Nanomed. J., 4(2): 98-106, Spring 2017

ORIGINAL RESEARCH PAPER

Green synthesis of silver nanoparticle using echinops extract and its antibacterial activity

Fatemeh Nakhjiri [1], Mahboubeh Mirhosseini [1], Malihe Alsadat Mozaheb [2]*

[1]Department of Biology, Payame Noor University, Iran
[2] Technical and Vocational University of HazratRoghayeh, Yazd, Iran

ABSTRACT

Objective(s): Silver nanoparticles (Ag NPs) are not only specific physical and chemical properties but also are considered for their antibacterial activity and ecofriendly.

Materials and Methods:In this study a simple, cost effective biologically method for Ag^{+}reducing to Ag NPs using Echinops extractas a stabilizer, and reducing agent.Ag NPs were analyzed using UV-Vis spectrometry,TEM, XRD and FTIR. The role of Echinops concentration, silver nitrate concentration, pHand reaction time on the synthesis of nanoparticles were studied. Antibacterial activity of the Ag NPs werecarried out by disc diffusion method against *Staphylococcus aureus* and *Escherichia coli*. Alsothe amount of MBC and MIC for AgNPs against bacteria wereinvestigated.

Results: The AgNPs formation were observed as a color change of the mixture from colorless to dark-brownish. The UV-Vis spectroscopy absorbance peak at 420 nm confirmed the presence of Ag NPs. TEM analysis, showed Ag NPs were spherical, triangle and bar particles in shape with size range within 1.32-36.41 nm. XRD study showed particles were crystalline in nature. FTIR analysis detected that Ag NPsare functionalized with biomolecules that are present in the aqueous Echinops extractact as the reducing agents and stabilizing the nanoparticles. The results showed that the time of reaction, temperature, pH, Echinops extract concentration and $AgNO_3$concentration could accelerate the formation of AgNPs.

Conclusion: In this study, synthesized Ag NPs havethe efficient antibacterial activity against pathogenic bacteria. Ag NPs havean importantfunction in the field of nanotechnology and nanomedicine.

Keywords: *Antibacterial activity, Echinops extract, Green synthesis, Silver nanoparticle*

How to cite this article
Nakhjiri F, Mirhosseini M, AlsadatMozaheb M. Green synthesis of silver nanoparticle using echinops extract and its antibacterial activity. Nanomed J. 2017; 4(2): 98-106. DOI:10.22038/nmj.2017.21570.1228

INTRODUCTION

Silver nanoparticles among noble metallic nanoparticles have received enormous consideration due to antibacterial activity, low toxicity; and *in vitro* and *in vivo* applications [1]. Some drugs are accessible in industry principle on silver such as silver sulphadiazine, etc. for the therapeutic of burn and the chronic injury infected by microorganisms. Silver nano gels/sprays are also worthy referring to their application in cosmetic [2] and drug industries for treatment [2-4].

*Corresponding Author Email: *nakhjiri7520@gmail.com*
Tel: (+98) *03536235715*
Note. This manuscript was submitted on January 6, 2017; approved on February 17, 2017

Among the different synthesis methods, physical and chemical methods require poisonous materials, [5] higher pressure, energy and temperature [6]. They are also costly [7] and inflammable [8] with unfavorable influence to the ecology [9, 10]. Bio-inspired synthesis applying microorganisms, plant extracts and different plant products for silver nanoparticles have been indicated as worthy alternate to chemical methods as it prevents applicationof poisonous materials and use of higher pressure and temperature. The plant products have become nano manufactory for producing metal nanoparticles of gold and silver. Its applicationfor the productionof nanoparticles is potentially

favorable over microorganisms due to the assistance of scale up, slight health hazard, eco-friendship and elaborate procedure of keeping cell cultures [11]. It is regarded to be the best level for production of nanoparticles being unbound from poisonous materials as well as giving biological capping agents for stabilization of silver nanoparticles.

Moreover, utilizing of plant products has drawn specific regards because it decreases the cost of microorganisms separation and culture media increasing the price competitor quality over nanoparticles production by microorganisms [4].

In the present study, we demonstrated that an aqueous extract of *Echinops* were applied in reduction of Agand in the production of stable silver nanoparticles and examined the effect of antibacterial activities.

Echinops is a genus of about 120 species of flowering plants in the Asteraceaefamily, commonly known as globe thistles. They have spiny foliage and produce blue or white spherical flower heads. They are native to east Europe to central Asia and south to the mountains of tropical Africa [12]. Manna aresweet products that made naturally or by the action of insects from leaves, bark, branches or trunks of some trees by splitting the leaks. Echinopswere made by the action of a weevil insect species with the scientific name *Larinusvulpesolivier*. On the leaves and stems of this plant, insects hold their eggs and baby cells, the size of a hazelnut spinner which is white. After theBaby, he became an adult insect cocoons and out of the hole.This insect cocoon is made of plant fluids. Echinopscombination are of starch and cellulose and the amount of nitrogen and high (about 25 percent) special sugar called trehalose [13]. Echinops have many important medical applications and have been used extensively in traditional medicine since ancient times. This sweet flavor and glazes that we have a laxative effect and severe cough deep housing and boil it for examination by the stimulation of bronchial, the elimination of violent breasts, smooth sound, relieve irritation of the esophagus and is used for relieving acute respiratory disorders. Also, it is beneficial to eliminate dry throat and stomach [14]. Echinops contains 27% of our chemical composition of the cellulose, 8.5% of mucilage, and 25% of the sugar trehalose, 2.8% ash, 17.5% starch, 13.5% albumin material and minor amounts of fat, tannin and chlorophyll. The method used is easy, clean and requires only harmless materials like plant extract, water and silver nitrate, and is beneficial in large-scale production of silver nanoparticles [14].

MATERIALS AND METHODS

Synthesis of silver nanoparticles

*Echinopspersicus*was purchased from the local market, Yazd, Iran. White cocoon separated of insect inside it. White cocoons were washed thoroughly first with water followed by distilled water to clean all the dust particles and dried in room temperature. Then were powdered with the aid of mixer grinder. 0.5 gram of powder was mixed in with 100 ml of distilled water and heated up at 60°C for 1 hour. The solution was then filtrated and centrifuged at 7000g for 5 min to throw away the indissoluble substances. The extract was subsequently applied as the reducing agent for Ag NP production. Meanwhile, the color alter of the solution from light yellow to brownness to chocolate-brown to colloidal brown was observed periodically. Complete reduction of Ag^{+1} ions to Ag^{0} was proved by the change in color from colorless to colloidal brown.

The production of Ag NPs were accomplished by applying 45mL the extract in 45mL distilled water and 10 mL 0.1M aqueous $AgNO_3$ solution. The solution was stirred and heated at 75°C for different time.

Factors affecting synthesis rate of silver nano particles

The effect of concentration of Echinops (1-5 gl^{-1}) and reaction time (0-48 h) and pH (5, 7, 8, 9, 10 and 11) and silver nitrate concentration (1.0×10^{-3}, 1.0×10^{-4}, 1.0×10^{-5} mM) and temperature (0 °C, 25 °C, 50 °C, 75°C, 100 °C) was studied on nanoparticle synthesis. The absorbance of the resulting solutions was recorded spectrophotometry[16].

Characterization of synthesized silver nanoparticles

In order to investigate the production of silver nanoparticles, the UV visible absorption spectra of the prepared colloidal solutions were measured by applying a two beams spectrophotometer, UNIC Model 4802 made in America, from 300 to 800 nm, against water blank. The size and shape of the nanoparticles were determined with Brookharen/90 Plas/BI-MAS (New York, America).;PANalytical's X-ray diffraction system, transmission electron microscopes, operating at 80 and 200 kV.The X-ray diffraction analysis was determined by a Panalytical, Pert Pro

diffractometer (America). The intensity information for the lyophilized nano silver powder was measured θ range of 30–80° with a scan rate of 1°/min. The IR spectra of the lyophilized samples were recorded using a, spectrum two FTIR spectrometer (PerkinElmer, America); over a spectral range of 400–4000 cm^{-1}.

Determination of the antibacterial activity of NPs

Antibacterial activity was carried out on *Staphylococcus aureus (PTCC1431);* and *Escherichia coli (PTCC1394)*. For testing antibacterial activity of Ag NP, disc diffusion method was utilized. Triptic soy agar (TSA, Merck, Germany) seeded with 10^7 cell/mL*E. coli* and *S. aureus*, respectively. Sterile paper disc of 5 mm diameter were loaded with double distilled water (as control), Echinops extract and Ag NPs (20 – 80 µL) solution were placed in each agar plate. Each plate was incubated at 37 for 24 hours. Then the inhibition zone around the disc was assigned to exhibit antibacterial activity for each concentration of Ag NPs [4].

MIC determination of NPs

Minimal concentration of an antimicrobial agent inhibits the growth of microorganisms known as MIC [15]. Ag NPs suspension were provided with a concentration of $5gl^{-1}$. The first, 1 ml of media broth was added to the tubes. 1 ml of each nanoparticle was supplied one by one to first tube. In the next, two-fold dilution of the sample was done from tube 1 to tube 2 and then continued down 7 tubes (the 8th was as a control tube). Then 100 µL of *staphylococcus aureus* or *Escherichia coli* bacteria suspension was added to all tubes. Finally, tubes were incubated at 37 °C for 24 h and growth of bacteria was recorded by measuring optical density at 620 nm in a spectrophotometer. The NP amount that lead to growth inhibition of bacteria was regarded as MIC [15, 18].

MBC determination of NPs

Minimal concentration of an antibacterial agent inhibits the majority (99/9%) of inoculated bacterial population known as MBC. MBC was assessed by sub-culturing 50 µl on to TSA, from each tube in which no growth was viewable.

After incubation for 24 h at 37°C, the number of grown colonies will be enumerated. The growth of one colony showed a 99.8% fall for in viable numeration [15].

RESULTS AND DISCUSSION

The presence study describes the green synthesis of Ag NPs applying widely Echinops extract as its reducing agent. While there, have been many techniques on nanoparticles synthesis, most of the techniques apply costly chemicals and therefore are not cost-efficient. Moreover, the productionresidues are dangerous and poisonous. This will result in contamination, which could conduct to unfortunate effects on our ecology. Lately the application of plant extracts and various plant products has become a concern due to its cleaness and easy approaches [14].

AgNPswere successfully produced from the aqueous silver nitrate solution applying Echinops extract in a constantly heated and stirred solution. The color reaction suspension slowly altered to a yellow suspension after some minutes of reaction (Fig. 1*).* This color alteration is in agreement with other reports of green synthesis applying various types of extracts [20, 21].

Factors affecting production rate of silver nano particles

The production of silver nanoparticles was recorded by measuring the absorption spectra of produced silver nanoparticles.

The AgNPssynthesis wereassessedby different $AgNO_3$ concentration (1.0×10^{-3}, 1.0×10^{-4}, 1.0×10^{-5} mM). With addition in $AgNO_3$ concentration, the SPR peaks became sharper and sharper for the brownishsuspension. An absorption band at 420 nm was reached

Fig. 1. Echinops extract, and produced AgNPs (from right to left)

which indicated the production of silver nano particles. The optimum concentration of $AgNO_3$needed for the completion of reaction was studied to be 1.0×10^{-3}mM (Fig. 6).

The role of Ehinops concentration on the AgNPs productionwas investigated by the various concentrations (1-5 gl^{-1}) of Ehinopssuspension containing 1mM of silver nitrate for 2hours (Fig. 2).

Ehinopssuspension contain silver nitrate, the appearing of yellow color in the reaction solution was determined. This is a clarity indication for the production of silver nanoparticles by the Ehinops. In the UV-vis spectra strength peaks with maxima about 405–425nm were determined.

Fig. 3 demonstrates the influence of temperature on synthesis of AgNPs. A wide peak was determined for the colloidal suspension acquired after heating the reaction solution at 25 °C for 30 min.

With addition in temperature from 25 °C to 100 °C, the SPR peaks became sharper and sharper. An absorption band at 420 nm (for the brown dishsuspension) wasdetermined after 10min of

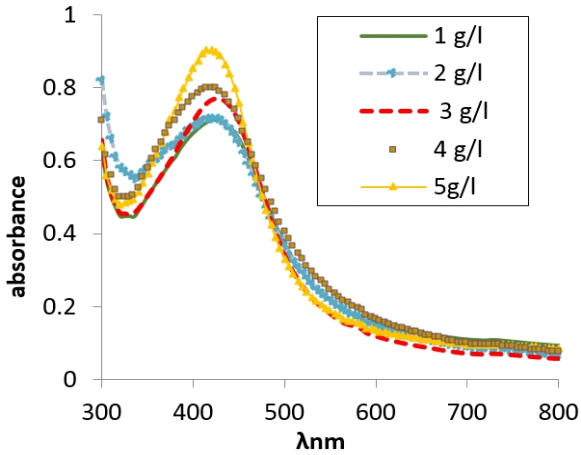

Fig. 2. The UV-vis absorption spectra of silver nanoparticles produced by various concentrations of Echinops extract at 1mM $AgNO_3$ concentration

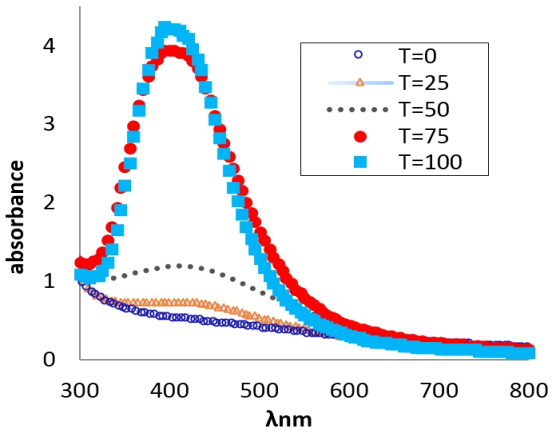

Fig. 3. The UV-vis absorption spectra of silver nanoparticles produced with $5gl^{-1}$ Echinops extract at 1mM $AgNO_3$ concentration for various temperature

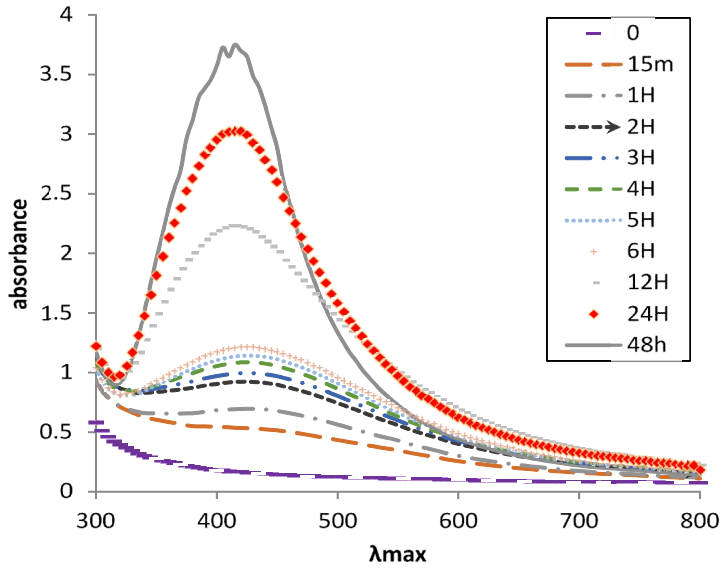

Fig. 4. The UV-vis absorption spectra of silver nanoparticles produced with $5gl^{-1}$Echinops extract at 1mM $AgNO_3$ concentration for various durations

stirring at 75°C, which indicated the productionof silver nanoparticles. It can be determined that an optimum temperature is needed for the completion of reaction, due to the inconstancyproduction silver nanoparticles.

The optimum temperature needed for the completion of reaction was 75 °C. It was determined that reduction rate of silver ions enhanced by raising temperature. Same results were described by Pastoriza-Santos and Liz-Marzan[22]. This sharpness in absorbance peak counts on the size of the produced nanoparticle, as with high temperature particle size may representlesser, which consequences in sharpness of the UV-Vis peak of AgNPs[23].

Effect of time on the AgNPs production was investigated by UV-Vis spectroscopy. An absorption band of very weakshowed at 420 nm for the colloid after 10 min of stirring, Fig. 4 indicatedpeak alteredinto a definite viewable peak after 1h at the similar absorbance, showing the present of spherical AgNPs. The absorption peak intensity enhanced quickly with enhance in reaction time from 15 min to 6h. It was, therefore, determined that an optimum time is needed for the completion of reaction due to the instabilityof synthesizedsilver nanoparticles. The optimum time needed for the completion of reaction was determined to be 1h (Fig. 4).

PH is another significant factor influencing the production of Ag NPs. The influence of pH on the production of Ag NPs was investigated by UV-Vis spectroscopy and is exhibited in Fig 5. At pH 7.0, no absorption peak was determined in the range of 400–450 nm for the colloidal solution of all samples even

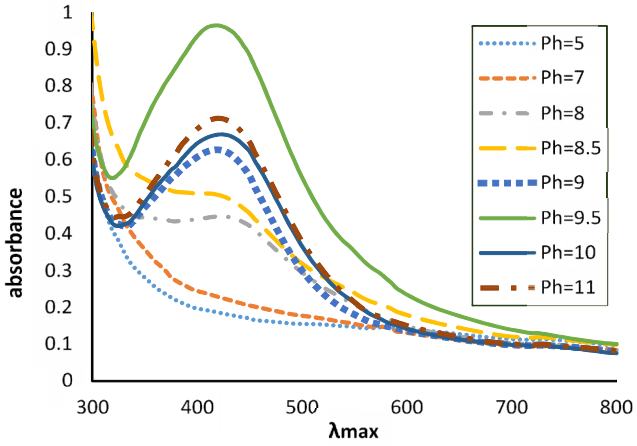

Fig. 5. The UV-vis absorption spectra of silver nanoparticles produced with $5gl^{-1}$ Echinops extract at 1mM $AgNO_3$ concentration for various pH

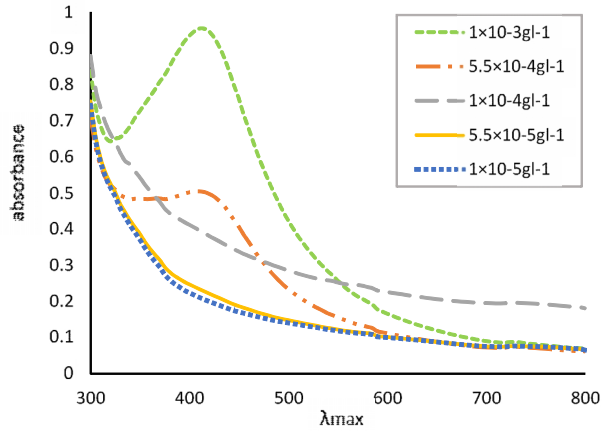

Fig. 6. The UV-vis absorption spectra of silver nanoparticles produced by various concentrations of $AgNO_3$

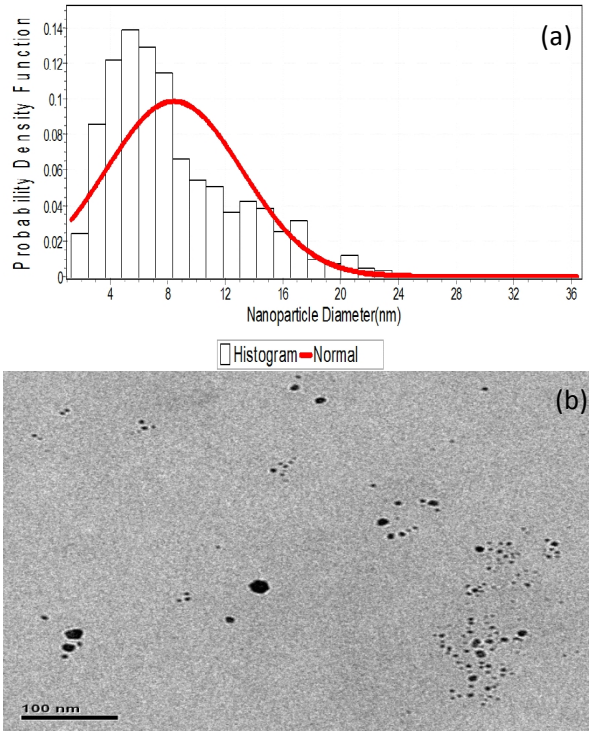

Fig. 7. images of silver nanoparticles synthesized with 5 gl^{-1}Ehinops and 1mM $AgNO_3$TEM (b) particle size distribution

after 24 h. However, an absorption band showed at around 420 nm when pH enhanced from 7 to 8 exhibiting the production of AgNPs, Fig. 5. It was determinedthat the absorption peak streng then hancedstep-by-step with an enhance in pH,suggesting that the production rate of Ag NPs enhances with an enhance in pH. The production of AgNPs wereincreased by basic conditions. At lower pH 8, were produced larger nanoparticles, whereas, at higher pH (pH 9.5) were produced small and highly dispersed nanoparticles.

AgNP characterization

Transmission Electron Microscopy

Fig 7(a) exhibits the TEM images of the silver nanoparticles produced with 5 gl^{-1}Ehinops and 1mM AgNO3. It was detected that the nanoparticles are sometimes spherical and rarely triangular and rod and alsodeterminedabnormal distribution of particles. The size of the particles extended from 1.32-36.41 nm, and the mean particle size was around8 nm (Fig. 7(b)).Suriya et al 2012 described particles size between 3-44 nm with mean of 30 nm. A same result was described by Rahimi and et al 2014applying*Ulva flexousa* reducing as well as capping agent [3].

X-Ray Diffraction

The XRD spectrum demonstrated the crystalline structure of the precipitant as Ag (Fig 7). The peak values at 25ØßÞ =38.24° 49.22°, 54.08°, and 65.13° equal to the (111), (200), (220), (311), and (222) lattice planes of the facecentered cubic crystal structure of AgNPs(Fig. 8).

Unassigned peaks were also determined; indicate that the crystallization of bio-organic phase develops onthe surface of the nanoparticles. Same results were described in silver nanoparticles produced byusing geranium leaf extract [24].

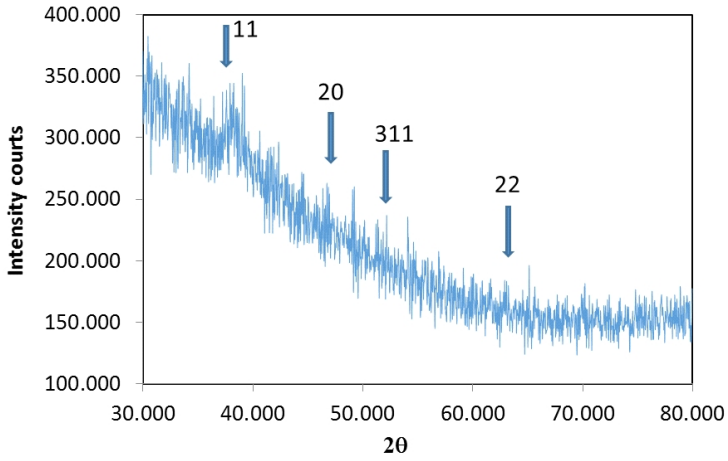

Fig. 8. XRD spectrum of AgNPs green-synthesizedusing Ehinops extract

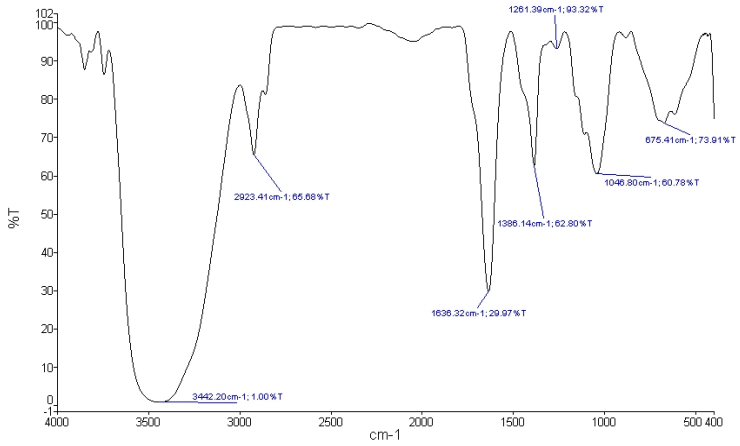

Fig. 9. FTIR spectra of silvernanoparticle

Fourier Transform-Infrared Spectroscopy (FTIR)

The FTIR spectra were showed silver nanoparticles produced with Ehinops extract notice the functional groups of Echinopsincluded in the reduction of silver ions (Fig. 9).

The majorityabsorbance bands existing in the spectrum of Echinops were at 1636.3, 2923.41, 3442.20, 1386.14, 1046.80, and 675.41cm^{-1}. Designate the wide band determined at 3442.20 cm^{-1}could to stretching vibrations of O–H groups in the Echinops. The bands at 2923.41 cm^{-1} represent to asymmetric and symmetric stretching vibrations of methylene groups. The strength band found at 1636.3 cm^{-1} could bedesignated to attribute asymmetrical stretch of carboxylate group. The symmetrical stretch of carboxylate group can be assigned to the bands existing at 1386.14 cm^{-1}. The peaks at 1046.80cm^{-1} were due to alcoholic groups.

This proves that the reduction of the silver ions is matched to the oxidation of the hydroxyl and carbonyl groups. Foundation of these band shifts, it can be concluded that both hydroxyl and carbonyl groups of Echinops are included in the production of silver nanoparticles [25]. The spectrum exhibits a sharp band at 675.41 cm^{-1}, assigned to the stretching vibrations of Ag–N [24] and Ag–O bonds [26, 27]. This peak shows the production of a chemical bond between silver and amino nitrogen [21] and silver and carboxylate groups [26, 27]of the Echinops molecules. It proves that the Echinops is bounce to the silver nanoparticle surface either through amino or carboxylate group or both [25]. As determined in IR spectra (Fig. 9), the Echinops is rich in different functional groups [17]; their capping on silver nanoparticles prepares surface reactivity.

Antibacterial assay

In this study, the antibacterial activities of the AgNPsagainst both Gram positive (*S. aureus*) and Gram negative (*E. coli*) bacteria were examined, and resultsare represented in Fig 10.

According to the acquired results, the inhibition zonesenhanced instantly at one time in association with the percentcontent of nano-sized Ag for all samples (Fig. 10).

The determined MIC and MBC values for the AgNPs were represented (Fig. 10).The result showed that MIC against *S. aureus*and*E.coli bacteria* for Ag NPs were 1.67µgml^{-1}and 1.41µgml^{-1}respectively.

Also, results showed that MBC against *S. aureus*and*E. coli bacteria* for Ag NPs were 1.91 µgml^{-1}and 1.87 µgml^{-1}respectively. Foundation of these results, it can be inferred that the productiond silver nanoparticles had considerable antibacterial action on both Gram classes of bacteria.Shahverdi et al[28]described that the silver nanoparticles have an antibacterialactivity on *S. aureus* and *E.coli*[28].

The mechanism of the antimicrobial action of silver ions is nearlyassociated to their interaction with sulfhydryl groups [29, 30] although other target determinants stay a possibility [31].

Several studies [31-34] indicated that the AgNPs could produce Ag ions, which will injure the cell membrane, disturb the metabolic action, and afterwardconduct to denaturation of protein and finally cell death. AgNPs could also induceactivated

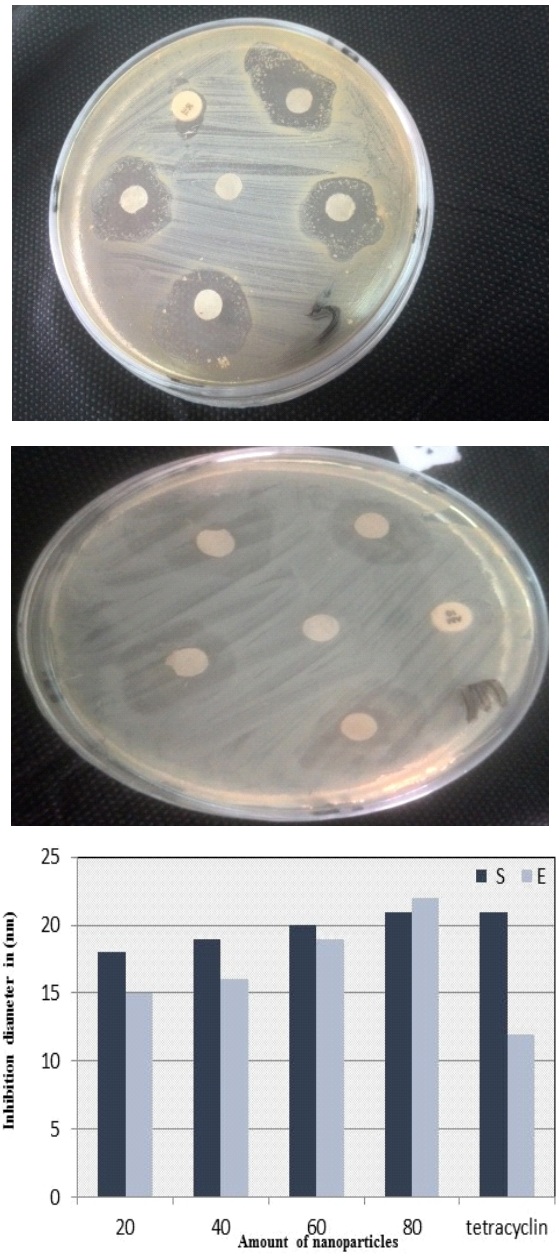

Fig .10. Inhibition zones (mm) determined with different bacterial culture plates loading with silver nanoparticles and the antibiotic tetracycline. (a): *E.coli* ,(b):*S.aureus,* (c):Diagram ofinhibition zones (mm) against 2 bacteria

oxygen species (ROS) such as singlet oxygen O_2, hydroxyl radical $^{\cdot}OH$, and peroxide radical $O_2^{\cdot-}$ which are poisonous to the microorganisms[35]. Also, nanoparticles would interact with the bacterial growth signaling pathway by regulating tyrosine phosphorylation of putative peptides substan ceimportant for cell viability and division [36, 37].

CONCLUSION

Green synthesis of silver nanoparticle using Ehinops extracthas been described. Silver nanoparticle has been successfully produced by this easy, quick, efficient, ecofriendly, efficient cost-effective techniqueapproved by the physicochemical characterization as.

TEM, XRD, FTIR and UV-Vis analysis which are guessed through the plant extract as capping and reducing agents. It was nominated that higher pH value, temperature, time and concentration of Ehinops extractand $AgNO_3$ could accelerate the production rate of AgNPs.

The production silver nanoparticles exhibited efficient antibacterial activities. The silver nano-particles produced by this green synthesis is able to use medical technologies

ACKNOWLEDGMENTS

Financial support of this research by Taft Payme Noor University of Iran gratefully acknowledged.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

REFERENCES

1. Okuda M, Kobayashi Y, Suzuki K, Sonoda K, Kondoh T, Wagawa A, Kondo A, Yoshimura H. Self-organized inorganic nanoparticle arrays on proteinlattices. Nano Lett. 2005; 5(5): 991–993
2. Banerjee P, Satapathy M, Mukhopahayay A, Das P. Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. Bioresour Bioprocess. 2014; 1:3.
3. Rahimi Z, Yousefzadi M, Noori A, Akbarzadeh A.Green Synthesis of silver nanoparticles using *Ulvaflexousa* from the Persian Gulf. J PG.2014; 5(15): 9-16.
4. Ahmed S, Ikram S. Silvernanoparticles: one pot green synthesis using *Terminaliaarjuna* extract for biological application. J Nanomed Nanotechnol. 2015; 6: 4.
5. Ingle A, Rai M, Gade A, Bawaskar M. *Fusarium solani*: A novel biological agent for theextracellular synthesis of silver nanoparticlesý.J Nanopart Res. 2009; 11: 2079-2085.
6. Gnanadesigan M , Anand M, Ravikumar S, Maruthupandy M, Vijayakumar V. Antibacterial potential of biosynthesised silver nanoparticles using *Avicennia marina* mangrove plant. Appl Nanosci.2012; 2(2): 143-147

7. Nabikhan A,Kandasamy K, Raj A, Alikunhi NM. Synthesis of antimicrobial silver nanoparticles by callus and leaf extracts from saltmarsh plant, *Sesuviumportulacastrum* L. Colloids Surf B Biointerfaces. 2010; 79(2): 488–493.
8. Sahayaraj K, Rajesh S. Bionanoparticles: synthesis and antimicrobial applications. Science against microbial pathogens: communicating current research and technological advances. A Mendez-Vilas (Ed). 2011; 228-244
9. Jagtap U, Bapat V. Biosynthesischaracterization and antibacterial activity *Annona Squamosal* L. leaf extract at room temperature. J Plant biochem Biotech. 2013; 22(4): 434-444
10. Shams S ,Pourseyedi S, Hashemipour Rafsanjani H. Green synthesis of silver nanoparticles: eco-friendly and antibacterial. Int J Nano sci Nanotechnol. 2014; 10(2): 127-132.
11. Ahmed S, Ahmad M, Swami B, Ikram S. Plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. J Adv Res. 2016; 7(1): 18-28.
12.RHS A-Z encyclopedia of garden plants.United Kingdom: Dorling Kindersley. 2008; 1136.
13. Nasirzadeh A, Javid- Tash I. Determination of plant species of Manna Echinops production in Fars province. Med Plants. 2005: 389 - 90.
14. Samsam-Shariat H. Pharmacognosy.Iranian Instituete of Medicinal Plants. 2004: 9-93
15. Stoimenov P, Klinger R, Marchin G, KlabundeK J. Metal oxide nanoparticles as bactericidal agents. Langmuir. 2002; 18(17): 6679-6686.
16. Amin M, Anwar F, Saeed A, Janjua M, Awais M, Rashid U. Green synthesis of silver nanoparticles through reduction with *Solanum xanthocarpum* L. Berry Extract: characterization, antimicrobial and urease inhibitory activities against *Helicobacter pylori***.** Int J Mol Sci. 2012; 13(8): 9923-9941.
17. Bharati K, Gujarathi D, Godage N, Dhage RD. Antimicrobial activity of biomediated silver nanoparticles of *Echinopsechinatus* and its characterization. IOSR-JAC. 2016; 9(10): 41-44.
18. Xie Y, He Y, Irwin P, Jin T, Shi X. Antibacterial activity and mechanism of action of Zinc Oxide nanoparticles against *Campylobacterjejuni*. Appl Environ Microbiol. 2011; 77(2): 2325–2331.
19. Ali Z, Yahya R, Sekaran S, Puteh R .Green synthesis of silver nanoparticles using apple extract and its antibacterial properties. Adv Mater Sci Eng. 2016. 2016: 6 pages
20. Sinha S, Pan I, Chanda P,Sen S, Nanoparticles fabrication using ambient biological resources. J Appl Biosci. 2009; 19: 1113–1130.
21.Stensberg M, Wei Q,McLamore ES,Porterfield DM., Wei A,Sepúlveda MS. Toxicological studies on silver nanoparticles: challenges and opportunities in assessment, monitoring and imaging. Nanomed J. 2011; 6(5): 879–898.
22.Pastoriza-Santos I, Liz-Marzan LM. Formation of PVP-protected metal nanoparticles in DMF. Langmuir. 2002; 18(7): 2888–2894.
23. Daizy P. Green synthesis of gold and silver nanoparticles using *Hibiscus rosa*sinensis. Physica E Low Dimens SystNanostruct. 2010; 42(5): 1417–1424.
24.Shankar SS, Ahmad A, Sastry M. Geranium leaf assisted biosynthesis of silver nanoparticles. J Biotechnol Progress. 2003; 19(6): 1627-1631.
25.Kora AJ, Sashidhar RB,Arunachalam J. Gum kondagogu (*Cochlospermumgossypium*): A template for the green synthesis and stabilization of silver nanoparticles with antibacterial application. Carbohydr Polym. 2010; 82(3): 670–679.
26.Biswas N, Kapoor S, Mahal HS, Mukherjee T. Adsorption of CGA on colloidal silver particles: DFT and SERS study. Chem Phys Lett. 2007; 444(4-6): 338–345.
27. Hou X, Fang Y. Investigation of p-hydroxybenzoic acid from a new surface-enhanced Raman scattering system. J Colloid Interface Sci. 2007; 316(1): 19–24.
28. Shahverdi AR, Fakhimi A, Shahverdi HR, Minaian S. Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against *Staphylococcus aureus* and *Escherichia coli.* Nanomed J. 2007; 3(2): 168-171.
29. Silver S. Bacterial silver resistance: molecular biology and uses and misuses of silver compounds. FEMS Microbiol Rev. 2006; 27(2-3): 341-353.
30. Kumar R, Munstedt H. Silver ion release from antimicrobial polyamide/silver composites. Biomaterials.2005; 26(14): 2081–2088.
31. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria.J Colloid Interface Sci. 2004; 275(1): 177–182.
32. McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev. 2001; 14(1): 227.
33. Pal S, Tak YK, Song JM. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium *Escherichia coli*. Appl Environ Microbiol. 2007; 73(6): 1712–1720.
34. Mukherjee P, Roy M, Mandal BP, Dey GK, Mukherjee PK, Ghatak J, Tyagi AK. Green synthesis of highly stabilized nanocrystalline silver particles by a non-pathogenic and agriculturally important fungus *T. asperellum*. J Nanotechnol. 2008; 19(7): 075103
35. Carlson C, Hussein SM, Schrand AM, Braydich-Stolle LK,Hess, KL, Jones RL, Schlager JJ. Unique cellular interaction of silver nanoparticles: size-dependent generation of reactive oxygen species. J Phys ChemB. 2008; 112(43): 13608–13619.
36. Duran N, Marcato PD, Alves OL, De souza G, Esposito E. Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains. J Nanobiotechnol. 2005; 3(8): 1.
37. Shrivastava S, Bera T, Roy A, Sing G, Ramachandrarao P, Dash D. Characterization of enhanced antibacterial effects of novel silver nanoparticles. J Nanotechnol. 2007; 18(22): 225103–225111.