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RESEARCH ARTICLE

BIOSYNTHESIS OF SILVER NANOPARTICLES AND ITS ANTIBACTERIAL ACTIVITY USING BLACK PEPPER (PIPERNIGRUM)

Ebtihal Ahmed Mergani[1] and Ali.A.Ibrahim[2]

[1]Department of Chemistry, College of Art and Science (Samtah), Jazan University. Saudi Arabia
[2]Department of Chemistry, College of science and Humanities, Prince Sattam Bin Abdulaziz University, Alkharj, Saudi Arabia

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ABSTRACT

In this work, the synthesis of stable silver nanoparticles by the bioreduction method was investigated.Aqueous extract of crude black pepper (Piper nigrum) extracted at room temperature was used as reducing. UV-Vis absorption spectroscopy was used to monitor the formation of silver nanoparticles the UV–vis absorption shows a characteristicabsorption peak of silver nanoparticles at 410 nm. Thecharacteristics of the obtained silver nanoparticles were studied alsoby usingFourier transform infrared spectroscopy. The FT-IR spectra of the purified silver nanoparticles were compared withspectra ofcommercially available black pepper. X-ray diffraction analysis revealed the distinctive facets ((1 1 1),(2 0 0), (2 2 0), and (2 2 2) planes) of silver nanoparticles.The synthesized silvernanoparticles had significant antibacterial action on both the Gram classes of bacteria namely: *Staphylococcus aureus, Escherichia coli ,Pseudomonas aeruginosa*and *Bacillus subtilis.*

INTRODUCTION

Nanotechnology is science, engineering, and technology conducted at the nanoscale, which is about 1 to 100 nanometers Nanoscience and nanotechnology are the study and application of extremely small things and can be used across all the other science fields, such as chemistry, biology, physics, materials science, and engineering. Nanotechnology is not just a new field of science and engineering, but a new way of looking at and studying.

Noble metal nanoparticles are being widely used nowadays in the fields of medicine, biology, material science, physics and chemistry [1,2]. Metal nanoparticles have been shown to possess enormous application potential in the areas such as photography, catalysis, biological labeling, photonics, optoelectronics and surface-enhanced Raman scattering (SERS) [3,4]. Traditionally nanoparticles have been prepared and stabilized by a number of physical and chemical methods; of them, chemical reduction, electrochemical techniques and photochemical reduction are being most widely used [5,6]. Of the noble metal nanoparticles, silver is of particular interest because of distinctive properties, such as good electrical conductivity, chemical stability, catalytic and antibacterial activity.

Many techniques of synthesizing silver nanoparticles, such as chemical reduction of silver ions in aqueoussolutions with or without stabilizing agents, thermal decomposition inorganic solvents, chemical reduction and photoreduction in reverse micelles, and radiation chemical reduction [7-10] have been reported in theliterature.

Most of these methods are extremely expensive and also involve the use of toxic, hazardous chemicals,which may pose potential environmental and biological risks.Since noble metal nanoparticles are widely applied to areas of human contact [11], there is a growing need to develop environmentally friendlyprocesses for nanoparticle synthesis that do not use toxic chemicals. A quest for an environmentally sustainablesynthesis process has led to a few biomimetic approaches. Biomimetics refers to applying biological principles inmaterials formation. One of the fundamental processes in biomimetic synthesis involves 2000; bioreduction. Biologicalmethods of nanoparticle synthesis using microorganisms [12], enzymes [13], fungus [14], and plants or

Corresponding author:* **Ebtihal Ahmed Mergani
Department of Chemistry, College of Art and Science (Samtah), Jazan University. Saudi Arabia

plantextracts Shankar *et al*. reported the synthesis of gold and silver nanoparticles by the reduction of aqueous $AuCl_4$and Ag+ ions using extracts from geranium, neem and lemongrass plants and they revealed that the gold nanotriangles showed that ketones/aldehydes present in the extract may play an important role in directing the shape evolution in these nanostructures. Ankamwar *et al*. reported the synthesis of gold nanotriangles using tamarind leaf extract as the reducing agent and identified that tamarind plant as a potential candidate for shape-controlled synthesisof gold nanoparticles due to tartaric acid (–COOH) [15–19].(Chandran *et al*., 2006; Jae and Beom, 2009)[20] have been suggested as possible eco-friendly alternatives to chemical and physical methods. Sometimes the synthesis of nanoparticles using plants or parts of plants can prove advantageous over other biological processes by eliminating the elaborate processes of maintaining microbial cultures [16].

Production of nanoparticles can be achieved through different methods. Chemical approaches are the most popular methods for the production of nanoparticles. However, some chemical methods cannot avoid the use of toxic chemicals in the synthesis protocol. Since noble metal nanoparticles such as gold, silver and platinum nanoparticles arewidely applied to human contacting areas, Biological methods of nanoparticles synthesis using microorganisms [21].

If biological synthesis of nanoparticles can compete with chemical methods, there is a need to achieve faster synthesis rates. The exact mechanism of silver nanoparticles synthesis by plant extracts is not yet fully understood. Only participation of phenolics, proteins and reducing agents in their synthesis has been speculated [22]

In this present study, the synthesis of silver NPs was reported on by using black pepper seed extract at room temperature. This synthesis approach is simple, cost effective, stable for long time, reproducible, eco-friendly and promising for the biosensing and nanoelectronic applications. In addition, the antibacterial activity of the prepared nanoparticles was also demonstrated on Gram-positive and Gram-negative bacteria for finding out the potential of the generatednanoparticles for various environmental and biomedical.

Experimental

MATERIALS

Deionized water, $AgNO_3$, NaOH, Black pepper seeds, were used with high purity and the sample was characterized by SHIMADZU UV spectrophotometer (model Uv-1800) in the range 200 to 600 nm, Fourier transform infrared SHIMADZU (FTIR-8400S) and X-ray diffraction analysis.

METHODS

Preparation of extract

10 g black pepper seeds were boiled in 50 ml deionized water for 10 min. The cooled filtrate was adjusted at pH 12 by adding molar solution of NaOH.

Synthesis of silver nanoparticles

100 ml solution of 0.10 g$AgNO_3$was prepared in a conical flask; to this solution 10.0 ml of the prepared extract was added drop by drop. The above solution was stirred on magnetic stirrer for 2 h and centrifuged at 3000rpm for 10 min, supernatant decanted off and residue collected. Residue was washed three times with deionizedwater. Finally, residue was characterized by various spectroscopic techniques.

The proposed synthesis was illustrated in Fig. 1.

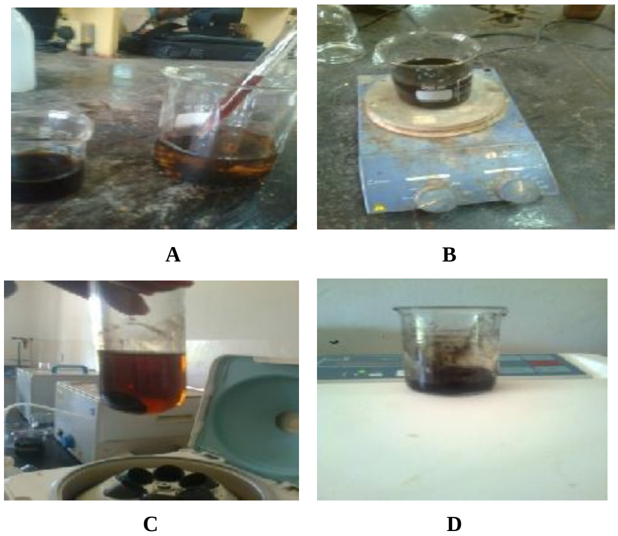


Fig.1. Shows photos representation of silver nanoparticles synthesis: (A)mixing of extract in $AgNO_3$ solution, (B) Stirring process,(C) Centrifugation and (D) formation of silver nanoparticles.

Characterization

Fourier transforms infrared spectroscopy

The powders of the silver nanoparticles were then subjected to FTIR spectroscopy measurements. The measurements were carried out on a SHIMADZU (FTIR-8400S) instrument in the diffuse reflectance mode at a resolution of KBr pellets. For comparison, black pepper powders used as control. **Figure: 3.**

UV–Visible spectroscopy

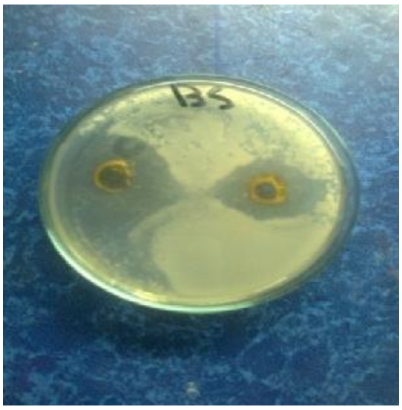
Preliminary characterization of the silver nanoparticles was carried out using UV–Visible spectroscopy. The reduction of silver ions to the nanoparticle form was monitored by measuring the UV–Visible spectra of the solutions after diluting the sample with deionized water 20 times. The spectrawere recorded on SHIMADZU UV spectrophotometer (modelUv-1800) from 200 to 600 nm. Deionized water was used as blank. **Figure: 4.**

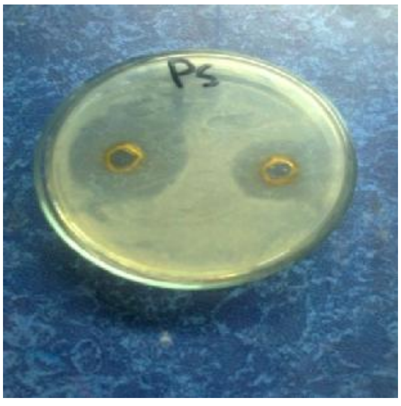
X-ray diffraction

The purified powders obtained after 2h of interaction under laboratory conditions was subjected to X-raydiffraction analysis (PW304160-X pert proconscale, PHLIPS) and the anode material was Cu with a wavelength of 1.54060A˚. The generator was operated at 40 kV and with a 50mA current. The scanning range was selected between 0.0◦ and 70◦.**Figure: 5.**

Antibacterial assay

The cup-plate agar diffusion method was adopted to assess the anti-bacterial activity of the prepared silver nanoparticles solution. *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Bacillus subtilis* were used for antibacterial study. 0.6 ml of standardized bacterial stock suspensions (10^8-10^9) colony-forming units per ml was thoroughly mixed with 60ml of sterile nutrient agar. 20 ml of the inoculated nutrient agar were distributed into sterile Petri dishes. The agar was left to set and in each of this plates 2cups, 10 mm in diameter, were cut using a sterile cork borer No.4 and the agar disk were removed. Alternate cups were filled with 0.1 ml (10mg/ml) of silver nanoparticles solution using micro-titer pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at $73^{O}C$ for 24 hours. Two replicate were carried out for silver nanoparticles against each of the test organism. After incubation the diameters of growth inhibition zones were measured, as in Fig. 2.





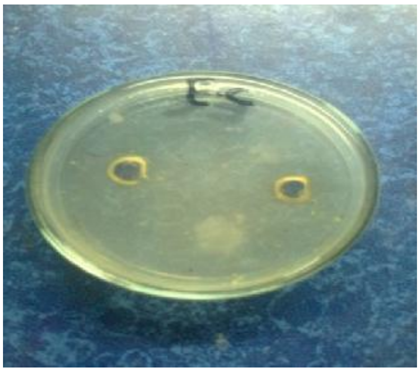


Fig. 2 Bacterial culture plates showing the inhibition zones around wells loaded with silver nanoparticles.

RESULTS AND DISCUSSION

The development of green processes for the synthesis of nanoparticles is evolving into an important branch of nanotechnology, because biological methods are considered safe and ecologically sound for the nanomaterial fabrication as an alternative to conventional physical and chemical methods. The green synthesis techniques are generally synthetic routes that utilize relatively non-toxic chemicals to synthesize nanomaterials, and include the use of non-toxic solvents such as water, biological extracts, biological systems and microwave assisted synthesis.

Commercially available black pepper seeds have been used for the synthesis of silver nanoparticles at room temperatures.

Proposed mechanism of reduction

Black pepper broth is known to contain several biomoleculessuch as alkaloids, proteins, polysaccharides, amino acids and vitamins. These biomolecules could be used as bioreductants to react with metal ions and they could also be used as scaffolds/template to direct the formation of nanoparticles in solution. The mechanism responsible for the reduction may postulate as follow: the silverions were trapped on the surface of proteins/or predominated alkaloidpiperine in the extract via electrostatic interactions. This stage was the recognition process. The silver ions were then reduced by the proteins leading to changes in their secondary structure and the formation of silver nuclei. The silver nuclei subsequently grew by the further reduction of silver ions and their accumulation of the nuclei.

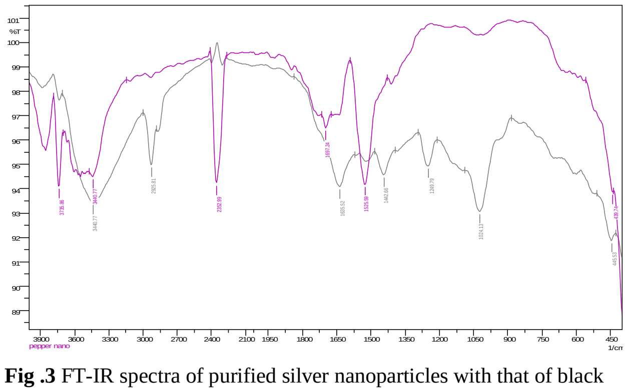


Fig .3 FT-IR spectra of purified silver nanoparticles with that of black pepper powder.

Fig. 3shows the FT-IR spectra of the purified silver nanoparticles and commercially available black pepper.

The major absorbance bands present in the spectrum of black pepper were at 3440, 2925, 1635, 1442, 1249 and 1024cm^{-1}. While, the spectrum of silver-nanoparticles showed characteristic absorbance bands at 3735, 3440, 2352, 1696 and1525 cm^{-1}.

Characteristic bands found in the infrared spectra of proteins and polypeptides include the Amide I and Amide II. These arise from the amide bonds that link the amino acids. The absorption associated with the Amide I band leads to stretching vibrations of the C=O bond of the amide at (1635), absorption associated with the Amide II band leads primarily to bending vibrations of the N—H bond . Because both the C=O and the N—H bonds are involved in the hydrogen bonding that takes place between the different elements of secondary structure, the locations of both the Amide I and Amide II bands are sensitive to the secondary structure content of a protein.

This concludes that the compounds attached with silver nanoparticles could be protein and bound amide region.

Fig. 4 shows UV–vis absorption spectrum of the sample, recorded in the wavelength range of 300–800 nm. The shape of the plasmon band is almost symmetrical, suggesting that the nanoparticles are well-dispersed and uniform in shape. If nanoparticles are not uniform, then it leads to a broad absorption peak at higher wavelength and splitting of a plasmon band into two bands [18].

Silver nanoparticles are known to exhibit a size-dependent characteristic surface plasmon resonance band that can be measured using UV–vis spectroscopy. We observed a characteristic surface plasmon absorption band at 410nm in our silver nitrate solution incubated with the black pepper broth at 2 h. The plasmon bands are broad with an absorption tail in the longer wavelengths. This broadening of the plasmon band could be in principle due to the size and shape distribution of the particles.

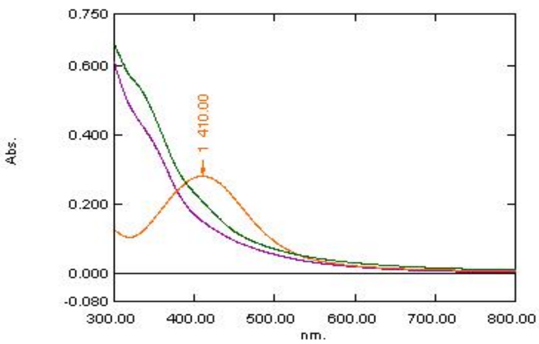


Fig. 4 ShowsUV–vis absorption spectra of silver nanoparticles formation. (▬)AgNPs after termination of reaction, i.e., powdered sample,

(▬)NaOH treated black pepper extract (pH 12) and
(▬) black pepper extract without NaOH

As expected, black pepper exhibited silver to precipitation.[23]The main biochemical difference between the silver precipitating black pepper and alone is the overall charge of the biomolecules. Based on these assumptions, the silver-surfacingbiomolecules including predominant alkaloids (especially piperine and piperidine) are responsible for precipitating silver from an aqueous reaction solution medium of silver ions. Several basic amino acids as well as other non-silver surfacing bioconstituents may also be responsible for the precipitating silver from the same.

UV–vis spectra in Fig. 4revealed as: After termination of reaction or collected powder solution show speak suggesting desired Ag NPsformation via black pepper assisted approach (▬). Spectrum (▬) shows the black pepper broth with NaOH treated (maintained atpH 12), suggesting many peaks at UV wavelengths suggesting the basic amino acids or alkaloids, they might play role for AgNPsformation. Whereas, spectrum (▬) shows the black pepper broth without NaOH treated, no peak appeared.

Fig. 5shows XRD pattern of the dried sample which confirms the face centered-cubic (fcc) lattice with strong diffraction peaks at 38.2,44.5, 64.7 and 77.7 degrees of 2θ which corresponds to (1 1 1), (2 0 0),(2 2 0) and
(3 1 1) crystal planes.

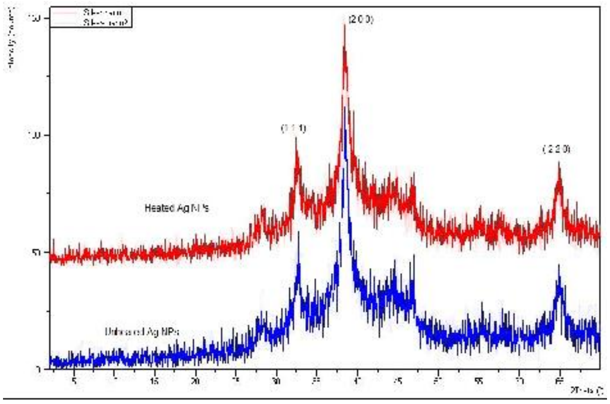


Fig.5 Shows XRD pattern of silver nanoparticles synthesized by biomimeticmethod using black pepper.

There was no significant change in the peak position of silver nanoparticles formation before and after heating the sample as depicted in Fig. 5. No broad amorphous peak in between 20◦ and 35◦ of 2θ was appeared before and after heating of the sample, suggesting that the black pepper extract acts as a reducing agent not as a template (substrate). Even though, in the heated sample, no other diffraction peaks could be detected, i.e., phase-pure metal silver formation. In the XRD pattern, onesharp and strong diffraction peak centered at 38.2◦ was appeared, which can be indexed to the (1 1 1) reflection of the metallic silver with fcc structure. The weak diffraction peaks at 44.5◦, 64.7◦ and 77.7◦ in the pattern agrees well with the(2 0 0), (2 2 0) and (3 1 1) reflections. Thus, the XRD pattern suggesting that the product is purely silver nanoparticle with highcrystallinity.

The size of the particle was calculated by means of the Debye–Scherrer equation. The Debye–Scherrer equation was used to derive the particle size from the XRD data by determining the width of the (1 1 1) Bragg reflection (the facet with maximum intensity) according to **Fig. 5.**The equation:

$$L=\frac{k\lambda}{\beta\cos\theta}$$

Where L is the particle size (nm), k is the Scherrer constant, β is the Full Width Half Maximum, θ is half of Bragg angle and λ is the wavelength of X-ray [24]. The size of the particles was determined to be 25 nm

Antimicrobial activity

The antibacterial activity of silver nanoparticles we reanalyzed by well diffusion assay. The(2o-21 mm) clear inhibitory zone appeared around silver nanoparticles against S. aureus after incubation for 24 h followed by Bacillus subtilis (20-21mm), and E. coli (33mm), suggesting that synthesized nanoparticles showed phenomenal bactericidal effect (Figure 2).Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Bacillus subtilis were used for antibacterial studythis observation is in excellent agreement with earlier studies [25].

The mechanism of the bactericidal effect of silver colloid particles against bacteria is not very well-known. Silver nanoparticles may attach to the surface of the cell membrane and disturb its power function such as permeability and respiration. It is reasonable to state that the binding of the particles to the bacteria depends on the surface area available for interaction. Some nanoparticles penetrate into the cell and bind with DNA interrupting some gene expression necessary for important metabolism. Smaller particles having the larger surface area available for interaction will give more bactericidal effect than the larger particles [25]. This suggests the possibility that the silver nanoparticles may also penetrate inside the bacteria and fungi, causing damage by interacting with phosphorus- and sulphur-containing compounds such as DNA. One more possibility would be the release of silver ions from nanoparticles, which will have an additional contribution to the antimicrobial properties of silver nanoparticles. Currently, the increase of bacterial resistance to antimicrobial agents poses a serious problem in the treatment of infectious diseases as well as in epidemiological practice. Increasingly, new bacterial strains have emerged with dangerous levels of resistance, including both of Gram-positive and Gram-negative bacteria. Dealing with bacterial resistance will require precautions that lead to prevention of the emergence and spreading of multi-resistant bacterial strains and the development of new antimicrobial substances [26].

Our findings could be targeted for the promising potential applications including water purification, recording media, biosensing devices, nanoelectronic, and catalysis. Silver nanoparticles are finding a variety of applications starting from biological tagging to electronic devices

CONCLUSIONS

An interesting room temperature green synthesis method has been developed for the preparation of silver nanoparticle using black pepper extract at room temperature. The formation of silver nanoparticle by biomimetic route opens the new avenues over chemical routes because of its cost effective and eco-friendly nature. The characteristics of the obtained silver nanoparticles were studied using UV-Vis, FTIR and XRD techniques. The results confirmed the reduction of silver nitrate to silver nanoparticles with high stability and without any impurity. The alkaloid piperine (or few basic amino acids or both) of the black pepper seed extract plays a leading role for the formation and stabilization of Ag NPs, respectively. The formed silver nanoparticles are highly stable and had significant antibacterial action on both the Gram classes of bacteria.

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