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RESEARCH ARTICLE

Study of antibacterial performance of synthesized silver nanoparticles on Streptococcus mutans bacteria

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ABSTRACT

Green Synthesis is a method for synthesizing nanoparticles using protein, carbohydrates, plant extracts, and similar structures, which has made it a simple, cost-effective, environmentally friendly, and repeatable method compared to chemical methods. This study was conducted to investigate the antibacterial effect of silver nanoparticles (Ag-NP), synthesized by the extract fruit of *Prosopis fracta*, on *Streptococcus mutans* bacteria as the cause of tooth decay. The synthesized nanoparticles were identified through the UV-Vis spectrophotometer, transmission electron microscope (TEM), and powder X-ray diffraction (PXRD) methods, while their antibacterial effect was evaluated by the usage of microbroth dilution test. UV-Vis spectroscopic analysis displayed the presence of a peak in the range of 425-445 nm and indicated the successful synthesis of nanoparticles in the extract. Meanwhile, the average size of our product was determined by the TEM image to be about 30 nm. According to the investigation results, the synthesized nanoparticles exhibited satisfying antibacterial effects against the studied bacteria.

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INTRODUCTION

The science of nanotechnology is based on nanoparticles, which are recognized as materials with three-dimensional structures and the size variance of 1 to 100 nm[1, 2]. Nano particles can be produced through the application of green and non-green methods. There are non-green chemical and physical approaches[3]. The chemical routes require the application of toxic chemicals for the preparation and stabilization of nanoparticles, which lead to the production of by-products that are incompatible with the environment. Additionally, chemical manufacturing often implicates the adsorption of some toxic substances by the surface of nanoparticles and therefore, the products impose the threat of inducing harmful effects in the course of their medicinal usage. Also, there are also some disadvantages to the application of physical methods, among which one can mention the requirement of sufficient space, energy, and time. However, the benefits of plants exertion for the synthesis of nanoparticles include biological safety, non-toxicity, cheapness, and the available wide variety of metabolites that are involved in ion reduction[3].

Nowadays, nano technology facilitated the possibility of creating metallic silver in the

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form of particles with a size of less than 100 nm and accommodation of 10,000 to 15,000 silver atoms, which are labeled as silver nanoparticles or nanosilver[4]. The tremendous revolution of nano silver production technology in antibacterial materials, which is the main direction of these products development, provided many advantages over the chemical materials[4, 5]. These particles proved to contain special physico-chemical properties and biological activities antibacterial agents against a wide range of antibiotic-resistant bacteria [6]. Due to their surface charge and high surface-to-volume ratio, Nanomaterials, especially metal nanomaterials, can inactivate the enzymes and DNA of microorganisms with an electron balance between electron-donating groups such as thiol, carboxylate, amide, imidazole, indole, and hydroxyl[6]. Tooth decay is an infectious-microbial disease that stands responsible for the dissolution and destruction of dental calcareous tissues[7, 8]. The failure of symptomatic and restorative treatments is inevitable regardless of the underlying cause of a disease. Treatment and prevention of caries with antibiotics and steroids can alter the oxidation-regeneration potential of saliva, weaken the activity of lysozyme, facilitate the conditions for allergic reactions, and reduce the body's resistance to pathogenic factors[7].

The antimicrobial properties of silver compounds have been known for many years[9-11]. However, their recent discovery in the form of nanoparticles extended the contact surface of silver and has increased and itsempowered its antimicrobial property up to more than 99%. it has been shown the dependency of the antibacterial effect of silver nanoparticles on their size and shape[12]. The research of Espinosa *et al* displayed the strong antibacterial properties of silver nanoparticles against *Streptococcus mutans* bacteria. According to their results, the minimum inhibitory concentration of silver nanoparticles can be halved by reducing their size from 100 nm to 16 nm, which expresses the effect of smaller sizes on increasing the antibacterial properties of this product[13].

Considering the resistance of studied pathogenic strains to many common antibiotics, it is necessary to find alternative substances with the ability to inhibit their growth. Since the oral environment contains many bacterial species, the application of chemical drugs in the oral cavity can be associated with side effects such as the occurrence of changes in microbial flora. Therefore, this study attempted to synthesize silver nanoparticles (Ag-NP) through the usage of *Prosopis fracta* fruit and also evaluate its antimicrobial effects on *Streptococcus mutans* bacteria.

MATERIAL AND METHODS

Extraction of fruit of P. farcta

To prepare the extract, the fruits of *P. farcta* were washed and dried at room temperature to be crushed, weighed, and added to distilled water at a ratio of 1 to 10. The obtained mixture was placed in a shaker for 20 h, which was then filtered by the usage of Whatman filter paper No. 1 to achieve a light brown extract. The resultant was exerted for the preparation of nanoparticles.

Synthesis of Ag-NP

To begin the synthesis of Ag-NP, 10 mL of fruit extract was added to 40 mL of $AgNO_3$ (1, 3, and 5 mM) solutions. The solutions were stirred separately for 2 hours at room temperature. The formation of nanoparticles was indicated by observing a change in the color of reaction solution from light brown to dark brown. As the last step, the solutions were centrifuged at 10,000 rpm for 10 min and the obtained precipitations were dried at 70 ˚C for 20 hours. The prepared black powder was confirmed to be silver nanoparticles.

Characterization

The physical-chemical properties of synthesized Ag-NP were characterized through the employment of UV-vis, TEM, and PXRD devices. The UV-vis spectra were recorded on double beam spectrophotometer (Shimadzu, model UV-1800) in a range of 300 to 900 nm, while the image of Ag-NP was presented through the transmission electron microscopy (TEM, model LEO 440i). The crystallin structure of our product was identified by the application of PXRD diffraction pattern (Philips, X'pert, Cu Kα radiations).

Antibacterial test

Minimum Inhibitory Concentration

We determined the minimum inhibitory concentration (MIC) of synthesized nanoparticles against *Streptococcus mutans* bacteria through the performance of Micro-broth dilution method, which required the usage of sterile 96-well microplates. Initially, the serial concentrations of nanoparticles (3.12, 6.25, 12.5, 25, 50, 100, and 200

μg/mL) were prepared, and then, 100 μL of each sample was poured into the wells of a microplate. In the following, 90 μL of Mueller Hinton Broth (MHB) medium and 10 μL (Merck) of microbial suspension, which was equivalent to 0.5 McFarland, were added to the microplate wells that contained the nanoparticles. A culture medium without bacteria was considered as the negative control and a culture medium with bacteria and without nanoparticles was considered as the positive control. Subsequent to the cultivation, the microplates were placed on a shaker for three seconds to obtain a completely uniformed mixture and go through an incubation process at 37 ˚C for 24 hours. Once the experiment was repeated 3 times for each of the nanoparticles concentrations, the turbidity of the wells was measured by an ELISA Reader (ELX 800, BioTek USA) at the wavelength of 620 nm after the incubation period. The lowest nanoparticles concentration that contained zero bacterial growth (lack of any turbidity inducement by bacterial growth) was considered as the minimum inhibitory concentration or MIC.

Minimum Bactericidal Concentration

The minimum bactericidal concentration (MBC) was determined in accordance to the MIC values. In this regard, 10 μL of MIC dilution and several dilutions above this volume were cultured on Mueller Hinton Agar culture medium (Merck) and placed in an incubator for 24 hours at a temperature of 37 ˚C. Then, the plates were checked for bacterial growth and the lowest concentration of nanoparticles that lacked the growth of 99.9% of bacteria was considered as the minimum bactericidal concentration (MBC).

Statistical Analysis

The statistical analysis of data was gathered by the usage of SPSS version 21 software and One way ANOVA statistical test. All of the results were reported as the averages of minimum inhibitory concentration and the minimum lethal concentration of nanoparticles, while implicating three repetitions of the test. The P values less than 0.05 was considered as significant in all of the cases.

RESULTS AND DISCUSSION

UV-Vis analysis

In order to prove the presence and stability of nanoparticles in the samples, UV-Vis spectrophotometer was used to read their absorption spectrum in the range of 300-800 nm after facing a color alteration towards dark brown. Considering the light absorption of Ag-NP among the range of 400 and 500 nm [10], Fig. 1 displays the occurrence of characteristic absorption band of surface plasmon resonance at 425, 436, and 441 nm for the cases of synthesized Ag-NP at 1, 3, and 5 mM, separately. As it is evident in this Figure, λmax value was shifted towards higher wavelengths as a result of increasing the silver concentration, which can be due to the growth of silver particles.

TEM analysis

The size and morphology of synthesized Ag-NP

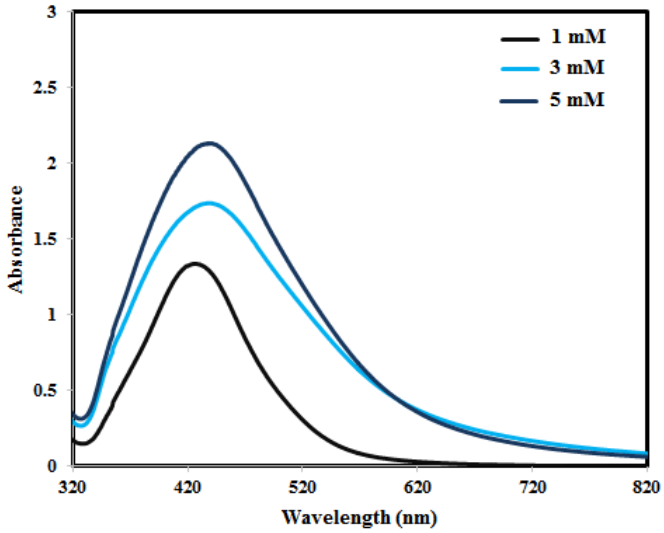

Fig. 1. UV-Vis of synthesized Ag-NP with *P. fracta* fruit extract

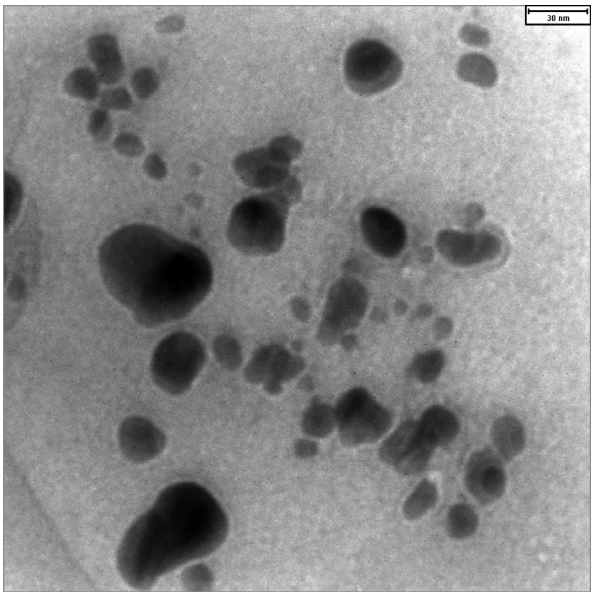

Fig. 2. TEM image of Ag-NP at 1mM concentration

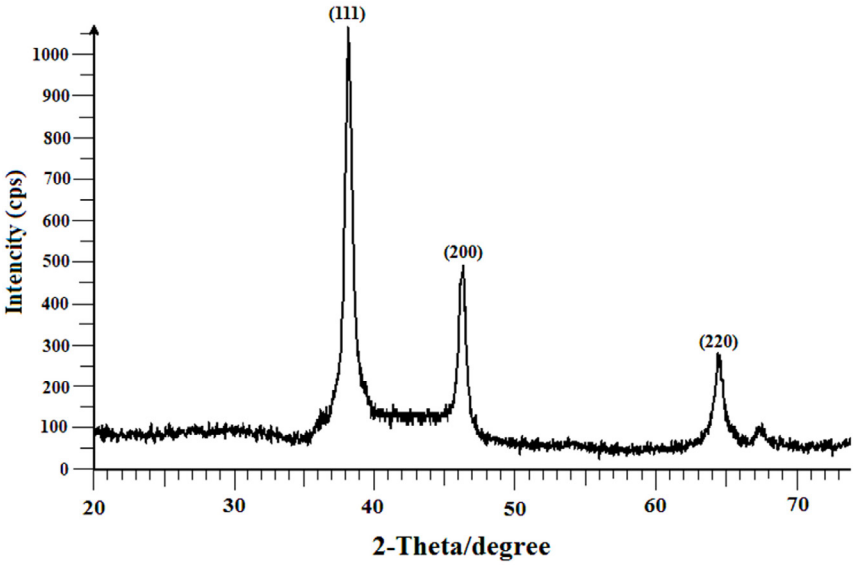

Fig. 3. PXRD pattern of Ag-NP at 1mM concentration

were surveyed by the application of TEM analysis. In conformity to Fig. 2, next to containing a spherical morphology, the prepared Ag-NP at the concentration of 1 mM were detected in the range of 30 nm.

PXRD analysis

The PXRD analysis was exerted to study the crystalline nature and purity of synthesized Ag-NP. According to the data of Fig. 3, the PXRD pattern exhibited some major peaks at the 2θ values of 38.8˚, 46.8˚, and 64.9˚ that can be indexed to (111), (200), and (220), respectively [14]. This pattern indicated the crystalline nature and face centered cubic (FCC) structure of our synthesized nanoparticles. The main crystalline size of synthesized Ag-NP was calculated through the Scherrer formula ($D = 0.9\lambda/\beta\cos\theta$; D: crystallite size, λ: wavelength of the X-ray source (0.1541 nm), β: FWHM, and θ: angle of diffraction) [15], and obtained to be about 27 nm. This outcome was in accordance with the

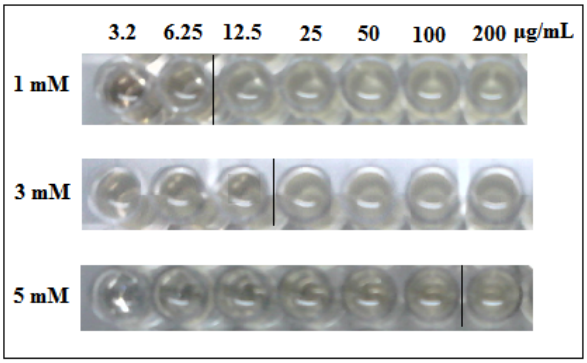

Fig. 4. Minimum Inhibitory Concentration (MIC) of Ag-NP at 1, 3, and 5 mM concentration on *Streptococcus mutans* bacteria

Table 1. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of synthesized Ag-NP

Ag-NP	***Streptococcus mutans* bacteria**	
	MIC (µg/mL)	**MBC (µg/mL)**
1 mM	6.25	3.25
3 mM	12.5	12.5
5 mM	100	50

presented mean diameter of nanoparticles in their TEM image (Fig. 3).

Antibacterial activity

The obtained results on the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Ag-NP, synthesized by the fruit extract of *P. fracta, regarding* the bacteria that induce tooth decay indicated the growth inhibitory and bactericidal effects of each nanoparticle on *Streptococcus mutans* bacteria. Some growth of bacteria was observed at the nanoparticles concentration of 5 mM (Fig. 4); however, this rate of growth was decreased at the concentration of 1 mM (Table 1).

Therefore, it is indicated that the occurrence of antibacterial effects is directly related to the size of silver nanoparticles, meaning that smaller particles are capable of exhibiting higher antibacterial impacts. The work of C Krishnaraj *et al* reported the value of 10 µg/mL as the lowest inhibitory concentration of silver nanoparticles on two standard strains of *Escherichia coli* and *Vibro cholera*[16]. The effect of silver nanoparticles with the average sizes of 70 and 7 nm on *Escherichia coli*, *Pseudomonas aeruginosa,* and *Bacillus cereus* was investigated by Khaydarov *et al*, which reported the lowest inhibitory concentration of Ag-NP on all three strains with an average diameter of 7 nm to be equalled to to 3, 2, and 19 µg/mL[17].

In conformity to previous studies, the size and shape of silver particles stand as important factors for the antibacterial properties of Ag-NP, since a reduction in the size of particles causes a suitable conflict with bacteria and increases this property. Considering this fact, decreasing the size of particles leads to increasing the rate of released silver ions from the surface and provides more antibacterial features[10, 18]. The main mechanism behind the antibacterial properties of Ag-NP is the release of silver ions, while the other mechanisms involve the damaging of cell membrane, production of reactive oxygen species and cell attack by silver ions (or even silver nanoparticles due to the presence of membrane holes), as well as the further damaging of ATP products and inhibition of DNA replication. Many studies reported the inducement of damages to the cell membrane by silver ions, which were mainly based on observing the holes or large holes of bacterial membrane through the TEM analysis. Silver ions may interact with sulfur-containing membrane proteins (for example the thiol group of respiratory chain protein) and cause physical damages to the membrane [19]. Based on the gathered outcomes, it can be suggested that the obtained Ag-NP by using the fruit extract of *P. fracta* can be applicable throughout the field of oral and dental infections treatment.

CONCLUSION

In the present study, silver nanoparticles were produced by the extract of rattle fruit without requiring any energy or expensive raw materials. Colloidal nanoparticles can easily penetrate into bacterial cells due to being distributed in the form of microscopic particles. In this regard, our produced nanoparticles exhibited a satisfying antimicrobial effect on the oral bacteria and therefore, their application based on their biological impacts can be effective in dealing with infections that are induced by the studied oral bacteria.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare

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