

Full Length Research Paper

Noble silver nanoparticles (AgNPs) synthesis and characterization of fig *Ficus carica* (fig) leaf extract and its antimicrobial effect against clinical isolates from corneal ulcer

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Nanotechnology is rapidly growing with nanoparticles produced and utilized in a wide range of pharmaceutical and commercial products throughout the world. In this study, fig (*Ficus carica*) leaf extracts were used for ecofriendly extracellular synthesis of stable silver nanoparticles (AgNPs) by treating an aqueous silver nitrate (1 mM) solution and using the plant *F. carica* leaf extracts as reducing agents. The bioreduced silver nanoparticles were characterized by ultra violet visible (UV-Vis) spectrophotometer, Fourier transform infra-red (FTIR) spectroscopy and transmission electron microscopy (TEM). The average particle size ranged from 5 to 40 nm. The particle size could be controlled by changing the reaction temperature, leaf broth concentration and AgNO₃ concentration. Further, these biologically synthesized nanoparticles concentration of 50 µl were found to be highly effective and exhibited maximum microbial activity with mean zone of inhibition 20.33±1.00 mm and 18.00±1.00 against *Pseudomonas aeruginosa* and *Aspergillus fumigatus* isolated from human corneal ulcer patients. This environmentally friendly green synthesis is an eco-friendly approach to conventional chemical synthesis and can potentially be used in various areas such as food, cosmetics, and medical applications and hope the recent technology can provide next generation of antimicrobials.

Key words: *Ficus carica*, silver nanoparticles, characterization, antimicrobial activity.

INTRODUCTION

In 21st century, the development of green processes for the synthesis of nanoparticles is evolving into an important branch of nanotechnology (Raveendran et al., 2006; Armendariz et al., 2002). Today, nanometal particles especially Silver have drawn the attention of

scientists because of their extensive application in the development of new technologies in the areas of material sciences, electronics, medicine and biolabelling as well as antimicrobials (Magudapathy et al., 2001; Panacek, et al., 2006). Silver has been used as an antimicrobial agent

for centuries, the recent resurgence in interest for this element particularly focuses on the increasing threat of antibiotic resistance, caused by the abuse of antibiotics (Panaek et al., 2006; Sambhy et al., 2006). The use of environmentally benign materials like plant leaf extract, bacteria and fungi for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and biomedical applications as they do not use toxic chemicals in the synthesis protocols (Upendra Kumar et al., 2009). Synthesis of nanoparticles provides advancement over chemical and physical methods as it is a cost effective and environmentally friendly and in this method there is no need to use high pressure, energy, temperature and toxic chemicals (Goodsell, 2004).

Corneal ulceration continues to be one of the most important causes of ocular morbidity and blindness worldwide, bacterial keratitis is considered a leading cause of monocular blindness in the developing world (Solomon et al., 2006). The incidence of infection by specific organisms varies by region, and practitioners should be aware about the local epidemiological patterns of corneal infection. Suppurative corneal ulcers may be caused by bacteria, fungi and protozoa. Moreover, the increasing resistance of many bacteria and the side effects to the currently used antibiotics are documented (Yang et al., 2009), although there were a significant proportion of corneal ulcers reported from Saudi Arabia (Khairallah et al., 1992).

The genus *Ficus* constitutes about 750 species found in tropical and subtropical regions (Subramanian et al., 2013). *Ficus carica*, commonly called "fig" plant, is known to harbor diverse chemical compounds with proven medicinal importance figs (*F. carica*) are cultivated in the Kingdom of Saudi Arabia and the leaf has been reported to have health benefits including anti-diabetic property of *F. carica* are traditionally used to cure throat diseases, constipation, hemorrhoid and high cholesterol. Several researchers demonstrated the medicinal importance of fig plant as an antioxidant (Gond and Khadabadi, 2008), antidiabetic (Patil et al., 2010), hepatoprotective (Jeong et al., 2009), antipyretic (Rubnov et al., 2001), and antimicrobial (Aref et al., 2011). Latex of fig suppresses cancer cell proliferation and has an antiviral potential (Shankar, 2004). Several plants have been utilized for the production of silver nanoparticles (Parashar, 2009; Tripathi et al., 2009). Much attention is now required for synthesis of nanoparticles using biological sources due to limitations associated with chemical and physical methods of nanoparticle synthesis.

In the present study, reducing silver ions present in the

aqueous solution of silver nitrate by the help of *F. carica* extract and their antibacterial assessment was performed to produce novel drugs to overcome drug resistance and adverse reaction. This research study was undertaken to determine the effect of fig leaf extract as antimicrobial against local bacterial and mycotic infectious agents in corneal ulcer, it will be helpful in planning of corneal ulcer management strategy.

MATERIALS AND METHODS

Collection of *F. carica* leaf

F. carica leaves were collected from Riyadh market, and the species was identified with the authenticated specimen from the Department of Agriculture, Qassim University, Kingdom of Saudi Arabia.

Preparation of fig leaf extract

The silver nitrate (AgNO_3) was purchased from Sigma-Aldrich chemicals and the fresh leaf extract used for the reduction of Ag^+ ions to Ag^0 was prepared by taking 20 g of thoroughly washed finely cut leaves in 500 ml Erlenmeyer flask along with 100 ml of distilled water and then boiling the mixture for 5 min before decanting it. Further, the extract was filtered with Whatman No. 1 filter paper and stored at 4°C and used for further experiments.

Synthesis of silver nanoparticles

In a typical experiment, *F. carica* leaf extract (0.5 ml) was added to 10 ml of 1 mM AgNO_3 aqueous solution. The bioreduced aqueous component (0.5 ml) was used to measuring UV-Vis spectra of the solution. The particle suspension was diluted 10 times with distilled water to avoid the errors due to high optical density of the solution.

UV-Vis spectral analysis

Synthesized silver nanoparticles was confirmed by sampling the aqueous component of different time intervals and the absorption maxima was scanned by UV-Vis spectrophotometer at the wavelength of 300 to 800 nm on Perkin-Elmer Lambda 25 spectrophotometer.

FTIR spectral analysis

The bioreduced silver nitrate solution was centrifuged at 10,000 rpm for 15 min and the dried samples were grinded with KBr pellets used for FTIR measurements. The spectrum was recorded in the range of 400 to 4000 cm^{-1} using Thermo Nicolet Nexus 670 spectrometer in the diffuse reflectance mode operating at resolution of 4 cm^{-1} .

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Abbreviations: UV-Vis, Ultra-violet visible; FTIR, Fourier transform infra-red; TEM, transmission electron microscopy.

TEM analysis of silver nanoparticles

Morphology and size of the silver nanoparticles were investigated by TEM images using Phillips, TECHNAI FE 12 instrument. Thin film of the sample was prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid and drying under lamp.

In-vitro antibacterial and fungal activity of silver nanoparticles

Test microorganisms

Microbial cultures of six different strains from bacterial and fungal isolates were used for determination of antimicrobial activity. Gram-positive: *Staphylococcus aureus*, *Streptococcus pneumoniae*, Gram-negative: *Pseudomonas aeruginosa*, *Proteus vulgaris* as well as *Aspergillus fumigates* and *Fusarium spp.* clinical isolates were used. All the strains were sub-cultured at 37°C on Mueller-Hinton agar and potato dextrose agar (Oxoid, Hampshire, UK) every 15 days and stored at 4°C. The isolates were obtained during parallel studies from clinical cases suffered corneal infections and have been subjected to several hospitals at Qassim region during 2012. Sampling, culturing, isolation and identification were done in the Department Medical Laboratories, Qassim University, Kingdom of Saudi Arabia.

Antibiotic susceptibility testing

The test microorganisms were also tested for their sensitivity against the bacterial and fungal drugs Ciprofloxacin (5 µg) and Ketoconazole (30 µg). The cultures were enriched in sterile Mueller-hinton broth for 6 to 8 h at 37°C. Using sterile cotton swabs, the cultures were aseptically swabbed on the surface of sterile Mueller-hinton agar plates and potato dextrose agar (Guzmán et al., 2008). Using an ethanol dipped and flamed forceps, the antibiotic discs were aseptically placed over the seeded agar plates sufficiently separated from each other to avoid overlapping of inhibition zones. The plates were incubated at 37°C for 24 h for bacteria and seven days for fungi the diameter of the inhibition zones was measured in mm. All media used in the present investigation were obtained from Oxoid, Hampshire, UK.

Antibacterial activity by well diffusion method

Antibacterial activity of AgNPs was carried out by agar well diffusion method. Each microorganisms were grown overnight at 37°C in Mueller-Hinton Broth. 100 µl of standardized inoculum (0.5 MacFarland) of each test bacterium were inoculated on molten Mueller-Hinton agar, homogenized and poured into sterile Petri dishes. The Petri dishes were allowed to solidify inside the laminar hood. Standard cork borer of 16 mm in diameter were used to make uniform wells into which was added (50 µl) synthesized silver nanoparticles. Zones of inhibition for control, AgNPs and silver nitrate were measured. The experiments were repeated thrice and mean values of zone diameter were presented.

Antifungal activity by well diffusion method

Potato dextrose agar plates were prepared, sterilized and solidified. After solidification, fungal cultures were swabbed on these plates. Three cavities were made using a cork borer (10 mm diameter) at an equal distance and were filled with the Silver nanoparticle solution (50 µl), then were incubated at 37°C After seven days zone of inhibition was measured, the formation of a clear zone (restricted growth) around the cavity is an indication of antifungal activity.

RESULTS AND DISCUSSION

Synthesis of silver nanoparticles (AgNP's)

After the addition of the extract to the silver nitrate solution, the solution changed from colourless to pale yellow within 2 min, the final colour deepening to brown within 30 min. Figure 1 shows the *F. carica* leaf extract with silver nitrate at initial point of time and after 30 min reaction end point, similar results were reported (Balaji et al., 2008). The brown colour indicated surface plasmon vibrations, typical of silver nanoparticles (Saxena, et al., 2010).

Characterization of silver nanoparticles (AgNP's)

UV-vis is the most widely used technique for the structural characterization of nanoparticles, so the sizes of the synthesized nanoparticles were provisionally predicted on the basis of UV-vis spectrum in the range of 200 to 00 nm. A distinct peak with smooth shoulder was observed at 432 nm (Figure 2). Thus, the UV-vis absorption spectrum reveals the formation of nanoparticles by showing surface Plasmon absorption maxima at 432 nm. Plasmon resonance in nanoparticles is strongly depends on the shape, size and dielectric constant. Noble silver nanoparticles exhibit a strong absorption band in the visible region and giving specific color to the solution (Khandelwal et al., 2010).

Fourier transform infrared spectroscopy (FTIR) measurements are carried out to identify the possible biomolecules responsible for the reduction of the Ag⁺ ions and capping of the bio-reduced AgNP's synthesized by *F. carica* leaf extract. The FTIR spectra of *F. carica* leaf extract and biosynthesized nanosilver are depicted in Figure 3. The appearance of peaks in the amide I and amide II regions is the characteristic of proteins/enzymes that have been found to be responsible for the reduction of metal ions. FTIR analyses confirm that the larger size of the nanoparticles might be due to the capping of nanoparticles by proteins (Warisnoicharoen et al., 2011).

Antibacterial and antifungal analysis

The antimicrobial activity of synthesized silver nanoparticles was investigated using the well diffusion method against different bacterial and fungal such as *S. aureus*, *S. pneumoniae*, *P. aeruginosa*, *P. vulgaris*, *A. fumigates* and *Fusarium spp* these pathogens are treated with 50 µl of AgNP.

Determination of mean zone of inhibition

The mean zone of antibacterial activity of AgNP is

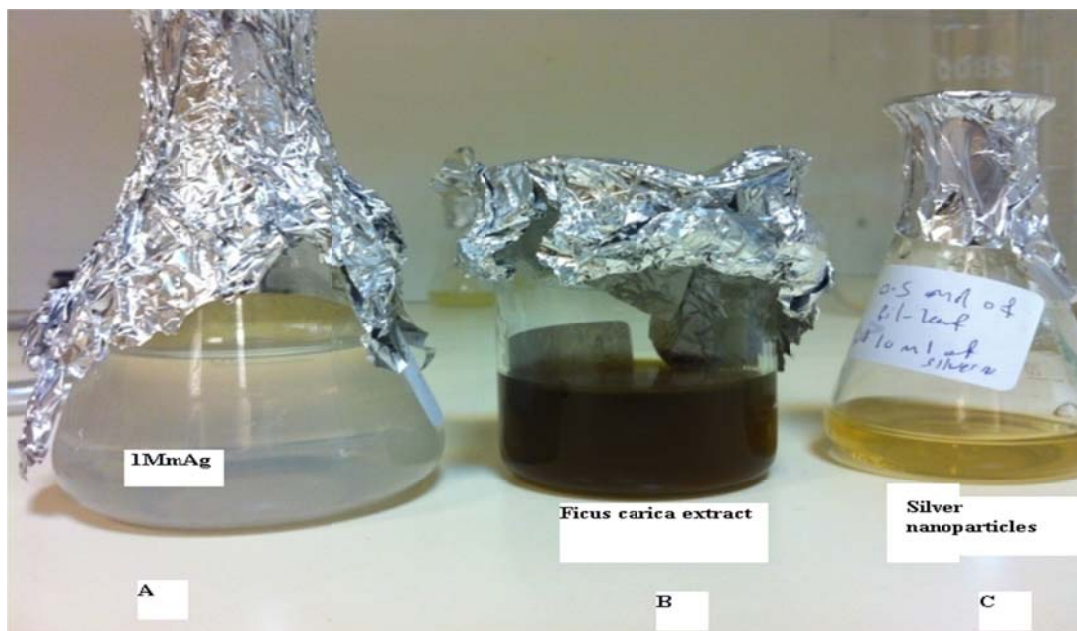


Figure 1. The colour change of plant extracts after addition of silver nitrate (a) 1 mM silver nitrate (b) plant extract (c) silver nanoparticles.

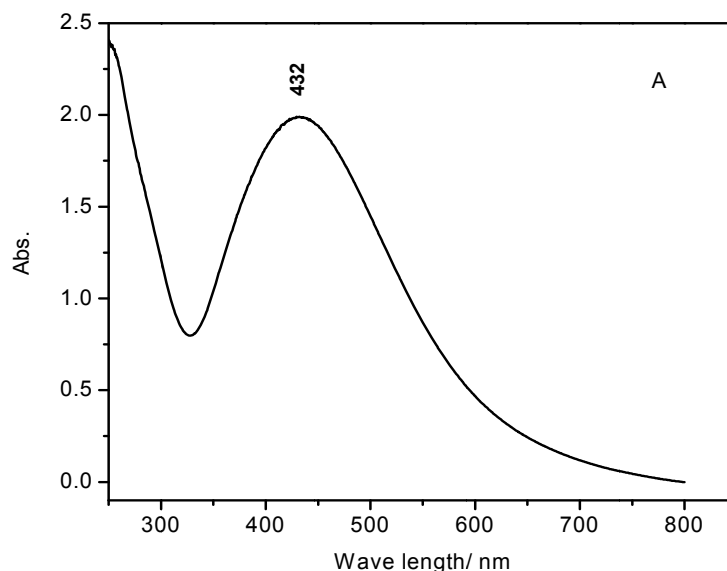


Figure 2. UV absorption spectra of silver nanoparticles. A peak was observed at 419 nm.

presented in Table 1. *P. aeruginosa*, *S. aureus* and *Aspergillus* spp. exhibited highest rate of sensitivity to aqueous extract with mean zone of inhibition of 20.33 ± 1.00 , 19.00 ± 1.00 and 15.33 ± 0.57 mm, respectively, at the test concentration of 50 μ l, which was comparable to standard antibiotic (Ciprofloxacin 5 μ g /disc). The AgNP exhibited lowest activity against *P. vulgaris* and *Fusarium* spp. with mean inhibition zone of, 15.33 ± 0.57 and 14.66

± 0.57 mm, respectively. The biologically synthesized silver nanoparticles were found to be highly effective against different bacteria and fungi of selected species. It shows that, they have great potential in biomedical applications. Similar observation was found in *Allium cepa* (Shahverdi, et al., 2007), indication that the silver nanoparticles have an ability to interfere with metabolic pathways. The result shows the potential biocidal effect

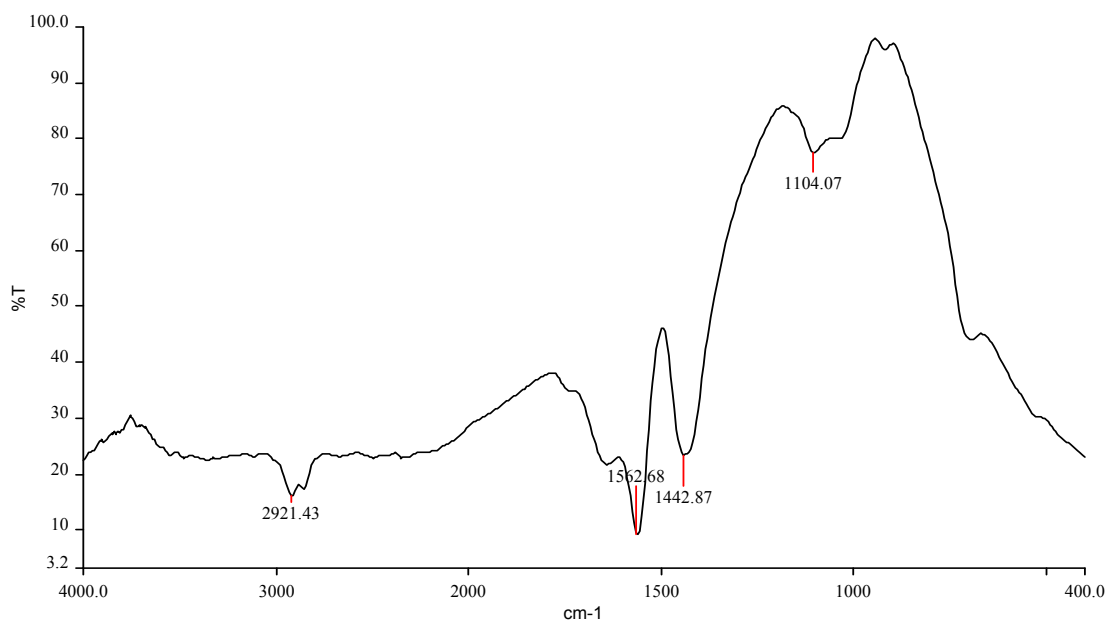


Figure 3. FTIR Spectra of nanoparticles synthesized from *Ficus carica* leaf extract.

Table 1. Mean zone of inhibition (mm) of silver nanoparticles against bacterial and fungal isolates in comparison with standard antibiotic.

Fungus	Mean zone of inhibition (mm) (mean \pm SD)	
	Silver nanoparticles (50 μ l)	Standard antibiotic (ciprofloxacin 5 μ g)
<i>Staphylococcus aureus</i>	19.00 \pm 1.00	27.33 \pm 0.57
<i>Streptococcus pneumoniae</i>	15.33 \pm 0.57	28.66 \pm 1.52
<i>Pseudomonas aeruginosa</i>	17.00 \pm 1.00	30.33 \pm 1.52
<i>Proteus vulgaris</i>	15.33 \pm 0.57	26.00 \pm 1.52
<i>Aspergillus fumigates</i>	18.00 \pm 1.00	27.66 \pm 0.57
<i>Fusarium spp.</i>	14.66 \pm 0.57	28.33 \pm 1.52

against clinical bacterial and fungal isolates.

Transmission electron microscope (TEM) analysis

TEM image of silver nanoparticles derived from *F. carica* leaf extract is shown in Figure 4. The morphology of the nanoparticles was spherical in nature. Under careful observation, it is evident that the silver nanoparticles are surrounded by a faint thin layer of other materials, which we suppose are capping organic material from *F. carica* leaf broth. The obtained nanoparticles are in the range of sizes approximately 5 to 40 nm and few particles are agglomerated. Figure 5 shows the histogram of silver nanoparticles, it is evident that there is variation in particle sizes and the average size estimated 9.5 nm. It may be noted that, the size of the silver nanoparticle obtained from TEM is similar with the size obtained from the FTIR determination. Same phenomenon was reported for the silver nanoparticles synthesized using *P.*

graveolens leaf broth.

Conclusion

Simple, efficient and stable silver nanoparticles were synthesized by using *F. carica* leaf extract. These particles are of uniform size and shape has the potential to kill a broad range of bacteria and fungi. The bioreduced silver nanoparticles were characterized using UV-Vis, FTIR, and TEM techniques and estimated approximately as 5 to 40 nm. These particles may be useful in pharmaceutical area with potential of future development in nano preparations.

Conflict of Interests

The author(s) have not declared any conflict of interest.

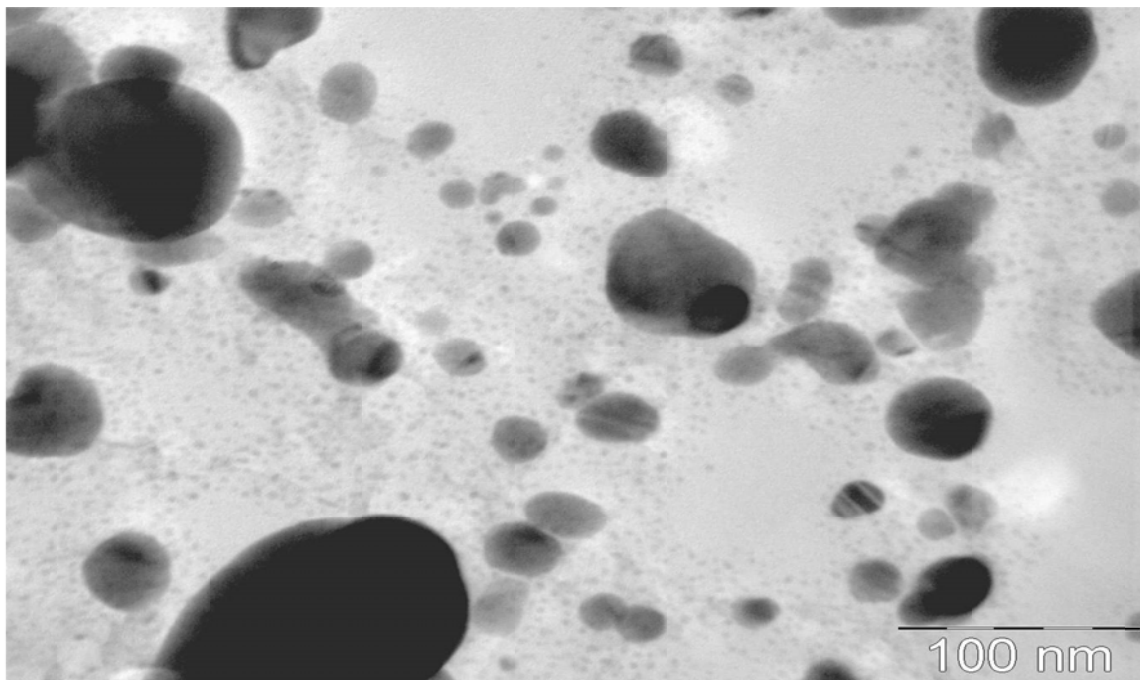


Figure 4. TEM Images of silver nano particles.

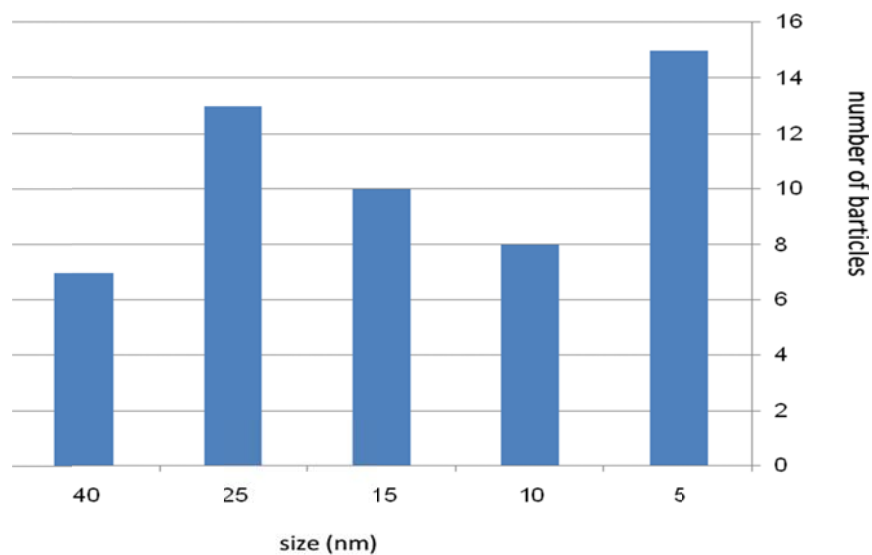


Figure 5. Histogram of synthesized silver nanoparticles.

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