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RESEARCH ARTICLE

Silver nanoparticles Synthesis by coffee residues extract and their antibacterial activity

Mahnaz Baghaienezhad[1*], Meshkat Boroghani[2], Romina Anabestani[3]

[1] Department of Nanotechnology, Asrar student research center. Sabzevar, Iran

[2] Department of Pharmaceutics, Faculty of Pharmacy, Mazandaran University of Medical Sciences, sari, Iran

[3] Asrar student research center. Sabzevar, Iran

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ABSTRACT

Objective(s): Nowadays, nanotechnology is growing rapidly due to its high application in science and industry. Particularly in recent years, the synthesis of green nanoparticles has been of great interest with plant extracts. It is a simple, inexpensive and environmentally friendly method. The possibility of synthesizing nanoparticles from vegetable wastes has been investigated in this article.

Methods: coffee powder was used to produce nanoparticles. Silver ions were found to decrease with coffee extract and resulted in the formation of silver nanoparticles crystals. The properties of nanoparticles synthesized by coffee powder were analyzed by various methods such as UV spectroscopy, scanning electron microscopy (SEM) and X-ray diffraction. In addition, antibacterial activity of synthesized nanoparticles against two bacteria *Escherichia coli and Pseudomonas aeruginosa* was done by paper disk and optical absorption method.

Results: The formation of silver nanoparticles was confirmed (by the presence of an absorption peak at 460 nm) using a spectrophotometer. The images of the electron microscope showed that the nanoparticles were spherical and had an average size about 50 nm. The X-ray diffraction has clearly proven the field of silver nanoparticles. The results of microbial tests also indicated that the synthesized nanoparticles had an appropriate effect on the two tested bacteria

Conclusions: This experiment showed that coffee residues extracts can be used for green synthesis of nanoparticles, which also have an appropriate antibacterial effect.

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INTRODUCTION

Nanotechnology is one of the most novel research area in modern material science which is based on nanoparticles [1]. Nanoparticles are materials with a three-dimensional structure, varying in size from 1 to 100 nm. These materials consist of dozens or hundreds of atoms or molecules that include various shapes such as crystalline, spherical, needle, shapeless, and so on [2]. Metal nanoparticles such as gold and silver have special optical, electrical and magnetic properties that make them widely used in research and industrial activities. Among the various properties of metallic nanoparticles, its optical properties are more prominent has taken. The optical properties of metal nanoparticles are due to phenomena called surface plasmonic resonance. Among the applications of metallic nanoparticles are: Their application in the field of electrical and thermal conductors, sensors and optical and electrochemical detectors, antibacterial materials, materials Superparamagnetic [3]. Mineral nanoparticles have many potential application for medical imaging and disease treatment, and they are used extensively for their diverse features, such as high availability, good environmental

* Corresponding Author Email: *m_baghaienezhad@yahoo.com*

compatibility, their ability to transfer drugs, and control of drug release[4,5]. Several commercial silver base medicines such as silver sulphadiazine are available for the treatment of burn and the chronic wound infected with microbes. Silver nano gels/sprays are used in cosmetic and drug industries for medical purposes.[6] Different methods such as chemical and photochemical reactions in reverse micelles, thermal decomposition of compounds using radiation, electrochemical, sonochemical, micro wave processing, etc. are used to produce nanoparticles[7]. Unfortunately, in the most nanoparticles production methods the use of hazardous materials is a coercion. Other disadvantages of these methods include low production of nanoparticles, high energy dissipation [8]. Hence, the need for a high-efficiency, low-cost, non-toxic and non-hazardous method is increasing. One of the production methods for nanoparticles is biodegradation, and the focus is on increasing the production of nanoparticles [9]. A huge inventory of resources used in the biological production of metallic nanoparticles is available. Plants, plant products, algae, fungi, yeasts, bacteria and viruses are used in the biological production of nanoparticles [9,10]. Herbal extracts prepared from leaves, stems, roots, flowers and seeds of plants, due to differences in compounds, have different effects on the amount and characteristics of produced nanoparticles[11]. Plants have a lot of natural regenerative and stabilizing substances. Plants are widely distributed, easily accessible and are the source of different metabolites [12]. Effective phytochemicals in the production of nanoparticles include terpenoses, flavonoids, ketones, aldehydes and carboxylic acid[13]. In addition, proteins, enzymes and other reducing agents have a key role in metal nanoparticles production by plants[14]. The biological synthesis methods produce nanoparticles of a better morphology and defined size as compared to some of other physicochemical production methods [15,16]. The bioaccumulation of nanoparticles is based on the presence of enzymes and proteins involved in their preparation. Nanotechnology plays a significant role in smart drug delivery systems. In these systems, the drug is protected and maintained from the site of entry to the tissue[17]. The goal is to use nanoparticles, reduce drug destruction, prevent side effects, increase drug access, and accumulate drug in the lesion. Examples of these biocompatible nano-scale drug carriers include liposomes, solid lipid nanoparticles (SLN), nanobubbles , micelles and polymers[18] .The use of protein nanoparticles as a drug carrier and protector has been of interest in recent years.[19] .These nanostructures can be synthesized by using protein like albumin, whey protein, gelatin, legumin, gliadin, soy protein, elastin, zein and milk protein. The techniques for their fabrication include emulsification, desolvation, complex coacervation, and electrospray [20]. The recovery of nanoparticles from plant tissues is tedious and expensive and requires enzymes to degrade the cellulosic tissue of the plant [20]. Hence, it is easier to use plant extracts in a large scale and processor to provide various metallic nanoparticles [21]. In recent researches, the use of plant extracts for the preparation of metal nanoparticles has been proposed as an easy and suitable method compared with chemical and physical methods [21]. For the first time, extracts of geranium from leaves, stems, and roots were extracted for the extracellular production of gold nanoparticles [22]. We first investigated various metabolites of coffee extracted from different sources. After determining the types of alkaloids in it, we made bio-nanoscale production of coffee powder. ***Coffea*** is a Flowering plant of Rubiace family that grows in tropical regions. The genus *Coffea* L. comprises more than 100 species of which only two species, that is, *C. arabica* (arabica coffee) and *C. canephora* (robusta coffee), are commercially cultivated [23]. Its height is between 3 and 10 meters and is 3 to 5 years after planting. The white and fragrant flowers of coffee are very similar to of jasmine flowers. The fruits of the egg are frozen and clustered. Each coffee tree has about 2,000 fruit [24]. Its seeds are called coffee beans and are used differently. Between 6 and 9 months it takes time to turn the fruit of the coffee from green to yellow, then red or violet, and reach perfectly. They take the fruit juice with the hand, they are sipped and usually dry in the sun [25]. There are, of course, industrial methods for drying coffee. Coffee beans contain two types of alkaloids, caffeine and trigonelline, as major components. In leaves and in fruits, in addition to caffeine, there are adenine, xanthine, hypoxanthine, and also guanine [26].

MATERIALS AND METHODS

Chemical and *Materials*

Silver nitrate from Merck was used for this study. Coffee powder was purchased from one of the reputable brands and its bulk was obtained. The bacteria used in this study were two gram

negative types of bacteria *Pseudomonas aeruginosa* and *Escherichia coli*, which were obtained from microbiological department of Hakim Sabzevari University.

Silver nanoparticles Synthesis (AgNps)

3 grams of coffee was dissolved in 30 ml of sterilized double distilled water and then centrifuged. The extract was filtered through whatman filter paper no1 and stored at -15 °C. 1 mM silver nitrate solution was prepared and stored in amber bottles. Then, 2 ml of the extract was added to 10 ml of 1 mM silver nitrate and was incubated at 37°C in a dark and stationary condition.

Characterization of synthesized silver nanoparticles
Spectrophotometer technique

In the study of the production of silver nanoparticles, after determining the color of the reaction solution to dark brown, the determination of the optical density of the solution containing nanoparticles was performed by a spectrophotometer apparatus at a wavelength of 300 to 700 nm.

Scanning electron microscope (SEM)

10 ml of an extract suspension containing synthesized silver nanoparticles were ultra-sonic for 5 minutes. Then some of it was poured onto the foil and exposed to the air at room temperature without using heat. The surface morphology and particle size was analyzed using SEM.

X-ray diffraction (XRD)

After the synthesis of silver nanoparticles was completed, the nanoparticulate colloidal solution was centrifuged for around 10,000 minutes to biosynthesize the nanoparticles. Then the supernatant was discarded and, in order to wash and disperse the deposited nanoparticles, adding the water twice ionized, the centrifuge was repeated 3 times. After each time the centrifuges of the supernatant phase were separated and water was added to the settled substance twice, ionized. After centrifugation, the remaining suspension was placed on a silicone plate and dried specimen was used for XRD analysis. The crystalline nature of silver nanoparticles was examined by XRD analysis.

Antimicrobial activity

In this study, a MacroFarland solution was first prepared, with 1: 1.175% of BaCl2.2H2O and 2: solution of 1% Sulphuric Acid (H2SO4), then 0.5 ml of solution 1 was added to 99.5 µl of solution 2. The solution is a semi-Fermented solution, and it can be used to estimate the number of bacterial cells during induction by comparing the pre-sampled opacity with this solution**.** In the laboratory, a bacterial concentration of 0.5 McFarland was prepared from the 24-hour culture of the bacteria. *Pseudomonas sp.* was cultured on a plate containing NA culture medium by loop with linear culture method. Then, a paper disk soaked in a solution of nanoparticles and several disks impregnated with chloramphenicol antibiotics, erythromycin and penicillin were also compared to the culture medium. By gradually releasing antibacterial agents from the disk, the bacteria around the disk are destroyed and the diameter of the zones of inhibition was measured around the discs after 3 to 4 days. For *Ecoli*, after initial culture, a dilution with different concentrations of nanoparticles (200 and 300 µg / ml) was prepared in liquid test tubes, and a tube without nanoparticles was cultured as control. Subsequently, these tubes were incubated in The temperature was 37 ° C for 24 hours, and at different times, the absorption rate of the cells was read and the growth pattern of the bacteria was obtained over time.

RESULTS

The development of pale yellow color in the sample is due to the reduction of silver ion nanoparticles. The active compounds in the plant extract reduce Silver nitrate solution, which is visually notable by color change (Fig1). Using UV–vis spectrophotometer analysis, the formation and stability of the reduced silver nanoparticles in the colloidal solution was observed. The UV–vis spectra showed maximum absorbance at 460 nm (Fig.2). Microscopic images of the nanoparticles produced by the coffee extract are shown in Fig 3. The images show that the shape of the nanoparticles is spherical and the nanoparticles have an average particle size of 50 nm. Of course, due to the presence of organic compounds in this plant, the impurities in the image are definitely observed. According to Fig. 4 of the XRD pattern, Four intense and sharp peaks at 2q = 38.16, 44.28, 64.51 and 77.45 can be indexed to the 111, 200, 220 and 300 planes of Bragg's reflection of silver, respectively. Thus the XRD pattern indicated that the AgNPs organized by the reduction of Ag+ ions by the coffee aqueous extract.

(A) *(B)*

Fig. 1. The color change of the reaction solutions before the production of silver (A) nanoparticles and after the production of nanoparticles(B)

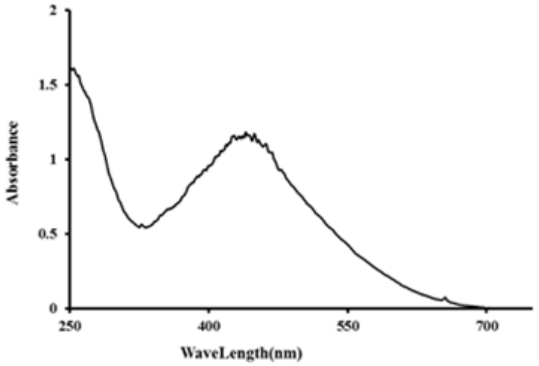

Fig. 2. UV–Vis spectra of synthesized AgNPs using coffee residues

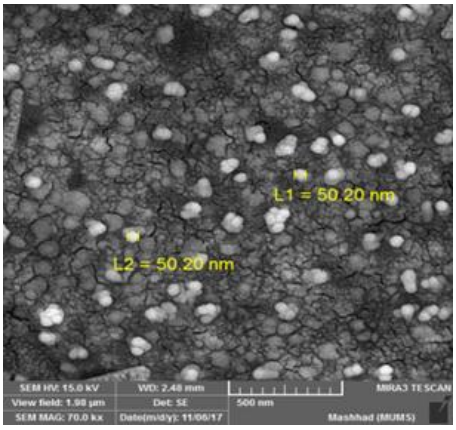

Fig. 3. SEM image of silver nanoparticles synthesized by coffe residues

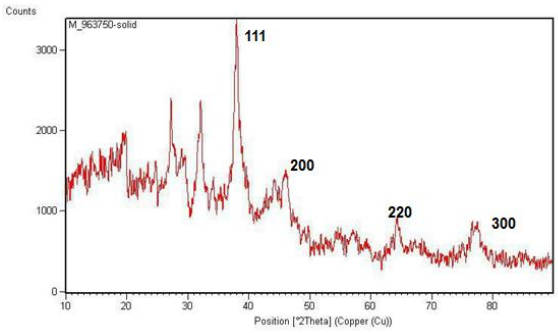

Fig. 4. X–ray diffraction patterns of synthesized AgNPs using coffee residues

Antibacterial assay

The results of antibacterial tests showed the proper effect of the silver nanoparticles produced against the target bacteria. The diameter of the zones of inhibition. is higher than that of other antibiotics in *Pseudomonas aeruginosa* (Fig. 5). The culture medium of bacterial cells of the *E. coli* treated with silver nanoparticles shows that nanoparticles can inhibit the growth and production of bacterial cells. And after 12 hours, almost all bacteria have been destroyed. It was shown that the concentration of 200 µg / ml and 300 µg / ml was better (Fig. 6)

DISCUSSION

The nanoparticle bio-production method is one of the new low-cost and low-risk new ways to produce nanoparticles. In this study, nanoparticles production and ways to diagnose and evaluate nanoparticles produced by aqueous extract of coffee were studied. Also, the antibacterial effects of nanoparticles produced were investigated**.** *Pseudomonas aeruginosa* is an opportunistic pathogen bacteria that is very common due to inherent and acquired resistance to common antibiotics and mortality due to infections [27]. By reviewing the articles and studying the history of *Pseudomonas aeruginosa*, It turns out that this bacterium is an important microbial component in Industrial and agricultural. This pathogen is one of the most common causes of pneumonia. Researchers have done a lot of research and have achieved good results but are still considered a medical challenge [28]. Increasing resistance of bacterial to antibiotics has led to an increase in antibiotics usage. The mechanism of the effect of this substance is how these particles bind to the level of bacteria and metabolism in the organism. However, the use of these particles also has certain

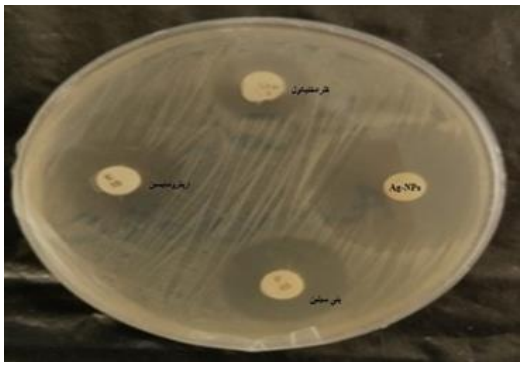

Fig. 5. Antibacterial activity of silver nanoparticles against Pseudomonas aeruginosa

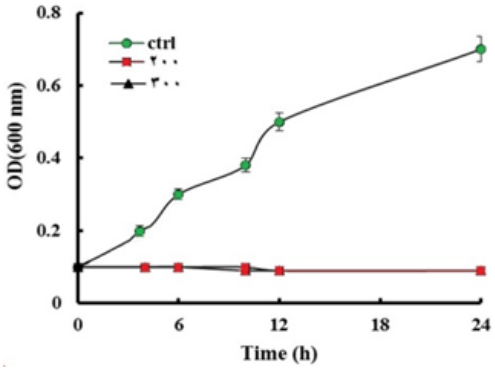

Fig. 6. The growth pattern of Escherichia coli in the presence and absence of silver nanoparticles (concentrations in µg / ml)

limitations [29]. The silver ion anti-explosion mechanism is well-defined. The positive charge of silver ion in this activity is very important because it can have an electrostatic reaction with the negative loads contained in the membrane and thus attach to the membrane [30]. Therefore, plasma and bacterial membranes, which are the enzyme accumulation center and DNA, are the target of silver ions [31]. The size of the cell decreases and the cellular structure is damaged [32]. The antibacterial property of silver nanoparticles in gram-negative bacteria depends on the concentration of nanoparticles and the accumulation of Pits in the cell wall [33]. These nanoparticles accumulated in membrane Causes the permeability of the cell membrane and gradual cell death. Of course, new reports by Danilczuk and colleagues about electron paramagnetic resonance signal the release of free radicals by silver nanoparticles, saying that membrane wall damage may be due to the destructive effects of these free radicals [34].The chemical methods of preparing these nanoparticles lead to the residual of some toxic reactants and the non-use of nanoparticles in bio applications. The production of nanoparticles by using the principles of green chemistry has found a special place in research, and for this purpose various types of biological systems are used; micronutrients, diatoms, including these systems, but these systems are less used due to their high cost of production and maintenance. Plants and crops have been given special attention as cheap and renewable resources for the production of biological nanomaterials [34].

CONCLUSION

In this research for the first time, the biosynthesis of stable and nearly spherical silver nanoparticles using the coffee residues as a reducing and capping agent was studied. The biosynthesized nanoparticles showed strong antimicrobial activity against Gram-negative (Pseudomonas aeruginosa and E. coli) bacteria. Moreover, the formation of silver nanoparticles using the coffea residues was confirmed using XRD, UV–Vis, and SEM. UV–Vis peak for AgNPs was observed at 460 nm. By XRD method, the synthesized AgNPs were found to have a crystalline structure. The average size of 50nm for the silver nanoparticles was observed .

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CONFLICT OF INTEREST

None declared.

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