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ANTIBACTERIAL CHARACTERISTICS OF Ag NANOPARTICLE EXTRACTED FROM OLIVE LEAF

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Abstract. In the presented research, silver nanoparticles have been synthesized with biological methods. In this method, an extract from an olive leaf, called green synthesis, has been used as a reducing agent. As a result, environmentally friendly, non-toxic nanoparticles have been obtained and their characteristics in SEM and UV-Vis have been analyzed. Then their antibacterial properties have been studied in bacterial cultures of *Bacillus subtilis* and *Staphylococcus Aureus*.

Keywords: *green synthesis, silver nanoparticles, plant extract, antibacterial property.*

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1. Introduction

Due to the small size of nanoparticles (1-100 nm) obtained by nanotechnological methods, they have several new physicochemical properties, in contrast to macro-sized structures, due to a large number of surface atoms relative to atoms. In this regard, many studies have recently been conducted on the production of nanoparticles, which have a wide range of applications, and substantial results have been obtained. Although the physical and chemical methods used in the synthesis of nanoparticles allow the production of nanoparticles of any size in a short period, their toxicity is high. For this reason, there is a need to use newer and more convenient methods for obtaining non-toxic forms of nanoparticles (Narayanan & Sakthivel, 2010). This biological method, called green synthesis, is more environmentally friendly and less expensive than conventional methods (Griffin *et al*., 2018). For this purpose, using plant extracts and microorganisms, they achieved the synthesis of various nanoparticles (Singh *et al.*, 2016). Biological methods are used more in the synthesis of metal nanoparticles of special importance, including Ag nanoparticles (Chaolupka *et al.*, 2010). The antibacterial and antioxidant effects of silver and its salts had been already known to science. Silver particles in the nanoscale range are used in the manufacture of several devices in electronics due to their high conductivity and catalytic, optical properties (Abou El-Nour *et al*., 2010). Besides, silver nanoparticles are currently used as an antibacterial agent in the treatment of infections that cause wounds and chronic ulcers (Chastre, 2008). Unlike the microbial synthesis of silver nanoparticles, their synthesis from plant extracts is shorter, and the resulting nanoparticles have a more stable form (Philip & Unni, 2011). Substances accumulated in various organs of plants act as reducing agents, resulting in the production of more environmentally friendly nanoparticles. In this case, it is possible to control the size and shape of the nanoparticles obtained by changing the pH, temperature, etc. of the environment (Wei *et al*., 2015).

Based on this information, we synthesized silver nanoparticles using an extract from olive leaves in our experiment and studied their antibacterial properties.

2. Materials and methods

As the object of the study, the leaves of an olive plant belonging to the *Oleaceae* family, collected from olive groves in the Absheron region have been used.

Image 1. Olive Leaves

The collected olive leaves have been cleaned with ordinary water and then with distilled water and kept at room temperature for some time. After the leaves were cut into small pieces, 2.0 g has been taken from them and the extract has been obtained by boiling in 100 ml of distilled water for 15 minutes. The extract has been filtered through filter paper and stored at room temperature for some time. In our experiments, Ag nanoparticles have been synthesized using silver nitrate salt ($AgNO_3$). For this purpose, a 2.10^{-2} M solution of $AgNO_3$ salt has been prepared in distilled water, added to the extract and heated on a magnetic stirrer in a 200 ml thermostatic flask. The synthesis of silver nanoparticles has been observed with a gradual change in the color of the solution. Thus, the color of the solution gradually changes to light yellow, brown-yellow and dark brown. The resulting solution has been cooled and stored at room temperature. These experiments have been carried out in the Nanoscience Laboratory of Baku State University.

Image 2. Discoloration of the extract due to the synthesis of Ag nanoparticles

3. Results and discussions

UV-Vis spectroscopy - UV-Vis spectroscopy has been used to determine the size of the nanoparticles in the sample. The resulting dark solution has been centrifuged at 10,000 rpm for 5 min. The top liquid obtained during sedimentation has completely been removed and the remaining sediment has been washed several times with distilled water. The sediment has been dried at 60^0C for 48 hours and stored for analysis.

UV-Vis spectroscopy is based on the absorption of a stream of light after passing through a sample. A decrease in light intensity indicates an increase in absorption. The UV-Vis spectrophotometer excites the surface plasmon resonance on the surface of the metal, creating a resonance at a certain wavelength, which causes a strong light at that wavelength. Reduction of the Ag^+ ion in the olive leaf extract-AgNP complex to Ag^0 has caused resonance and UV-Vis absorption spectra has been drawn. As the concentration of the solution increases, the peak of the absorption spectrum becomes apparent. The maximum of this peak is 455 nm and the minimum price is 431 nm.

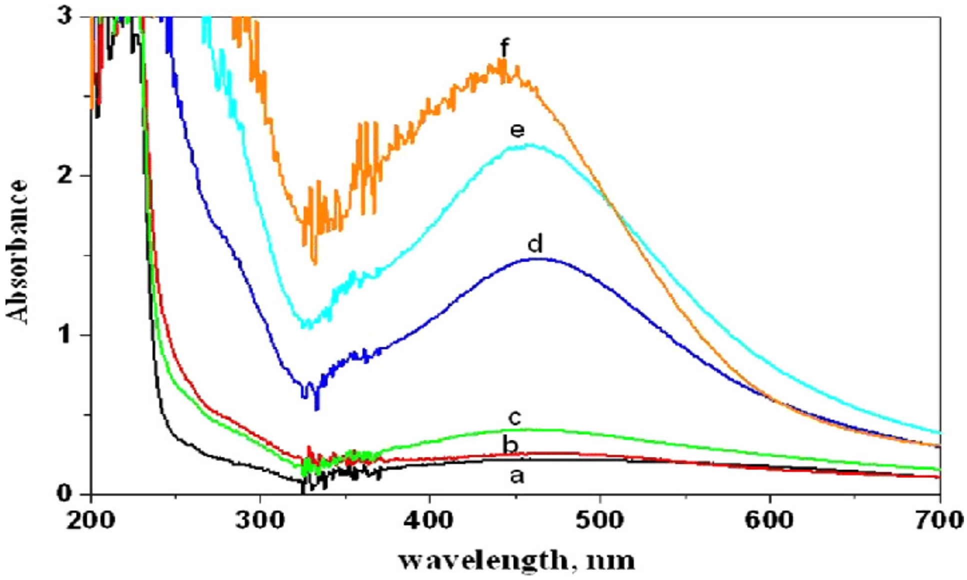

Figure 1. UV-Vis spectrum of silver nanoparticles synthesized in different concentrations of olive leaf extract

Scan Electron Microscope - The size of silver nanoparticles is determined in SEM. To do this, a drop of the solution is taken and evaporated at room temperature on a sample of the electron microscope. 24 hours later SEM images are taken. The size of Ag nanoparticles obtained during the chemical reaction varies in the range of 50-100 nm. The shapes are mostly spherical, in some cases ellipsoidal.

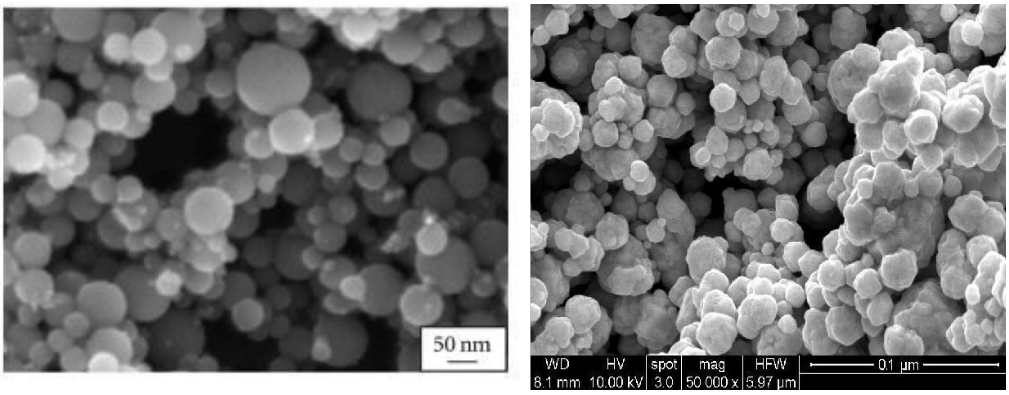

Image 3. SEM imaging of synthesized silver nanoparticles

Antibacterial activity of AgNPs

There are various opinions about the antibacterial properties of silver nanoparticles (Kim *et al*., 2007). It is believed that silver binds to the bacterial cell wall and membrane, binds to proteins in the thiol (-SH) groups, reduces membrane permeability, neutralizes them and kills bacteria. It can be used in 4 forms, including silver, Ag^0, Ag^+, Ag^{+2} and Ag^{+3}.Ag^{+2} and Ag^{+3} are considered to be unstable in the aqueous medium, while Ag^+ is considered to be in the free state (Wijnhoven, 2009). Particle size less than 10 nm results in a further expansion of the surface area, increasing the antibacterial effect. The mechanism of antimicrobial action of Ag^+ is explained by the cell of the microorganism to which it is applied, in the form of absorption of ions, accumulation inside the cell, contraction of the cytoplasmic membrane or retraction of the cytoplasm by the cell wall. For this reason, it has been found that DNA molecules are damaged and that cells lose their ability to reproduce due to the infiltration of Ag^+. It has already been proven that silver interacts with -SH groups to inactivate proteins (Feng *et al*., 2000). Besides, the role of silver as a catalyst in the oxidation of microorganisms in cultures with added oxygen has been studied (Cho *et al*., 2005).

Experiments on the antibacterial properties of synthesized silver nanoparticles have been performed on bacterial cultures of *Bacillus subtilis* and *Staphylococcus Aureus*. Different concentrations (1 mM, 8 mM, 16 mM, 25 mM) of the solution containing silver nanoparticles have been impregnated on 4 filter paper disks with a diameter of 5 mm. Then these filter papers have been placed in Petri dishes containing *Bacillus subtilis* and *Staphylococcus Aureus* bacterial cultures. Bacterial cultures have been studied at 37^0 C after a 1-day incubation period. It has been found that the division of bacteria in the area where the filter papers were located stopped and they lysed. Therefore, depending on the concentration of the solution in these areas, an inhibition zone has been formed in different measurement ranges. From the results obtained during the measurement of the inhibition area, it has been found that the size of this area was the maximum at the maximum concentration of the solution. Thus, in general, the size of the formed inhibition areas range from 4 to 12 mm. While lysis is not very noticeable in *Bacillus subtilis* cultures at minimal concentrations (1 mM), solutions at these concentrations have resulted in a markedly selective inhibition field in *Staphylococcus Aureus* cultures.

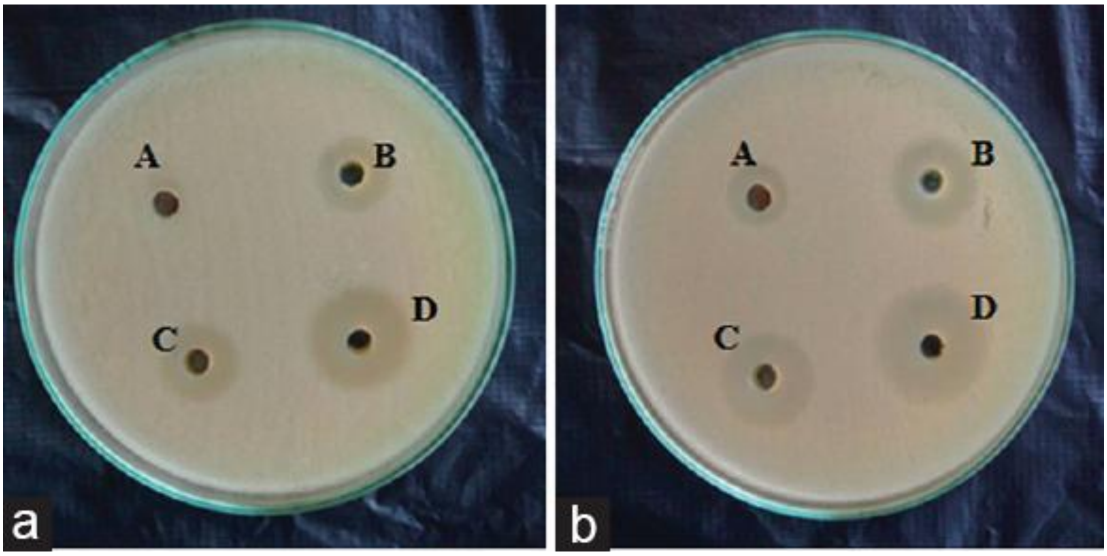

Image 4. Antibacterial properties of Ag nanoparticles at different concentrations of the solution (A- 1 mM, B-8 mM, C- 16 mM, D- 25 mM): A- *Bacillus subtilis* and B- *Staphylococcus Aureus*

4. Conclusion

The green synthesis method used in the synthesis of nanoparticles is currently the main area of interest for researchers. In the study, it has been studied that the maximum absorption spectrum of silver nanoparticles synthesized by an extract from an olive leaf is 455 nm, and their size is between 50-100 nm. Later, it has been found that silver nanoparticles at different concentrations lyse the bacteria in bacterial cultures, and thus have antibacterial properties. The broad-spectrum antibacterial activity of silver nanoparticles in small concentrations may be the most effective treatment in the future.

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