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Characterization of biosynthesized silver nanoparticles using *Hypericum perforatum* leaf and determination of their antibacterial activity

Arzu Ozgen, Erdi Bilgic, Sinem Gurkan Aydin, Mustafa Nizamlioglu

Istanbul Gelisim University, Vocational School of Health Sciences, Department of Medical Services and Techniques, Istanbul, Turkey

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Abstract

The silver nanoparticles of 1-100 nm size have the potential to be used in many medical fields, such as medical devices, therapies and molecular diagnostics, due to their antimicrobial properties. The biosynthesis of nanoparticles with numerous important advantages over chemical and physical methods has attracted attention today. In this study, the biosynthesis of the silver nanoparticles was performed using the *Hypericum perforatum* leaf extract to enhance the antibacterial effect of the silver nanoparticles. The morphological characteristic of the obtained mixture was determined using Transmission Electron Microscopy (TEM). Also the formation of the mixture was identified using UV-Vis spectrophotometer. The antibacterial activity of the synthesized Ag NPs using *H. perforatum* leaf extract was carried out in Gram (+) bacteria (*Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 95923, *Staphylococcus epidermidis* ATCC 12228) and Gram (-) bacteria (*Klebsiella pneumoniae* ATCC 700603, *Escherichia coli* ATCC 25922, *Proteus mirabilis* ATCC 27853, *Shigella*, *Acinetobacter baumannii* ATCC BAA 747) by disc diffusion method on solid media. The biosynthesized silver nanoparticles using the *H. perforatum* leaf showed higher antibacterial activity than the antibiotics.

Keywords: Biosynthesized, silver nanoparticles, antibacterial activity, *hypericum perforatum*

Introduction

Nowadays, the silver nanoparticles have become quite popular due to the wide range of applications such as numerous physical, biological, medical, pharmaceutical and engineering fields [1]. Due to their bactericidal, anti-fungal, anti-inflammatory and anti-angiogenic activities, the silver nanoparticles are widely used in medical field. For example, medical devices are coated with the silver ions as a result of their bactericidal effect. Also, silver is used in wound dressings. Furthermore, silver is used in optics, in textile engineering, in electronics. Examples of products containing silver are coatings of water filters, pillows, respirators, optical sensors, socks, wet wipes, detergents, soaps, shampoos, washing machines [2,1,3].

The silver nanoparticles having an antimicrobial structure in nature are used particularly in medical field [4]. For example, the surgical

meshes are frequently used for closing the large wounds and the repairment of the tissues. These materials are effective, but they are prone to microbial infections. In contrast, the polypropylene meshes coated with the silver nanoparticles have antimicrobial property [5]. The nanoparticles can be used to prevent the microbial infections of the medical devices such as intravenous catheters, endotracheal tubes, wound dressings, bone cements and dental fillings [6].

At present, the nanoparticles are restructured depending on their morphology, size and diffusion. Metal nanoparticles can be synthesized by physical, chemical and biological methods. The physical and the chemical methods which are used in the nanoparticle production have biological and ecological risks because they are expensive and particularly contain dangerous and toxic chemicals. Because of the risks mentioned, various plant extracts are used in the nanoparticle synthesis as an alternative to these methods [7, 8]. There are many plants used in the nanoparticle synthesis such as *Ocimum tenuiflorum*, *Spirogyra varians*, *Melia dubia*, olive [3].

H. perforatum is a member of the genus *Hypericum*, naturally

*Corresponding Author: Arzu Ozgen, Istanbul Gelisim University, Vocational School of Health Sciences, Department of Medical Services and Techniques, Istanbul, Turkey E-mail: ozgen@gelisim.edu.tr

grown in many parts of the world, mainly in Europe, West Asia, North Africa, North America and Australia. The plant is used in many medical applications including skin wounds, eczema, burns, diseases of the digestive tract and treatment of psychological diseases. The antimicrobial activity of *H. perforatum* with the effect of hyperforin, proanthocyanidins and xanthones compounds is supported by many studies [9].

In this study, we synthesized AgNPs using the *H. perforatum* leaf. The morphological characteristic of the obtained mixture was determined using Transmission Electron Microscopy (TEM) and particular size analyzer. Also the formation of kinetics of the mixture were identified using UV-Vis spectrophotometer. The antibacterial activity of the synthesized mixture was tested on various Gram (+) and Gram (-) bacteria strains.

Material and Methods

AgNPs synthesis

1.00 g of *H. perforatum* leaves, purchased from a local herbal store, were mixed with 100 ml of distilled water for 30 min using a magnetic stirrer. The resulting plant leaf extract was passed through a filter paper and then mixed with a 2 mM AgNO₃ solution at a ratio of 1:1. The final mixture was allowed to stand for about 6 hours under room conditions to reduce the colloidal nanoparticles.

Characterization of Ag NPs

UV-vis absorbance spectrums of final concentration were recorded in the wavelength range of 200-800 nm using Variant Spectrophotometer operating at a resolution of 1 nm. On the spectra, the *H. perforatum* extract diluted at a ratio of 1:1 with distilled water was taken as the reference solution, and the extract-AgNO₃ mixtures were examined in the presence of this reference solution. Quartz cuvettes were used in the spectrophotometer. Morphological characteristics of the Ag NPs were determined by the ZEIS-LEO 906E Brand-Model Transmission Electron Microscopy (TEM).

Antibacterial activity

The antibacterial activity of the synthesized Ag NPs using the *H. perforatum* leaf extract was carried out in Gram (+) bacteria (*S. aureus* ATCC 29213, *S. aureus* ATCC 95923, *S. epidermidis* ATCC 12228) and Gram (-) bacteria (*K. pneumonia* ATCC 700603, *E. coli* ATCC 25922, *P. mirabilis* ATCC 27853, *Shigella*, *A. baumannii* ATCC BAA 747) by disc diffusion method on solid media [10,11]. The bacterial strains were grown for 12 hours at 37 °C in Müller Hinton Agar (MHA). The densities of the bacterial suspensions were adjusted to 0.5 McFarland turbidity standard (1.5×10^8 CFU/mL) [12] by diluted 1:100 with Nutrient Broth. Subsequently, 100 µl of bacteria cells were spread onto the MHA Petri dishes using sterile spreader. Then, sterile Whatman filter papers (6-mm-diameters) were placed over the medium using sterile forceps and were impregnated with 10 (1.7 µg Ag/disc), 30 (5.1 µg Ag/disc), 50 (8.5 µg Ag/disc), 70 (11.8 µg Ag/disc), 100 (16.9 µg Ag/disc) µl of Ag NPs and the *H. perforatum* leaf extract called as the mixture. The *H. perforatum* leaf extract was used as the negative control and the doxycycline 30 µg/disc (doxycycline have a similar antimicrobial spectrum of activity against a wide range of Gram (+) and Gram (-) organism) were used as the positive control. The plates were incubated for 24 h at

37 °C in the incubator. The growth inhibition zones of each disc were measured in millimeters. All experiments were duplicated.

Results

Once, the *H. perforatum* leaf extract was mixed in aqueous solution of the silver ion complex, the reduction of pure Ag⁺ ions to Ag⁰ was monitored by measuring UV-vis spectrum of the reaction. The absorbance of the mixtures whose color is turning brown (Figs. 1A and 1B) in time showed SPR (Surface Plasmon Resonance) peaks at 511 nm and 291 nm after 6 hours (Figure 2). The morphological properties of the nanoparticles were determined by TEM. As shown in Figure 3, the size of the nanoparticles is between 8 and 35 nm and the shape of the nanoparticles is spherical.

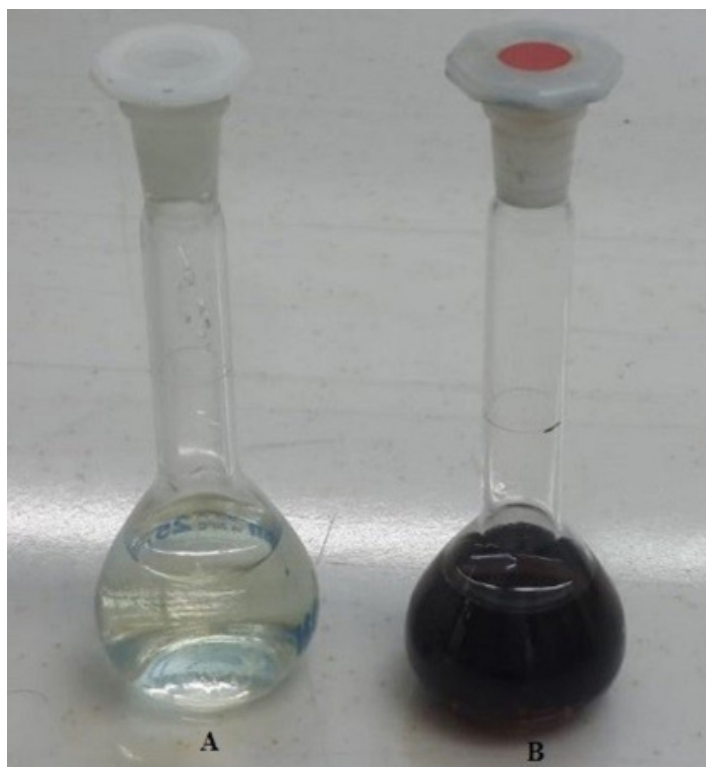


Figure 1. The images of synthesized silver nanoparticles, (A) *H. perforatum* extract, (B) Ag NPs and *H. perforatum* leaf mixture.

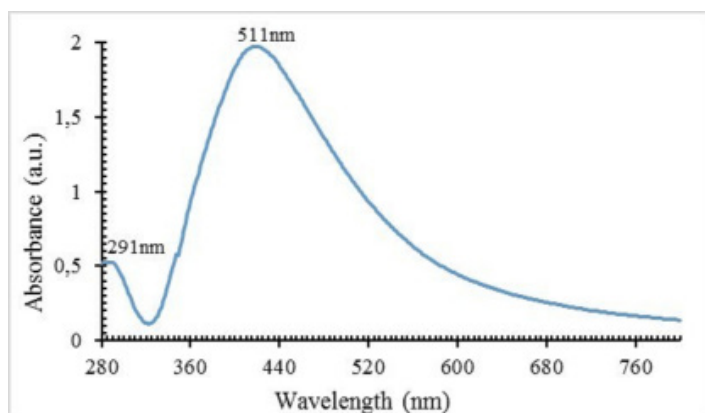


Figure 2. UV-Vis absorbance spectrums of Ag NPs and *H. perforatum* leaf mixture

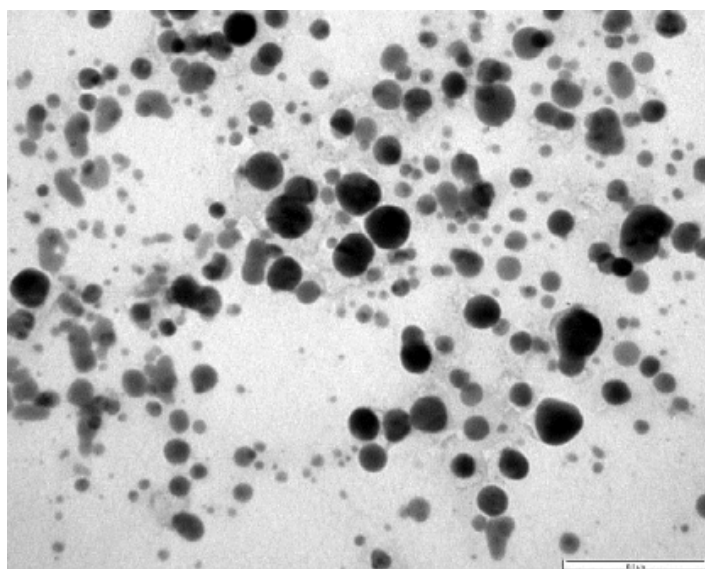


Figure 3. Transmission Electron Microscopy (TEM) imaging of Ag NP's

The antibacterial activity of the synthesized Ag NPs using the *H. perforatum* leaf extract called as the biological method was carried out in Gram (+) bacteria (*S. aureus* ATCC 29213, *S. aureus* ATCC 95923, *S. epidermidis* ATCC 12228) and Gram (-) bacteria (*K. pneumonia* ATCC 700603, *E. coli* ATCC 25922, *P. mirabilis* ATCC 27853, *Shigella*, *A. baumannii* ATCC BAA 747). The results are reported in Table 1 and Figure 4.

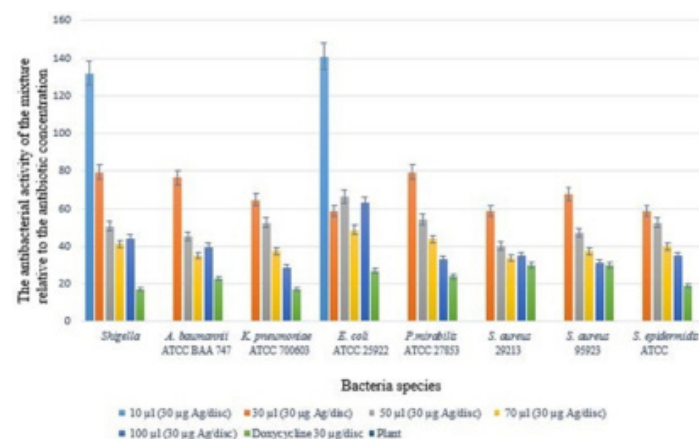


Figure 4. The antibacterial activity of the mixture relative to the antibiotic concentration

Table 1. The inhibition zone in diameter (mm) formed around the discs impregnated with mixtures in various volume

Bacterial species	The inhibition zone in diameter (mm) formed around the discs impregnated with mixtures in various volume						
	Plant extract	2 µg/disc	5 µg/disc	9 µg/disc	12 µg/disc	15 µg/disc	Doxycycline 30 µg/disc
<i>K. pneumonia</i>	-	-	11	15	15	16	20
<i>E. coli</i>	-	8	10	19	19.5	35	23
<i>P. mirabilis</i>	-	-	13.5	15.5	17.5	18.5	24
<i>Shigella</i>	-	7.5	13.5	14.5	16.5	24.5	17
<i>A. baumannii</i>	-	-	13	13	14	22	23
<i>S. aureus</i>	-	-	10	11.5	13.5	19.5	29
<i>S. aureus</i>	-	-	11.5	13.5	15	17.5	30
<i>S. epidermidis</i>	-	-	10	15	16	19.5	28

Discussion

The discovery of antimicrobial compounds in the treatment of infectious diseases has revolutionized modern medicine, and antibiotics, along with other antimicrobial compounds, have become one of the most important aspects of medical approaches. However, the resistance mechanism that bacterial pathogens have developed in a dramatic way against these compounds endangers the successful results of the medical interventions [13].

Infections caused by multidrug-resistant (MDR) organisms show a significant increase in mortality compared to infections caused by susceptible bacteria. In addition to threatening human life, it is also a burden on the economy of countries [14-16]. The reports of the Centers for Disease Control and Prevention show that approximately 23,000 people die in the United States every year as a result of infection with an antibiotic-resistant organism [17] and in a very recent report, antibiotic resistance would lead to approximately 300 million premature deaths by 2050; this

is estimated to cost \$ 100 trillion to the global economy [18]. Therefore, alternative solutions with alternative antibacterial activity to the antibiotics are needed. Especially the antibacterial coating is important for the devices that are used in the medical field. In this study, we investigated the antibacterial activity of Gram-positive and Gram-negative bacteria by performing biosynthesis of Ag NPs nanoparticles using the *H. perforatum* leaf extract for this purpose.

According to the obtained data, the peak which is seen at 511 nm can be related to the increase in the amount of the reduced silver in the mixture which is associated with the reduction of the Ag⁺ ions to Ag⁰. The other peak which is occurred at 291 nm can be associated with the decrease in the Ag⁺ ions caused by the ionic transition in a similar manner.

Plant extract had no antibacterial activity, as shown in Table 1. The antibacterial activity started in 10µl (2 µg/disc) concentration applications for *E. coli* and *Shigella*. In the case

of other bacteria, the antibacterial activity started in 30µl (5 µg/disc) concentration.

As the volume increases but the concentration is kept the same, the inhibition zone expands proportionally. It is associated with the increase in the surface area which is related to the volume caused by the fluidity of the mixture.

In the volume applications which cover more surface area, the nanoparticles react with the bacteria more which increases the inhibition zone. However, the antibacterial activity is the same for all volume values. As the concentration does not change, the number of nanoparticles reacting with the bacteria per unit surface area remains the same. When 30 µg of the antibiotic and 30 µg of the mixture were taken and the inhibition zones were calculated (Figure 4), the antibacterial activity of the mixture was found greater than the activity of the antibiotic.

The mechanism of the antibacterial effect of the silver nanoparticles are known and some theories are suggested regarding to that. One of these theories is about the bacterial killing activity of the nanoparticles. According to this theory, the silver nanoparticles are able to attach to the bacterial cell wall and pass easily into the cell. Therefore, the changes in the cell membrane increases the permeability and causes cell death [19].

Another theory states that the free radicals produced by the silver nanoparticles causes cell death. Electron spin resonance studies showed that the free radicals produced by this way damage the cell membrane making the membrane more permeable and this results in cell death [20-24].

Also it is proposed that the silver ions can be released by the nanoparticles. The released silver ions bind to the thiol groups of the essential enzymes of the cell to inactivate them [25].

The bacterial cells in contact with the silver ions take them inside the cell. The silver ions affect various metabolic events in the bacterial cell, therefore they damage the cell. After that, a respiratory enzyme is probably inhibited by the silver ions. This results in the production of harmful free oxygen radicals. Acids react with bases. Silver is a soft acid, therefore it reacts with soft bases. The cells are primarily made of sulfur and phosphorus that are soft bases. For that reason, the nanoparticles may interact with these soft bases and cause the death of the cell. Additionally, the cell DNA contains sulfur and phosphorus that react with the silver ions. This reaction causes a lethal damage to the DNA [26]. The damaging of the DNA prevents the replication of the genome and the bacterial cell that is unable to divide cannot continue its progeny [1].

The nanoparticles have been reported as the possible modulators of the signal transduction in the bacterial cells. The phosphorylation of the protein substrates in bacteria affects the signal transduction [27-30].

Dephosphorylation is only seen in the tyrosine residues of the Gram (-) bacteria. The nanoparticles can change the phosphotyrosine profiles of the bacterial peptides. Therefore, this inhibits the signal transduction and the bacterial growth [30]. To support these facts, new studies should be carried out.

Competing interests

The authors declare that they have no competing interest.

Financial Disclosure

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Ethical approval

Consent of ethics was approved by the local ethics committee.

Arzu Ozgen ORCID:0000-0003-2104-6019

Erdi Bilgic ORCID:0000-0002-2892-274X

Sinem Gurkan Aydin ORCID:0000-0002-5146-8936

Mustafa Nizamlioglu ORCID:0000-0002-2104-784X

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