Avicenna J Med Biochem. 2016 September; 4(2):e37504. doi: 10.17795/ajmb-37504.

Published online 2016 August 31. **Research Article**

Biosynthesis of Silver Nanoparticles Using *Carum carvi* Extract and its Inhibitory Effect on Growth of *Candida albicans*

Samira Nasiri,[1,*] and Sara Nasiri[2]

[1]Department of Mycology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, IR Iran
[2]Department of Biology, Faculty of Sciences, Neyshabur Branch, Islamic Azad University, Neyshabur, IR Iran

[*]*Corresponding author*: Samira Nasiri, Department of Mycology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, IR Iran. Tel: +98-9356683288, E-mail: samiranasiri3631@gmail.com

Received 2016 February 29; **Revised** 2016 May 06; **Accepted** 2016 June 25.

Abstract

Background: Biological synthesis of nanoparticles has emerged as a promising field of biotechnology. Various biological systems including fungi, yeasts, bacteria, and plants have been used for biosynthesis of nanoparticles. Silver nanoparticles have unique properties that make them ideal for various medical and industrial applications. Owing to high levels of organic reducing agents and ease of manipulation, plant extracts are widely used for biological generation of various types of metal nanoparticles.
Objectives: The objective of the present study was to evaluate efficacy of *Carum carvi* extract in biosynthesis of silver nanoparticles and to investigate antifungal effects of the biosynthesized nanoparticles.
Methods: Silver nanoparticles were synthesized by addition of silver nitrate solution into fresh extract of *C. carvi*. Characterization of the synthesized silver nanoparticles was performed by transmission electron microscopy (TEM), scanning electron microscopy (SEM), energy dispersive spectrometry (EDS), and X-ray diffraction analysis (XRD). Inhibitory effect of silver nanoparticles on *Candida albicans* growth was evaluated by serial microdilution method.
Results: The results revealed the formation of spherical silver nanoparticles with an average size of 10 nm. Moreover, concentration of SNPs in a 25 mL sample containing both SNPs and plant extract biomass was 2.934 mg/L on average. Serial microdilution test showed that SNPs at the concentration of 50 μg/mL can inhibit growth of the pathogen.
Conclusions: The present study extends the existing literature about green synthesis of nanoparticles using plant tissues and extracts.

Keywords: Biosynthesis, Nanoparticle, Silver, *Candida albicans*, *Carum carvi*

1. Background

Metal nanoparticles have attracted much attention during recent years because of their unique physicochemical properties, which make them ideal choices in a large variety of fields including electronics, medicine, textiles, sensing, etc. (1).

Nanoparticles can be synthesized by means of various methods, with chemical approaches being the most popular ones. These methods, though effective in production of metal nanoparticles with various types, suffer from limitations due to environmental and health considerations (2). In most cases, chemical approaches employ toxic chemicals as reducing agents, organic solvents, or non-biodegradable stabilizing agents. Since noble metal nanoparticles, such as gold and silver, are extensively applied to human contact areas, there is an urgent need to develop safe and green processes of nanoparticle synthesis that are free of toxic chemicals (3).

Biosynthesis of nanoparticles using microorganisms, enzymes, and plant extracts has emerged as a clean, cost-effective, and efficient alternative to chemical methods (4). Plant species appear to be the most suitable biological platforms for nanoparticle synthesis as they produce large biomass at low cost (5). Biosynthesis of nanoparticles using plant extracts is a biological reduction process in which functional groups of plant extracts including alkanes, hydroxyls, phenols, and other groups reduce metal ions into atomic particles. Next, the deionized particles are aggregated and accumulated to form so-called nanoparticles (2).

Black Zira (*Carum carvi* L.) is an annual herbaceous plant that belongs to the Umbelliferae family. The plant is native to some regions of Iran. Its fruit, commonly known as caraway, has been used as a condiment in foods and in pharmaceutical preparations as a part of herbal medicine (6). Caraway is rich in phenolics and other reducing groups (6), making this species an ideal platform for biosynthesis of nanoparticles. Thus, it is reasonable to

expect caraway extract to be effective in bioreduction and production of nanoparticles.

Nanosilver, in terms of product number, is one of the nanomaterials with the highest degree of commercialization, as roughly 30% of all products currently registered in nano-product databases claim to contain nanosilver (7). Perhaps the most popular application of nanosilver is in the field of medicine. Owing to their antimicrobial effects, silver nanoparticles are mainly used as antimicrobial agents in materials such as textiles, wound dressings, and biomedical devices (8).

The opportunistic yeast *Candida albicans* is a major pathogenic agent that profoundly invades various parts of the human body and causes cutaneous, mucutaneous and opportunistic infections. It has been reported that *C. albicans* invasions are among the most serious fungal infections in the world. Moreover, *C. albicans* accounts for approximately half of all yeasts isolated from clinical samples (9).

2. Objectives

The present study was conducted to evaluate the biosynthesis of silver nanoparticles by the herbal extract of the medicinal plant *C. carvi* and to investigate the inhibitory effects of the synthesized nanoparticles on the growth of *C. albicans*.

3. Methods

3.1. Biosynthesis of Nanoparticles

For biosynthesis of silver nanoparticles, we used the general method of SNP biosynthesis with some modification (2). Fresh young leaves of caraway were cut into very small pieces and ground in the presence of liquid nitrogen using mortar and pestle. The resulting frozen powder was transferred into a plastic 45 mL tube, then water was added, and after thorough agitation, the tube was centrifuged for 10 minutes at 5000 g. Supernatant was collected and used as a plant extract for biosynthesis of silver nanoparticles. For the bioreduction process, 2.5 mL of the 200 mM $AgNO_3$ solution was added to 47.5 mL of plant extract to get a final concentration of 10 mM. The experiment was carried out in triplicate at room temperature.

3.2. Characterization of Silver Nanoparticles

Color change in the culture was observed visually. Bioreduction of silver ions to silver nanoparticles was monitored by measuring the UV-Vis spectra of the solution over the range between 300 nm and 500 nm using Jenway 6715 UV/Visible Spectrophotometer (England). Morphology of the silver nanoparticles was studied using transmission electron microscopy (TEM). TEM images were taken using Leo 912 AB high-resolution transmission electron microscope (Germany) operating at an accelerating voltage of 120 kV. A sample of the aqueous biomass solution was placed on the carbon-coated copper grid and dried prior to microscopy. The biosynthesized SNPs were further studied by scanning electron microscopy (SEM) using LEO 1450VT microscope (Germany). For X-ray diffraction XRD measurement, a sample of cyanobacterial suspension was spread in a petri dish and oven-dried. The dried sample was taken for XRD analysis using PHILIPS PW1480 X-ray diffractometer (Netherlands). Concentration of biosynthesized SNPs was measured by high-resolution ICP-OES spectrometer (SPECTRO ARCOS, Germany). For this purpose, a 50 mg sample containing both biosynthesized SNPs and herbal extract was diluted and filtered through filter paper, and its final volume was adjusted to 25 mL. The concentration of SNPs was measured in this 25 mL sample.

3.3. Inhibitory Effects of Silver Nanoparticles

Standard strain of *C. albicans* ATCC10231 was obtained from a bio-bank of pathogenic fungi from Pasteur Institute (Tehran, Iran). Essential oil of caraway was also obtained from Pasteur Institute.

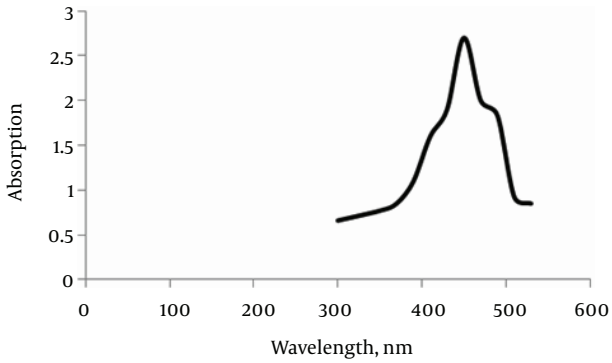
Minimum inhibitory concentration (MIC) of caraway essential oils was determined by serial dilution assay. In a microbroth dilution assay, 100 μL of RPMI along with various concentrations of silver nanoparticles (0, 12.5, 25, 50, 100, 200, and 400 μg/mL) were prepared in a 96-well microplate, and 1 μL of the yeast suspension (106 cell/mL) was added to each well. Broth medium with yeast suspension was used as control. MIC values were determined after 24h incubation at 35°C by counting the number of colony forming units (CFU) and reading the cultures' absorbance at 520 nm using a spectrophotometer. The lowest concentration, which resulted in 90% reduction of CFU compared to control, was assigned as MIC90. Various concentrations of the antifungal drug fluconazole were used in a parallel assay to compare antifungal activity of silver nanoparticles and that of the commercial synthetic medicines.

4. Results

Color change was immediately observed after adding silver nitrate into *C. carvi* extract. The dark green color of *C. carvi* was changed to ivory at first and then to brown, indicating biosynthesis of silver nanoparticles. UV-vis Spectroscopy, a surface plasmon resonance peak, was observed at about 450 nm, which confirmed the formation of SNPs

(Figure 1). This unique pattern of plasmon resonance is attributed to the optical properties of silver nanoparticles and has been reported by many authors as a reliable indicator of the presence of SNPs in a solution (4).

Figure 1. UV-Absorption Spectra of Caraway Extract Incubated With $AgNO_3$



The absorption peak at 450 nm indicates formation of silver nanoparticles.

Morphology of the biosynthesized SNPs was revealed by TEM microscopy (Figure 2). The TEM image was analyzed using imageJ software. Based on this analysis, some properties of the biologically synthesized SNPs were revealed that are presented in Table 1. In general, TEM microscopy revealed that the silver nanoparticles have a spherical shape with a size of about 10 nm. Such a small size provides SNPs with a large surface area, which subsequently enhances their reactivity and catalytic properties (10). Moreover, most of the SNPs had a circularity value of 1, confirming their spherical nature.

Table 1. General Properties of the SNPs Biosynthesized Using *C. carvi*

Parameter	Average Value
Spherical diameter, nm	10.95
Width, nm	6.05
Length, nm	7.38
Roundness	1.18
Circularity	1

The formation of silver nanoparticles was further confirmed by SEM imaging (Figure 3). Although TEM images are sufficient proof for confirmation of SNPs' biosynthesis, SEM images provide a general vision about crystallographic properties of the nanoparticles. Energy Dispersive Spectrometry (EDS) study indicated a sharp signal for Ag, confirming biosynthesis of silver nanoparticles. An absorption peak at 3 keV confirmed the presence of silver nanoparticles.

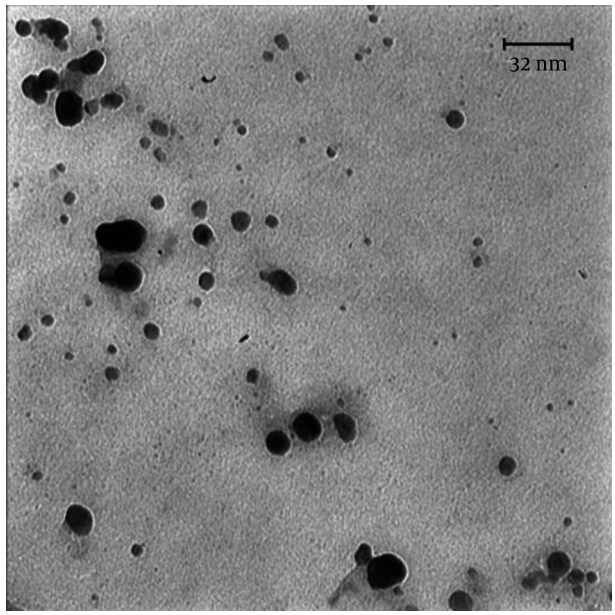


Figure 2. TEM Micrograph of Biologically Synthesized Silver Nanoparticles

Diffraction properties and crystalline structure of the biosynthesized silver nanoparticles were characterized by X-ray powder diffraction. XRD results showed peaks corresponding to (111), (200), (220), and (322) Bragg reflections. This pattern clearly shows presence of SNPs in the sample. Based on XRD results, particle size can be calculated using Debye-Scherrer formula: $d = k\lambda/\beta\cos\theta$, where d is the size of the nanoparticle, k stands for Scherrer constant (0.9), λ represents the X-ray wavelength (0.1541 nm), β is the full width at half-maximum (FWHM), and θ is diffraction angle. Using the Scherrer equation, the average crystallite sizes of the SNPs was found to be in the range of 9 nm to 11 nm; confirming particle size estimated by TEM images (Figure 4)

Concentration of the biosynthesized SNPs was determined using ICP method. Results showed that the concentration of SNPs in a 25 mL sample containing both SNPs and plant extract biomass was 2.934 mg/L on average (Table 2).

Serial microdilution was used to evaluate the inhibitory effect of SNPs on growth of *C. albicans*. Results of this test showed that SNPs at the concentration of 50 μg/mL can inhibit growth of the pathogen; the minimum inhibitory concentration (MIC) of the SNPs was therefore determined as 50 μg/mL. To study the influence of SNPs on *C. albicans*, growth kinetics of *C. albicans* in a 12 hours period was monitored under three concentrations of silver nanoparticles including 0 (control, non-treated), 50 μg/mL (MIC), and 400 μg/mL (the highest concentration in this study). A growth kinetics graph of the yeast under

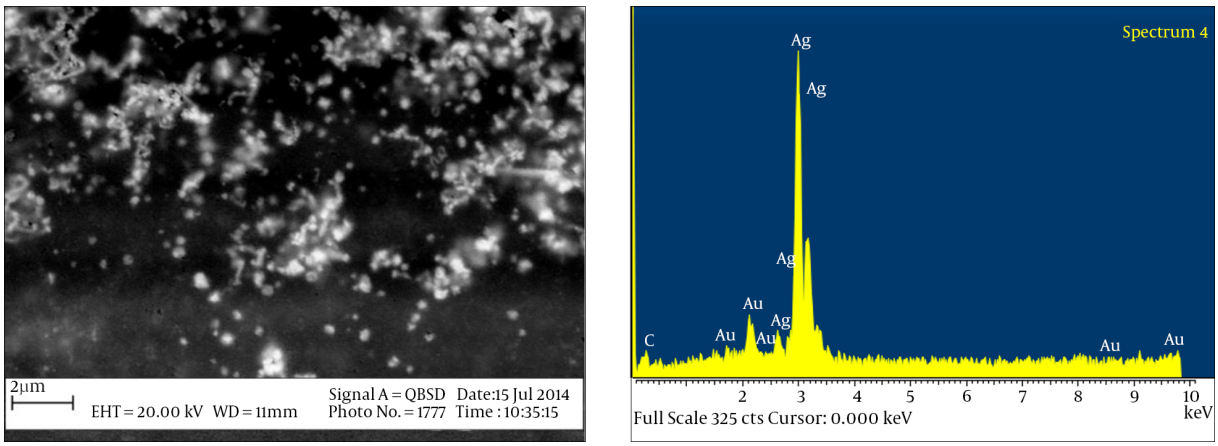


Figure 3. SEM Micrograph of Silver Nanoparticles (left), EDS Results Indicating Sharp Peak for Silver (Ag0)

Table 2. OD Values and CFU Number in Various Concentrations of SNPs (μg/mL)

SNP Concentrations	400	200	100	50	25	12.5	0
OD	0.001	0.002	0.011	0.020	0.030	0.034	0.039
CFU	-	-	160	9200	13300	17000	18200

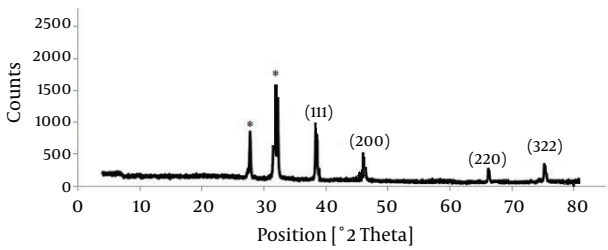


Figure 4. XRD Pattern of Silver Nanoparticles Biosynthesized by Spirulina Biomass

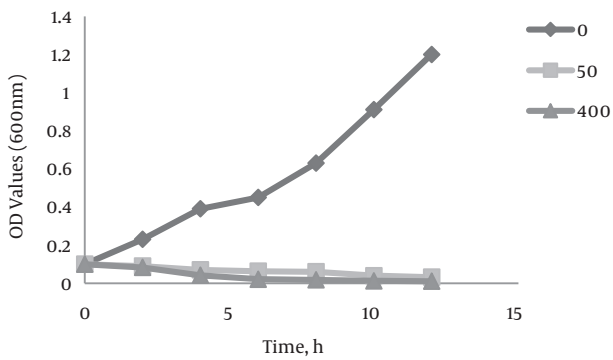


Figure 5. Growth Kinetics of *C. albicans* Under Treatment With Different Concentrations of Silver Nanoparticles

these SNPs treatments is presented in Figure 5. The control group showed a rapid growth pattern, which reached its maximum level about 12 hours after culture. The yeast sample treated with 50 μg/mL of SNPs showed a decreasing growth pattern, so that after 10 hours the growth level was nearly zero. Decrease in growth was more severe in the sample treated with 400 μg/mL of SNPs. A drop in growth was observed only after 4 hours, and the fungal growth was completely inhibited 6 hours after the treatment.

5. Discussion

Nanobiotechnology has created a link between nanotechnology and biotechnology in recent years. As a branch of nanobiotechnology, biosynthesis of metallic nanoparticles using a wide range of biological agents offers many advantages over conventional chemical and mechanical synthesis procedures, as noted earlier in this paper. Biological synthesis of nanoparticles is mediated by a wide range of herbal agents such as alkanes, phenolics, organic acids, hydroxyl groups, and others. These compounds act as reducing agents and aggregating nuclei that facilitate production of metal nanoparticles. It is believed that plant species rich in such reducing agents are better candidates for biosynthesis of metal nanoparticles (3).

In the present study, biosynthesis of silver nanoparti-

cles using extract of *C. carvi* was investigated. Plants are the main biological systems that have been widely used for biosynthesis of nanoparticles (11). So far, various nanoparticles including silver, copper, iron, and others have been synthesized by herbal extracts (12). We used *C. carvi* extract for production of silver nanoparticles, because this species has a high amount of phenolics and other reducing groups. Therefore, it was assumed that such a high level of reducing agents is an appropriate tool for efficient production of silver nanoparticles.

The first indicator of bioreduction of Ag ions to silver nanoparticle is the characteristic change in the color of the plant extract. Color change results from alteration in surface plasmon resonance, which occurs when positive ions (Ag^+) are transformed into nano-form (Ag0) (3).

Presence of a maximum peak at about 450 nm in UV-vis spectroscopy further confirmed biosynthesis of silver nanoparticles. Surface plasmon resonance peak in the range of 410 nm to 450 nm has been reported by other authors as an indicator of SNPs' biosynthesis (13).

The morphology of the biosynthesized SNPs was analyzed by TEM microscopy. Biologically synthesized nanoparticles can occur in various forms, including rectangular, cubic, spherical, and so on. TEM images of the present study revealed that the biosynthesized silver nanoparticles have a spherical shape, with an average diameter of 10.95 nm (Table 1). Moreover, circularity of the biosynthesized SNPs was estimated to be 1, reconfirming the spherical shape of the SNPs. This pattern of SNP biosynthesis has been reported by other authors. For example, Prasad et al. (2013) reported biosynthesis of spherical silver particles using brown marine algae (8). Presence of SNPs in caraway extract was further confirmed by SEM images, EDS graphs, and XRD analyses. An absorption peak at 3 keV in EDS study confirmed the presence of silver nanoparticles in the solution. Four peaks corresponding to (111), (200), (220), and (322) Bragg reflections were observed in XRD analysis. The XRD pattern obtained in this study was in alignment with previously determined Bragg reflections associated with silver nanoparticles (14).

After characterization of SNPs, their antifungal effect on *C. albicans* was studied by serial microdilution method. In vitro microdilution test showed that SNPs can inhibit the pathogen growth at a concentration of 50 μg/mL. The antimicrobial effect of silver nanoparticles has been reported by many authors (4, 10, 13). Silver nanoparticles produced in this study are about 10 nm, which makes them an ideal size for inhibitory effects on bacterial cells. The size of nanoparticles plays a critical role in their efficacy to inhibit microbial growth (15). It has been postulated that nanoparticles with smaller size have better antimicrobial effect, because they have larger surface area and higher percentage of interaction than bigger particles (10). The inhibitory effect of silver nanoparticles on bacterial growth can occur in many ways; for example, silver nanoparticles can interfere with sulfur-containing biomolecules residing on the bacterial membrane, or they may attack bacterial genome and respiratory chain. These interfering effects ultimately result in bacterial cell death (16).

The present study extends the existing literature about green synthesis of nanoparticles using plant tissues and extracts. Efficacy of plant extract in production of various forms of nanoparticles has been reported by many authors (16). The results obtained in this research showed biosynthesis of spherical silver nanoparticles; this finding is in agreement with those reported by other authors (16). Moreover, it was found that the biosynthesized SNPs have potent antifungal effects, which agrees with previous reports on antimicrobial properties of SNPs (16).

Footnote

Authors' Contribution: Study concept and design, analysis, and interpretation of data: Samira Nasiri; drafting of the manuscript, critical revision of the manuscript for important intellectual content, technical assistance: Sara Nasiri.

References

1. Cui H, Feng Y, Ren W, Zeng T, Lv H, Pan Y. Strategies of Large Scale Synthesis of Monodisperse Nanoparticles. *Recent Patents on Nanotech.* 2009;**3**(1):32–41. doi: 10.2174/187221009787003302.
2. Edison TJ, Sethuraman MG. Instant green synthesis of silver nanoparticles using Terminalia chebula fruit extract and evaluation of their catalytic activity on reduction of methylene blue. *Process Biochem.* 2012;**47**(9):1351–7. doi: 10.1016/j.procbio.2012.04.025.
3. Mittal AK, Chisti Y, Banerjee UC. Synthesis of metallic nanoparticles using plant extracts. *Biotechnol Adv.* 2013;**31**(2):346–56. doi: 10.1016/j.biotechadv.2013.01.003. [PubMed: 23318667].
4. MubarakAli D, Thajuddin N, Jeganathan K, Gunasekaran M. Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens. *Colloids Surf B Biointerfaces.* 2011;**85**(2):360–5. doi: 10.1016/j.colsurfb.2011.03.009. [PubMed: 21466948].
5. Parial D, Patra HK, Roychoudhury P, Dasgupta A, Pal R. Gold nanorod production by cyanobacteria—a green chemistry approach. *J App Phycol.* 2011;**24**(1):55–60. doi: 10.1007/s10811-010-9645-0.
6. Panda H. Handbook on Spices and Condiments (Cultivation Processing and Extraction). New Delhi, India: Asia Pacific Business Press Inc.; 2010.
7. Nowack B, Krug HF, Height M. 120 years of nanosilver history: implications for policy makers. *Environ Sci Technol.* 2011;**45**(4):1177–83. doi: 10.1021/es103316q. [PubMed: 21218770].
8. Prasad T, Kambala V, Naidu R. Phyconanotechnology: synthesis of silver nanoparticles using brown marine algae Cystophora moniliformis and their characterisation. *J App Phycol.* 2012;**25**(1):177–82. doi: 10.1007/s10811-012-9851-z.

9. Foxman B, Muraglia R, Dietz JP, Sobel JD, Wagner J. Prevalence of recurrent vulvovaginal candidiasis in 5 European countries and the United States: results from an internet panel survey. *J Low Genit Tract Dis.* 2013;**17**(3):340–5. doi: 10.1097/LGT.0b013e318273e8cf. [PubMed: 23486072].
10. Kannan N, Subbalaxmi S. A Current Perspective. *Rev Adv Mater Sci.* 2011;**27**:99–114.
11. Mehta SK, Gaur JP. Use of algae for removing heavy metal ions from wastewater: progress and prospects. *Crit Rev Biotechnol.* 2005;**25**(3):113–52. doi: 10.1080/07388550500248571. [PubMed: 16294830].
12. Mahdieh M, Zolanvari A, Azimee AS, Mahdieh M. Green biosynthesis of silver nanoparticles by Spirulina platensis. *Scientia Iranica.* 2012;**19**(3):926–9. doi: 10.1016/j.scient.2012.01.010.
13. Yasin S, Liu L, Yao J. Biosynthesis of Silver Nanoparticles by Bamboo Leaves Extract and Their Antimicrobial Activity. *JFBI.* 2013:77–84. doi: 10.3993/jfbi03201307.
14. Dhanalakshmi PK, Azeez R, Rekha R, Poonkodi S, Nallamuthu T. Synthesis of silver nanoparticles using green and brown seaweeds. *Phykos.* 2012;**42**(2):39–45.
15. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. *Biotech Adv.* 2009;**27**(1):76–83. doi: 10.1016/j.biotechadv.2008.09.002.
16. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramirez JT, et al. The bactericidal effect of silver nanoparticles. *Nanotechnology.* 2005;**16**(10):2346–53. doi: 10.1088/0957-4484/16/10/059. [PubMed: 20818017].