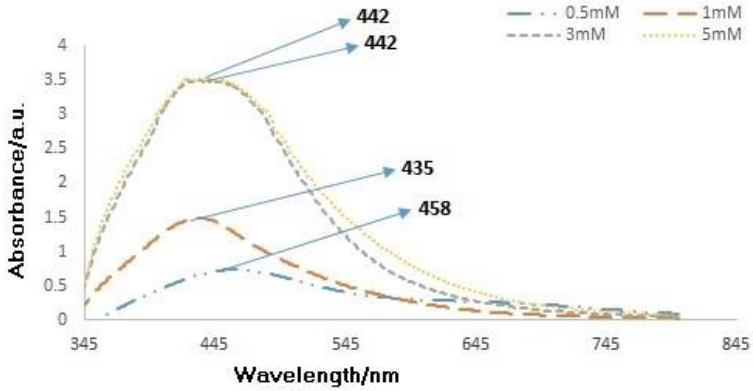

Figure 2. UV-Vis spectrum of the silver nanoparticles synthesized at 0.5, 1, 3 and 5 mM of silver nitrate with 5 ml of *Ephedra* sinica herb extract at 1 hour and environment temperature (25 °C)**.**

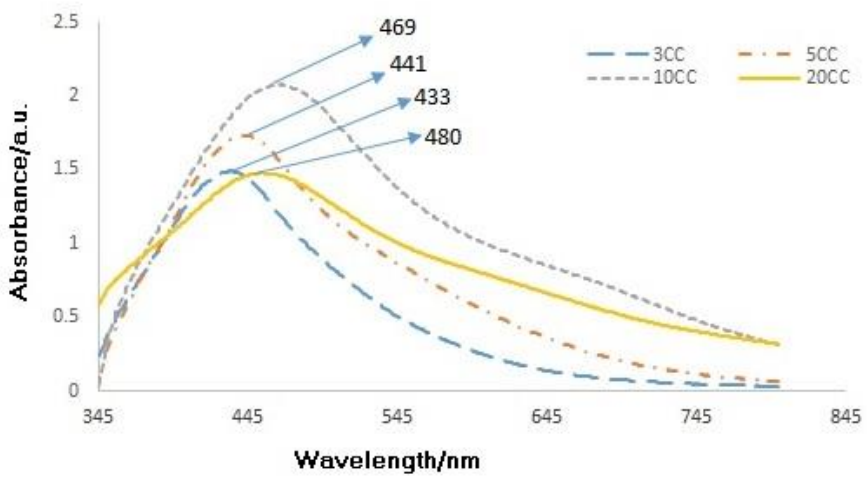

Figure 3. UV-Vis spectrum of the silver nanoparticles synthesized at 3, 5, 10 and 20 ml f *Ephedra sinica* herb extract with 1 mM concentration of silver nitrate, 1 hour and environment temperature (25 °C).

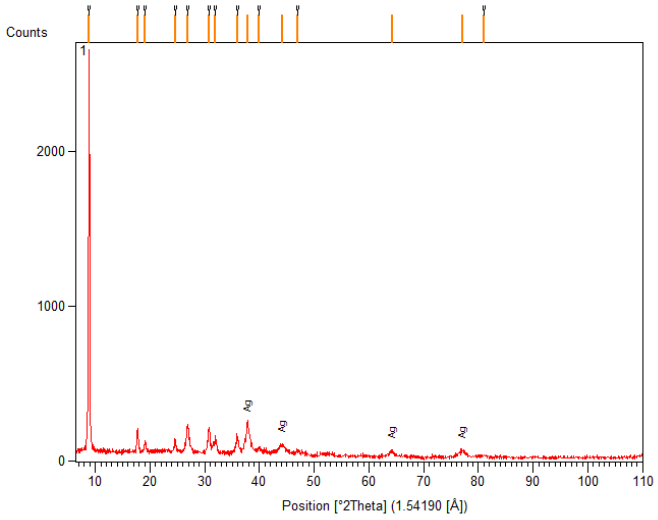

Figure 4. XRD spectrum of the silver nanoparticles at 1 mM concentration of silver nitrate, 5 ml of *Ephedra sinica* herb extract at 1 hour and environment temperature (25 °C).

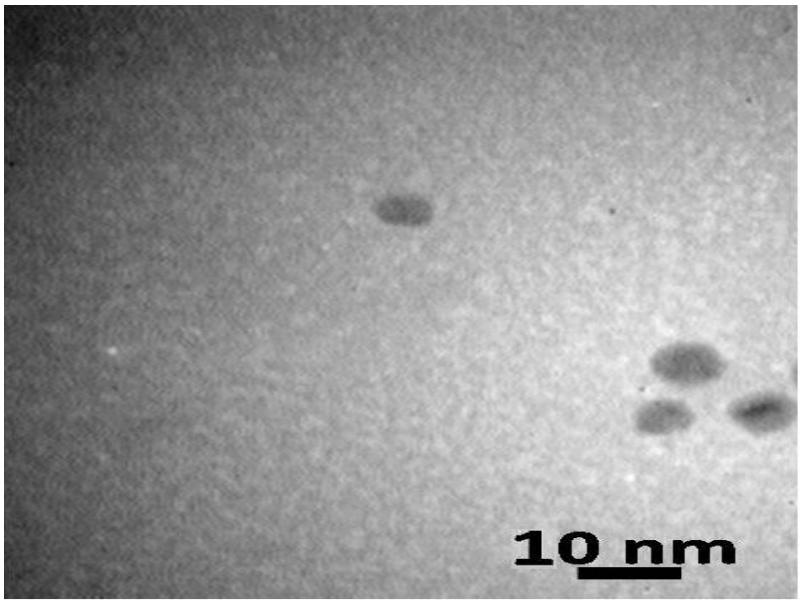

Figure 5. TEM image of the silver nanoparticles at 1 mM concentration of silver nitrate, 5 ml of *Ephedra sinica* herb extract at 1 hour and environment temperature (25 °C).

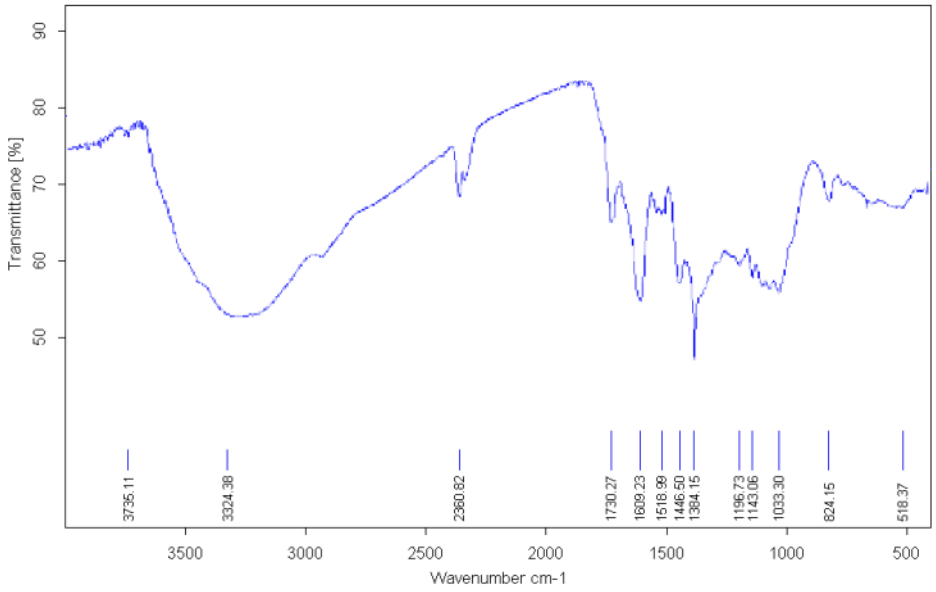

Figure 6. FTIR spectrum of the silver nanoparticles at 1 mM concentration of silver nitrate, 5 ml of *Ephedra sinica* herb extract at 1 hour and environment temperature (25 °C).

Table 1. The results obtained from the analysis of samples using inductively coupled plasma spectrometry (ICP).

Q (%)	CF (mg/L)	C0 (mg/L)	Plant extract
99.6	0.735	186.659	5%

Table 2. Results obtained from the analysis of samples using inductively coupled plasma spectrometry (ICP).

Bacterial strain	MIC (µg/ml)	MBC (µg/ml)
Listeria monocytogenes	25	50
Staphylococcus aureus	25	50
Shigella dysenteriae	25	50
Escherichia coli	25	50
Bacillus cereus	50	100

Table 3. Standard bacteria growth at different concentrations of the aqueous herb extract of *Ephedra sinica*.

Bacterial strain	Different concentrations of the aqueous herb extract of *Ephedra sinica* (µg/ml)					
	3.1	6.25	12.5	25	50	100
Listeria monocytogenes	++	++	++	+	-	-
Staphylococcus aureus	++	++	++	+	-	-
Shigella dysenteriae	++	++	++	+	-	-
Escherichia coli	++	++	++	+	-	-
Bacillus cereus	++	++	++	++	+	-

Table 4. Antimicrobial activity of silver nanoparticles synthesized from *Ephedra sinica* on standard of bacteria strains.

Bacterial strain	MIC (µg/ml)	MBC (µg/ml)
Listeria monocytogenes	12.5	25
Staphylococcus aureus	6.25	12.5
Shigella dysenteriae	6.25	12.5
Escherichia coli	12.5	25
Bacillus cereus	12.5	25

Table 5. Standard bacteria growth at different concentrations of silver nanoparticles synthesized from *Ephedra sinica***.**

Bacterial strain	Different concentration silver nano particle synthesized (µg/ml)					
	3.1	6.25	12.5	25	50	100
Listeria monocytogenes	++	++	+	-	-	-
Staphylococcus aureus	++	+	-	-	-	-
Shigella dysenteriae	++	+	-	-	-	-
Escherichia coli	++	++	+	-	-	-
Bacillus cereus	++	++	+	-	-	-

++ Complete growth of bacteria; + MIC; - MBC

Acknowledgment

It was conducted some experiments of the current study in another place such as FTIR in Zabol University of Medical Sciences and Health Services, XRD at Amirkabir University of Technology and TEM at the University of Science and Technology (School of mechanical engineering, dep. Of Aerospace Engineering), so the authors thank the centers.

Funding information

This article is related to research project No. PR-UOZ97-15, which is funded by the Research and Technology of the University of Zabol.

Ethics approval and consent to participate

Authors confirm the progress of the study, anything occurring in the course of the study, any revision in the protocol. Consent to publish were obtained from all the participants.

Conflict of interest

None declared.

References

1. Orejola J, Matsuo Y, Saito Y, et al. Characterizatio of proanthocyanidin oligomers of *Ephedra sinica*. *Molecules* 2017; **22**(8):1308.
2. Okada T, Takahashi H, Suzuki Y, et al. Comparative analysis of transcriptomes in aerial stems and roots of *Ephedra sinica* based on high-throughput mRNA sequencing. *Genom Data* 2016; **10**:4-11.
3. Organization W H. WHO monographs on selected medicinal plants: World Health Organization; 1999.
4. Morris JS, Groves RA, Hagel JM, et al. An N-methyltransferase from *Ephedra sinica* catalyzing the formation of ephedrine and pseudoephedrine enables microbial phenylalkylamine production. *J Biol Chem* 2018: jbc.RA118.004067.
5. Liang S, Meng X, Wang Z, et al. Polysaccharide from *Ephedra sinica* stapf inhibits inflammation expression by regulating Factor-β1/Smad2 signaling. *Int J Biol Macromol* 2018; **106**:947-54.
6. Wang LL, Kakiuchi N, Mikage M. Studies of *Ephedra* plants in Asia. Part 6: Geographical changes of anatomical features and alkaloids content of *Ephedra sinica*. *J Nat Med* 2010; **64**(1): 63.
7. Liu YM, Sheu SJ, Chiou SH, et al. A comparative study of commercial samples of *Ephedrae herba*. *Planta Med* 1993; **59**(04):376-8.
8. Westfall T, Westfall D. Goodman & Gilman's: The Pharmacological Basis of Therapeutics Adrenergic Agonists and Antagonists. In: New York, McGraw-Hill; 2012.
9. Caveney S, Charlet DA, Freitag H, et al. New observations on the secondary chemistry of world *Ephedra* (Ephedraceae). *Am J Bot* 2001; **88**(7): 1199-208.
10. Bruyne T, Pieters L, Deelstra H, et al. Condensed

vegetable tannins: biodiversity in structure and biological activities. *Biochem Syst Ecol* 1999; **27**(4):445-59.
11. Keikhaie KR, Fazeli-Nasab B, Jahantigh HR, et al. Antibacterial activity of ethyl acetate and methanol extracts of *Securigera securidaca*, *Withania sominefra*, *Rosmarinus officinalis* and *Aloe vera* plants against important human pathogens. *J Med Bacteriol* 2018; **7**(1-2):13-21.
12. Rezaei-Nasab M, Komeili G, Fazeli-Nasab B. Gastroprotective effects of aqueous and hydroalcholic extract of *Scrophularia striata* on ethanol-induced gastric ulcers in rats. *Der Pharm Lett* 2017; **9**(5):84-93.
13. Magos GA, Mateos JC, Páez E, et al. Hypotensive and vasorelaxant effects of the procyanidin fraction from *Guazuma ulmifolia* bark in normotensive and hypertensive rats. *J Ethno pharmacol* 2008; **117**(1):58-68.
14. Sanz M, Terencio M, Paya M. Isolation and hypotensive activity of a polymeric procyanidin fraction from *Pistacia lentiscus*. *Pharmazie* 1992; **47**(6):466-72.
15. Zeng X, Ma Y, Gu H, et al. The effect of oligomeric proanthocyanidin on airway microenvironment in asthma. *Eur Respir J* 2016; **48**(60): PA4100.
16. Zhou DY wanton, Fang SR, Zou CF, et al. Proanthocyanidin from grape seed extract inhibits airway inflammation and remodeling in a murine model of chronic asthma. *Nat Prod Commun* 2015; **10**(2):257-62.
17. Okawa M, Kinjo J, Nohara T, et al. DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scaveng-ing activity of flavonoids obtained from some medicinal plants. *Biol Pharm Bull* 2001; **24**(10): 1202-5.
18. Li L, Yu CH, Ying HZ, et al. Antiviral effects of modified Dingchuan decoction against respiratory syncytial virus infection in vitro and in an immunosuppressive mouse model. *J Ethno pharmacol* 2013; **147**(1):238-44.
19. Kim IS, Park YJ, Yoon SJ, et al. Ephedrannin A and B from roots of *Ephedra sinica* inhibit lipopolysaccharide-induced inflammatory mediators by suppressing nuclear factor-κB activation in RAW 264.7 macrophages. *Int Immunopharmacol* 2010; **10**(12):1616-25.
20. Kim SY, Son KH, Chang HW, et al. Inhibitory effects of plant extracts on adjuvant-induced arthritis. *Arch Pharm Res* 1997; **20**(4):313.
21. Zang X, Shang M, Xu F, et al. A-Type proanthocyanidins from the stems of *Ephedra sinica* (Ephedraceae) and their antimicrobial activities. *Molecules* 2013; **18**(5):5172-89.
22. Bagheri-Gavkosh S, Bigdeli M, Shams-Ghahfarokhi M, et al. Inhibitory effects of *Ephedra major* host on *Aspergillus parasiticus* growth and aflatoxin production. *Mycopathologia* 2009; **168**(5):249-55.
23. Park J, Lee H, Mun H, et al. Effect of Ultrasonification Process on Enhancement of Immuno-stimulatory Activity of *Ephedra sinica* Stapf and Rubus coreanus Miq. *Korean Soc Biotechnol Bioeng J* 2004; **19**(2):113-7.
24. Chen R, Zhu G and Xu Z. Effect of different extracts from *Ephedra* on cell immunity. *J Tradit Chin Med* 2001; **4**:1-15.
25. Nam NH, Lee CW, Hong DH, et al. Antiinvasive, antiangiogenic and antitumour activity of *Ephedra sinica* extract. *Phytother Res* 2003; **17**(1):70-6.
26. Tao H, Wang L, Cui Z, et al. Dimeric proanthocyanidins from the roots of *Ephedra sinica*. *Planta Med* 2008; **74**(15):1823-25.
27. Yokozawa T, Fujioka K, Oura H, et al. Decrease in uraemic toxins, a newly found beneficial effect of *Ephedrae herba*. *Phytother Res* 1995; **9**(5): 382-4.
28. Larue C, Castillo-Michel H, Sobanska S, et al. Foliar exposure of the crop *Lactuca sativa* to silver nanoparticles: evidence for internalization and changes in Ag speciation. *J Hazard Mater* 2014; **264**:98-106.
29. El-Chaghaby GA, Ahmad AF. Biosynthesis of silver nanoparticles using *Pistacia lentiscus* leaves extract and investigation of their antimicrobial effect. *Orient J Chem* 2011; **27**(3):929-36.
30. Veerasamy R, Xin TZ, Gunasagaran S, et al. Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities. *J Saudi Chem Soc* 2011; **15**(2):113-120.
31. Ahmed S, Ahmad M, Swami BL, et al. Green synthesis of silver nanoparticles using *Azadirachta indica* aqueous leaf extract. *J. Radiat. Res Appl Sci*

2016; **9**(1):1-7.
32. Ahmed S, Ahmad M, Swami BL, et al. A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. *J Adv Res* 2016; **7**(1):17-28.
33. Song JY, Kim BS. Rapid biological synthesis of silver nanoparticles using plant leaf extracts. *Bioprocess Biosyst Eng* 2009; **32**(1):79-84.
34. Klaus T, Joerger R, Olsson E, et al. Silver-based crystalline nanoparticles, microbially fabricated. *Proc Natl Acad Sci* 1999; **96**(24):13611-4.
35. Konishi Y, Ohno K, Saitoh N, et al. Bioreductive deposition of platinum nanoparticles on the bacterium *Shewanella algae*. *J Biotechnol* 2007; **128**(3):648-53.
36. Nair B, Pradeep T. Coalescence of nanoclusters and formation of submicron crystallites assisted by *Lactobacillus* strains. *Cryst Growth Des* 2002; **2**(4):293-8.
37. Willner I, Baron R and Willner B. Growing metal nanoparticles by enzymes. *Adv Mater* 2006; **18**(9):1109-120.
38. Shankar SS, Rai A, Ahmad A, et al. Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using Neem (*Azadirachta indica*) leaf broth. *J Colloid Interface Sci* 2004; **275**(2):496-502.
39. Mittal J, Batra A, Singh A, et al. Phytofabrication of nanoparticles through plant as nanofactories. *Adv Nat Sci*: *Nanosci Nanotechnol* 2014; **5**(4): 043002.
40. Jiang H, Manolache S, Wong ACL, et al. Plasma enhanced deposition of silver nanoparticles onto polymer and metal surfaces for the generation of antimicrobial characteristics. *J Appl Polym Sci* 2004; **93**(3):1411-22.
41. Becker RO. Silver ions in the treatment of local infections. *Metal-based drugs* 1999; **6**(4-5):311-14.
42. Silver S. Bacterial silver resistance: molecular biology and uses and misuses of silver compounds. *FEMS Microbiol Rev* 2003; **27**(2-3):341-53.
43. Padalia H, Moteriya P, Chanda S. Green synthesis of silver nanoparticles from marigold flower and its synergistic antimicrobial potential. *Arab J Chem* 2015; **8**(5):732-41.
44. Sadeghi B, Gholamhoseinpoor F. A study on the stability and green synthesis of silver nanoparticles using *Ziziphora tenuior* (Zt) extract at room temperature. *Spectrochi Acta A Mol Biomol Spectrosc* 2015; **134**: 310-315.
45. Salari Z, Danafar F, Dabaghi S, et al. Sustainable synthesis of silver nanoparticles using macroalgae *Spirogyra varians* and analysis of their antibacterial activity. *J Saudi Chem Soc* 2016; **20**(4):459-64.
46. Logeswari P, Silambarasan S, Abraham J. Ecofriendly synthesis of silver nanoparticles from commercially available plant powders and their antibacterial properties. *Scientia Iranica* 2013; **20**(3):1049-54.
47. Sre PR, Reka M, Poovazhagi R, et al. Antibacterial and cytotoxic effect of biologically synthesized silver nanoparticles using aqueous root extract of *Erythrina indica lam. Spectrochim*. *Acta A Mol Biomol Spectrosc* 2015; **135**: 1137-44.
48. Bindhu M, Umadevi M. Antibacterial and catalytic activities of green synthesized silver nanoparticles. *Spectrochim Acta A Mol Biomol Spectrosc* 2015; **135**:373-8.
49. Logeswari P, Silambarasan S, Abraham J. Synthesis of silver nanoparticles using plants extract and analysis of their antimicrobial property. *J Saudi Chem Soc* 2015; **19**(3): 311-17.
50. Khalil MM, Ismail EH, El-Baghdady KZ, et al. Green synthesis of silver nanoparticles using olive leaf extract and its antibacterial activity. *Arab J Chem* 2014; **7**(6):1131-9.
51. Krishnaraj C, Jagan E, Rajasekar S, et al. Synthesis of silver nanoparticles using Acalypha indica leaf extracts and its antibacterial activity against water borne pathogens. *Colloid Surface* B 2010; **76**(1):50-6.
52. Nabikhan A, Kandasamy K, Raj A, et al. Synthesis of antimicrobial silver nanoparticles by callus and leaf extracts from saltmarsh plant, *Sesuvium portulacastrum* L. *Colloid Surface* B 2010; **79**(2):488-93.
53. Davari A, Solouki M, Fazeli-Nasab B. Effects of jasmonic acid and titanium dioxide nanoparticles on process of changes of phytochemical and antioxidant in genotypes of Satureja hortensis L. *Eco-Phytochemical J medicinal plants* 2018; **5**(4): 1-20.
54. Dolatabadi S, Emrani S, Mehrafruz E, et al. Green synthesis and antibacterial effect of silver nanoparticles using *Eucalyptus camaldulensis*. *J*

Neyshabur University Med Sci 2017; **5**(3):74-85.
55. Etemadi M, Mohebbi - Kalhori D, Azizian Shermeh O, et al. Phytosynthesis of Silver Nanoparticles using aqueous extract of *Camellia sinensis* L. and study of their antibacterial activities. *J Fasa University Med Sci* 2017; **7**(1): 39-52.
56. de Jesús Ruíz-Baltazar Á, Reyes-López SY, Larrañaga D, et al. Green synthesis of silver nanoparticles using a *Melissa officinalis* leaf extract with antibacterial properties. *Results Phys* 2017; **7**:2639-43.
57. Yu J, Lei J, Yu H, et al. Chemical composition and antimicrobial activity of the essential oil of *Scutellaria barbata*. *Phytochemistry* 2004; **65**(7): 881-4.
58. Petrus E, Tinakumari S, Chai L, et al. A study on the minimum inhibitory concentration and minimum bactericidal concentration of nano colloidal silver on food-borne pathogens. *Int Food Res J* 2011; **18**(1):55-66.
59. Huang H and Yang X. Synthesis of polysaccharide - stabilized gold and silver nanoparticles: a green method. *Carbohydr Res* 2004; **339**(15):2627-31.
60. Mason C, Vivekanandhan S, Misra M, et al. Switchgrass (*Panicum virgatum*) extract mediated green synthesis of silver nanoparticles. *World J Nano Sci Eng* 2012; **2**(02):47.
61. Pourmortazavi SM, Taghdiri M, Makari V, et al. Procedure optimization for green synthesis of silver nanoparticles by aqueous extract of Eucalyptus oleosa. Spectrochim. *Acta A Mol Biomol Spectrosc* 2015; **136**:1249-54.
62. Ibrahim HM. Green synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative microorganisms. *J Radiat Res Appl Sci* 2015; **8**(3):265-75.
63. Bagherzade G, Tavakoli MM, Namaei MH. Green synthesis of silver nanoparticles using aqueous extract of saffron (*Crocus sativus* L.) wastages and its antibacterial activity against six bacteria. *Asian Pac J Trop Biomed* 2017; **7**(3):227-33.
64. Hoseinnejad M, Jafari SM, Katouzian I. Inorganic and metal nanoparticles and their antimicrobial activity in food packaging applications. *Crit Rev Microbiol* 2018; **44**(2):161-81.
65. Siddiqui MN, Redhwi HH, Achilias DS, et al. Green synthesis of silver nanoparticles and study of their antimicrobial properties. *J Polym Environ* 2018; **26**(2):423-433.
66. Tripathy S, Das S, Chakraborty SP, et al. Synthesis, characterization of chitosan–tripolyphosphate conjugated chloroquine nanoparticle and its in vivo anti-malarial efficacy against rodent parasite: A dose and duration dependent approach. *Int J Pharm* 2012; **434**(1-2):292-305.
67. Khan Z, Singh T, Hussain JI, et al. Starch-directed green synthesis, characterization and morphology of silver nanoparticles. *Colloid Surface* B 2013; **102**:578-84.
68. Zhang W, Qiao X, Chen J. Synthesis of silver nanoparticles—effects of concerned parameters in water/oil microemulsion. *Mater Sci Eng* B 2007; **142**(1):1-15.
69. Petit C, Lixon P, Pileni MP. In situ synthesis of silver nanocluster in AOT reverse micelles. *J Phys Chem* A 1993; **97**(49):12974-83.
70. Prathna T, Chandrasekaran N, Raichur AM, et al. Biomimetic synthesis of silver nanoparticles by *Citrus limon* (lemon) aqueous extract and theoretical prediction of particle size. *Colloid Surface* B 2011; **82**(1):152-9.
71. Miri A, Sarani M, Bazaz MR, et al. Plant - mediated biosynthesis of silver nanoparticles using *Prosopis farcta* extract and its antibacterial properties. Spectrochim *Acta A Mol Biomol Spectrosc* 2015; **141**:287-91.
72. Jancy ME, Inbathamizh L. Green synthesis and characterization of nano silver using leaf extract of *Morinda Pubescens*. *Asian J Pharm Clin Res* 2012; **5**(Suppl 1):159-62.
73. Vaidyanathan R, Gopalram S, Kalishwaralal K, et al. Enhanced silver nanoparticle synthesis by optimization of nitrate reductase activity. Colloids and Surfaces B: *Biointerfaces* 2010; **75**(1):335-41.
74. Sun Q, Cai X, Li J, et al. Green synthesis of silver nanoparticles using tea leaf extract and evaluation of their stability and antibacterial activity. *Colloids Surf A Physicochem Eng Asp* 2014; **444**:226-31.
75. Smitha S, Philip D, Gopchandran K. Green synthesis

of gold nanoparticles using *Cinnamomum zeylanicum* leaf broth. *Spectrochim. Acta A Mol Biomol Spectrosc* 2009; **74**(3):735-9.
76. Xie L, Yu Z, Islam SM, et al. Remarkable acid stability of polypyrrole-MoS4: a highly selective and efficient scavenger of heavy metals over a wide pH Range. *Adv Funct Mater* 2018; **28**(20):1800502.
77. Balashanmugam P, Kalaichelvan PT. Biosynthesis characterization of silver nanoparticles using Cassia roxburghii DC. aqueous extract, and coated on cotton cloth for effective antibacterial activity. *Int J Nanomed* 2015; **10**(Suppl 1): 87-97.
78. Devaraj P, Kumari P, Aarti C, et al. Synthesis and characterization of silver nanoparticles using cannonball leaves and their cytotoxic activity against MCF-7 cell line. *J Nanotechnol* 2013; **2013**: Article ID 598328.
79. Macdonald I, Smith W. Orientation of cytochrome c adsorbed on a citrate-reduced silver colloid surface. *Langmuir* 1996; **12**(3):706-13.
80. Van de Weert M, Hering JA, Haris PI. Fourier transform infrared. methods for structural analysis of protein pharmaceuticals: Chapter 4, pp. 131-166. In Biotechnology: Pharmaceutical Aspects, Volume III. Edited by W. Jiskoot and D. Crommelin. AAPS Press; 2005.
81. Bokaeian M, Fakheri BA, Mohasseli T, et al. Antibacterial activity of silver nanoparticles produced by *plantago ovata* seed extract against antibiotic resistant *Staphylococcus aureus*. *Int J Infect* 2015; **2**(1):1-3.
82. Balamanikandan T, Balaji S, Pandiarajan J. Biological synthesis of silver nanoparticles by using onion (*Allium cepa*) extract and their antibacterial and antifungal activity. *World App Sci J* 2015; **33**:939-43.
83. Kim SH, Lee HS, Ryu DS, et al. Antibacterial activity of silver-nanoparticles against *Staphylococcus aureus* and *Escherichia coli*. *Korean J Microbiol Biotechnol* 2011; **39**(1):77-85.
84. Fazeli-Nasab B and Mirzaei N. Evaluation of total phenol and flavonoid content in a wide range of local and imported plants. *Sci J Ilam University Med Sci* 2018; **26**(2):141-54.
85. Fazeli-Nasab B, Sirousmehr A, Mirzaei N, et al. Evaluation of total phenolic, flavenoeid content and antioxidant activity of Leaf and Fruit in 14 different genotypes of *Ziziphus mauritiana* L. in south of Iran. *Eco-Phytochem J Med Plants* 2017; **4**(4):1-14.
86. Tajkarimi M, Ibrahim SA, Cliver D. Antimicrobial herb and spice compounds in food. *Food control* 2010; **21**(9):1199-1218.
87. Burt S. Essential oils: their antibacterial properties and potential applications in foods (a review). *Int J Food Microbiol* 2004; **94**(3):223-53.
88. Pazos-Ortiz E, Roque-Ruiz JH, Hinojos-Márquez EA, et al. Dose-dependent antimicrobial activity of silver nanoparticles on polycaprolactone fibers against Gram-positive and Gram-negative Bacteria. *J Nanomater* 2017; 2017.
89. López-Esparza J, Espinosa - Cristóbal LNF, Donohue-Cornejo A, et al. Antimicrobial activity of silver nanoparticles in polycaprolactone nanofibers against gram-positive and gram-negative bacteria. *Ind Eng Chem Res* 2016; **55**(49): 12532-8.
90. Schrand AM, Braydich-Stolle LK, Schlager JJ, et al. Can silver nanoparticles be useful as potential biological labels? *Nanotechnol* 2008; **19**(23): 235104.
91. Braydich-Stolle LK, Lucas B, Schrand A, et al. Silver nanoparticles disrupt GDNF/Fyn kinase signaling in spermatogonial stem cells. *Toxicol Sci* 2010; **116**(2):577-89.
92. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. *Biotechnol Adv* 2009; **27**(1):76-83.
93. Singh LR, Ningthoujam R, Sudarsan V, et al. Luminescence study on Eu3+ doped Y2O3 nanoparticles: particle size, concentration and core–shell formation effects. *Nanotechnol* 2008; **19**(5):055201.
94. Gordon S, Teichmann E, Young K, et al. In vitro and in vivo investigation of thermosensitive chitosan hydrogels containing silica nanoparticles for vaccine delivery. *Eur J Pharm Sci* 2010; **41**(2):360-8.
95. Mickymaray S, Al Aboody MS, Rath PK, et al. Screening and antibacterial efficacy of selected Indian medicinal plants. *Asian Pac J Trop Biomed* 2016; **6**(3):185-91.