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Investigation of the Antimicrobial and Antibiofilm Effects of Silver Nanoparticles Obtained From Aloe Vera Plant by Green Synthesis

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ABSTRACT

Misuse of antibiotics globally has resulted in the development of resistant bacterial strains. Antimicrobial and antibiofilm effects of silver nanoparticles obtained by green synthesis from Aloe vera extract against *Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii* and *Staphylococcus aureus* were investigated. The synthesis of silver nanoparticles from the aloe vera plant was carried out by the green synthesis method. After the characterization of silver nanoparticles was evaluated by UV-Vis Spectroscopy, FT-IR, SEM, TEM, XRD its antimicrobial effect on pathogenic microorganisms was evaluated by microdilution method. The Minimum Inhibitor Concentration (MIC) values (µg/mL) of silver nanoparticles on *Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii,* and *Staphylococcus aureus* were found to be 32, 64, 32 and 32, respectively. In this study, the biofilm inhibition rate was evaluated by the crystal violet method. Green synthesis silver nanoparticles showed antibiofilm effect on bacteria.

Aloe Vera Bitkisinden Yeşil Sentez Yöntemiyle Elde Edilen Gümüş Nanopartiküllerin Antimikrobiyal ve Antibiyofilm Etkilerinin Araştırılması

Araştırma Makalesi

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ÖZ

Küresel olarak antibiyotiklerin yanlış kullanımı, dirençli bakteri suşlarının gelişmesine neden olmuştur. Aloe vera ekstraktından yeşil sentez ile elde edilen gümüş nanopartiküllerin *Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii* ve *Staphylococcus aureus*'a karşı antimikrobiyal ve antibiyofilm etkileri araştırıldı. Aloe vera bitkisinden gümüş nanopartiküllerin sentezi yeşil sentez yöntemi ile gerçekleştirilmiştir. Gümüş nanopartiküllerin karakterizasyonu UV-Vis Spektroskopi, FT-IR, SEM, TEM, XRD ile değerlendirildikten sonra patojenik mikroorganizmalar üzerindeki antimikrobiyal etkisi mikrodilüsyon yöntemi ile değerlendirilmiştir. Gümüş nanopartiküllerin *Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii* ve *Staphylococcus aureus* üzerindeki Minimum İnhibitör Konsantrasyon (MIC) değerleri (µg/mL) sırasıyla 32, 64, 32 ve 32 olarak bulundu. Bu çalışmada, biyofilm inhibisyon oranı kristal viyole yöntemi ile değerlendirilmiştir. Yeşil sentez gümüş nanopartiküller bakteriler üzerinde antibiyofilm etki göstermiştir.

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1. Introduction

Nanotechnology has a wide application area in science and technology by synthesizing new molecules in nano size (Ali et al., 2020). Today, interest in the synthesis and characterization of metal nanoparticles has increased. Nanoparticles can be produced especially by chemical, physicochemical, photochemical and electrochemical reduction methods. It can also be produced by many methods such as radiolysis and heat evaporation. Physical and chemical methods are costly, labor intensive and take a long time. In addition, a large amount of chemical waste is released in the precipitation and reduction processes. Recently, environmentally friendly, low-cost "green synthesis" methods that without using toxic solvents are preferred for the production of nanoparticles (Hatipoğlu, 2022). Since the green synthesis method uses plant material as a covering agent, no adverse effects are encountered during medical applications (Ali et al., 2020), Therefore, nanoparticles have become a relatively new trend in medicine (Selem et al., 2022).

Herbal extracts are used in the preparation of silver nanoparticles. Aloe vera leaves, in particular, are of interest as a medicinal plant due to their anti-inflammatory activity, antiarthritic properties, antibacterial properties, and promoting healing of wounds and burns. Aloe vera leaves have many biological properties such as lignin, hemicellulose, pectin, which can be used for the reduction of silver ions. The major enzymes and some proteins in aloe vera plant extract are believed to bind weakly to silver ions and act as a complexing agent. They have excellent reducing properties with their low cost and environmentally friendly nature (Tippayawat et al., 2016).

The antibacterial effect of silver has been known since ancient times. There is a lot of information that silver containers are preferred to preserve the properties of water and that silver ions bind to DNA and stop the growth of bacteria. Nanometric silver (less than 100 nm) nanoparticles have an effective large surface area, serious antibacterial effect and strong toxicity against a wide variety of microorganisms (Parvekar et al., 2020).

Many antimicrobial studies have been found with the isolates of *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC27833, *Escherichia coli* ATCC 25922, which are standard strains. However, studies on the antibacterial effect of silver nanoparticles on multi-drug resistant microorganisms that cause hospital-acquired infections are limited. For this reason, we aimed to see the antimicrobial effect of silver nanoparticles obtained from Aloe vera plant in both gram negative (*Acinetobacter baumannii, Escherichia coli, Pseudomonas aeruginosa*) and gram positive (*Staphylococcus aureus*) isolates resistant to multidrug. We chose the aloe vera plant because of its tremendous reducing properties, environmental friendliness and good supporting agent.

2. Material and Method

2.1. Aloe Vera Extraction

The mature leaves of the Aloe vera plant, which are now close to fruiting, were cut off. Then, firstly, it was weighed and then washed several times with pure water. Afterwards, these leaves were kept in an oven at low temperature for 5 days. At the end of day 5, the leaves were cut into small parts and taken into 100 ml of methyl alcohol and boiled for 20 minutes. During boiling, it was closed in such a way that it could not see the sun. At the end of this determined period, filtering was done with no:1-2 filter papers placed in 250ml flasks and the Aloe vera extract obtained was stored in the refrigerator at +4^{o}C for later use (Ellis, 2018).

2.2. Ag@AVNPs Preparation

10mM 100ml $AgNO_3$ solution was prepared in a 250 ml flask. To this prepared solution, 80 ml of Aloe vera extract, which was kept ready before, was added and mixed in a magnetic stirrer at 250 rpm at room temperature for 13 hours, covered. At the end of the determined time, centrifugation was started. In the centrifuge (5000 rpm, 5 minutes), the process was terminated by adding 2 times water and then 1 time methyl alcohol, respectively. Finally, the obtained solid sample was stored in an oven at 60 °C for 24 hours to be used in characterizations. Changing the color of the reaction mixture from light yellow to dark brown reveals the formation of Ag@AVNPs (Figure 1) (Ozturk et al., 2022; Gecer et al., 2022).

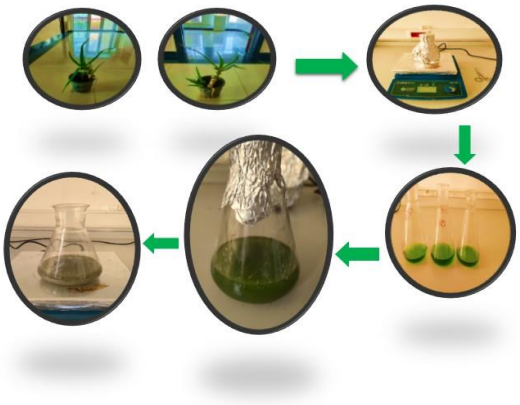

Figure 1. Image of obtaining silver nanoparticle from Aloe vera plant (Ag@AVNPs)

2.3. Ag@AVNPs Characterization

UV-Vis Spectroscopy: The optical properties of the nanoparticles were characterized using UV-Vis spectroscopy. The absorption spectra of the nanoparticle solution were recorded using a Agilent Cary 60 UV-Vis spectrophotometer in the range of 300-800 nm.

FT-IR: The synthesized nanoparticles were characterized using Fourier-transform infrared spectroscopy (FTIR). The FTIR spectra of the nanoparticle pellets were then recorded using a Cary 630 FTIR spectrometer in the range of 400-4000 cm^{-1}.

FE-SEM: The morphology and size distribution of the synthesized nanoparticles were analyzed using a scanning electron microscope (SEM) (Hitachi Regulus 8230 FE-SEM, 10kV, 1000X and 2000X magnification).

Transmission Electron Microscopy (TEM): The morphology and size distribution of the synthesized nanoparticles were analyzed using TEM. A drop of nanoparticle solution was placed on a carbon-coated copper grid and allowed to dry at room temperature. TEM (Hitachi HT7800 TEM, 100kV, 10000X magnification) was performed with the model device.

X-Ray Diffraction (XRD): The crystal structure and phase of the nanoparticles were produced using XRD. The nanoparticles were deposited on a glass substrate and analyzed using a Panalytical Empyrean, 2θ angle between 10-90°.

2.4. Identification of Clinical Specimens and Determination of Antimicrobial Susceptibility

In this study, 4 bacteria isolated in Iğdır State Hospital Microbiology Laboratory were used. Clinical samples were inoculated on blood and EMB agar. It was incubated at 37 °C for 24 hours. Growing cultures were evaluated in the VITEK 2 (bioMérieux, France) device for identification and antimicrobial susceptibility testing.

2.5. Determination of Antimicrobial Effects of Silver Nanoparticles

MIC (Minimum Inhibitory Concentration) of synthesized AgNPs on *Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumanii* and *Staphylococcus aureus,* were determined by microdilution method in 96-well ELISA plates. 100 microliters of Brain Heart Infusion Broth was added to the wells. 100 microliters of AgNP prepared at a concentration of 512 µg/ml was added to the wells and two-fold serial dilutions were made. 0.5 McFarland turbidity standard was prepared for each microorganism and inoculated into the wells. The first well without growth after incubation was accepted as the MIC value. In addition, an aqueous solution of 1 mM $AgNO_3$ and Aloe vera extract were used for comparison purposes.

2.6. Antibiofilm Effect of Silver Nanoparticles by Crystal Violet Assay Method

The inhibition of biofilm formation after treatment with Ag@AVNPs was quantitatively examined by microtiter crystal violet assay. Different concentrations of AgNPs were used in 48-well microplates against the antibiofilm activities of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumanii*. 400 microliters of Tryptic Soy Broth was added to the wells. Bacterial suspension (10^5 CFU/mL) was added to each well. Silver nanoparticles were added to 18, 9, 4.5 and 2.25 µg/mL concentrations. The microliter plate was maintained at 37°C for 24 h. Biofilms were washed twice with PBS. Then fixation was performed with 100% methyl alcohol for 15 minutes. The formed biofilms were stained with 0.1% crystal violet for 5 minutes. Then, they washed with

sterile distilled water. 400 µl of ethyl alcohol was added and measured at 630 nm using ELISA reader. Control consists of medium without added nanoparticles.

3. Results

3.1. Characterization

UV-Vis Spectroscopy: UV–Vis spectra of Aloe vera AgNPs sample. It is seen that Aloe vera AgNPs sample has peaks about 440 nm in the UV–vis spectrum. In the UV–Vis spectrum of the AgNPs study, however, the signal strength at approximately 360 nm is significantly reduced.

FT-IR: We think that some productive peaks were obtained in the sample analysis performed on the FT-IR device. In the FT-IR spectra of the obtained Ag@AVNPs (B), the 3000-3500cm^{-1} vibration band was matched with the –OH and –NH groups. The vibration bands obtained between 500-3000cm^{-1} are C-H in aromatic structure, C=C; C-N in the vibrational band amide group, C-O, -C-O- in the vibrational band ether group. In addition, it is clearly observed that some vibrational bands differ between (A) and (B) in the FT-IR spectra. It is predicted that the difference in (A) and (B) vibration bands between 500-1500cm^{-1} is due to Ag@AVNPs (Figure 2) (Bilgili et al., 2016; Öztürk et al., 2020; Öztürk et al., 2022).

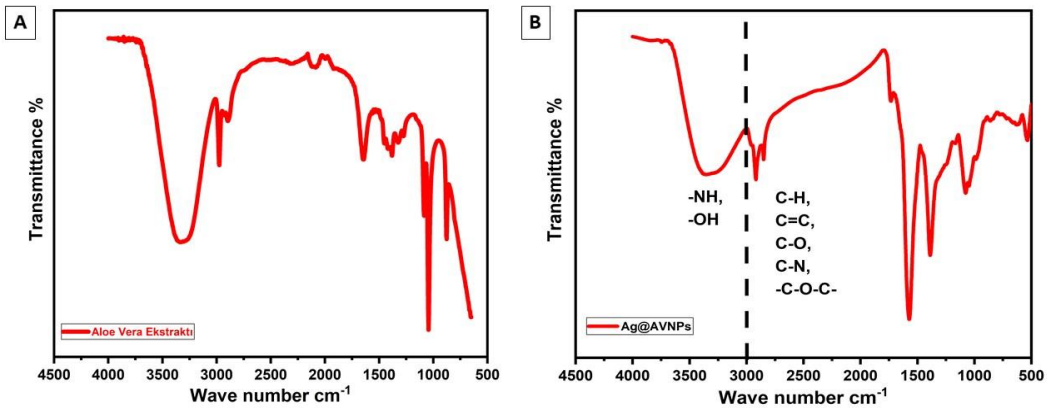

Figure 2. FT-IR image of Aloe Vera extract and Ag@AVNPs

FE-SEM: In addition, non-conductive samples for SEM imaging were obtained by coating gold/palladium (Au/Pd) at the desired coating thickness with the Leica EM ACE600 coating device. Morphological changes on the surface were observed with the help of FE-SEM. Agglomeration and good compatibility of repeating components are observed from FE-SEM images (Figure 3) (Huang et al., 2013; Jin et al., 2022).

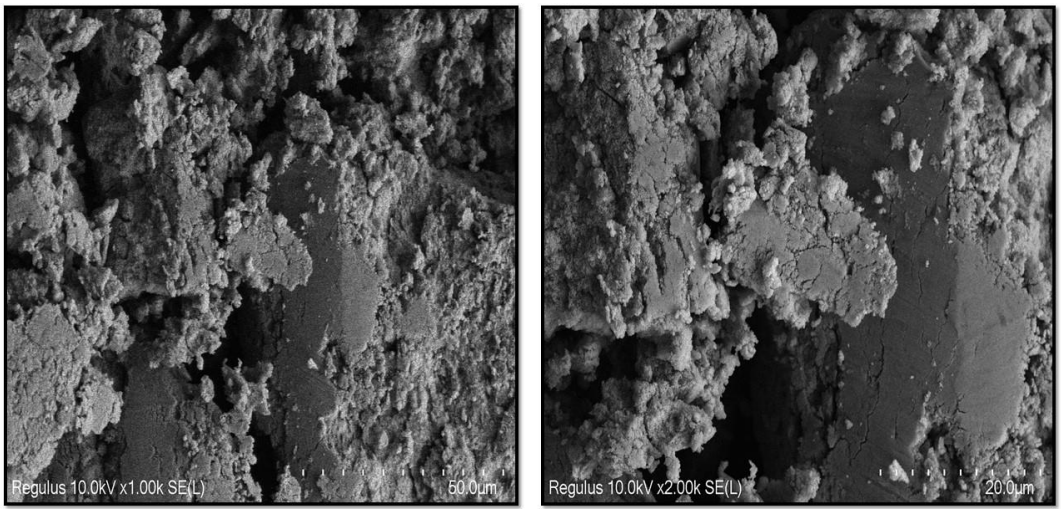

Figure 3. FE-SEM image of Ag@AVNPs

TEM: According to TEM imaging; The mean particle size was determined as (42.553 ±12.855) nm (Figure 4). In addition, Ag@AVNPs were observed to be spherical and scattered (Phongtongpasuk et al., 2016).

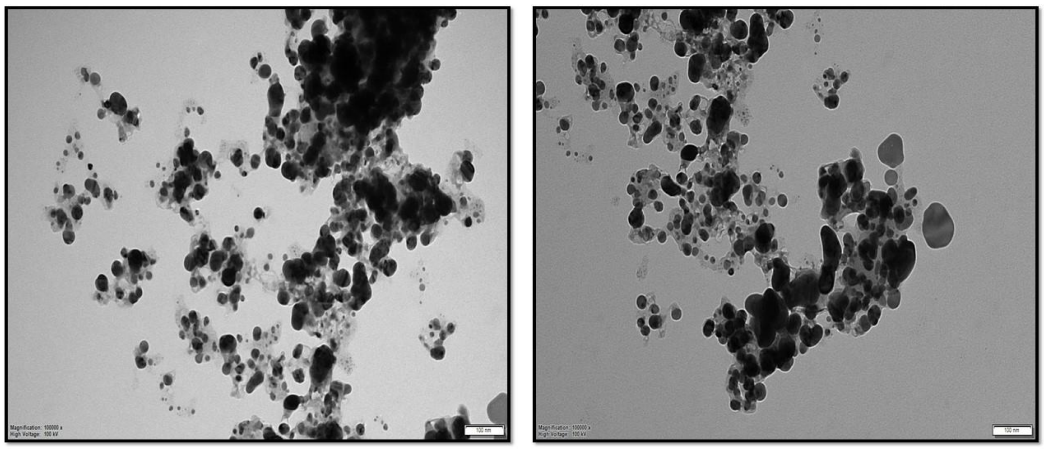

Figure 4. TEM image of Ag@AVNPs

Crystal size analysis was performed with the aid of a device with In the XRD analysis (27,60; 32,03; 46,93;58,24) peaks were obtained (Figure 5).

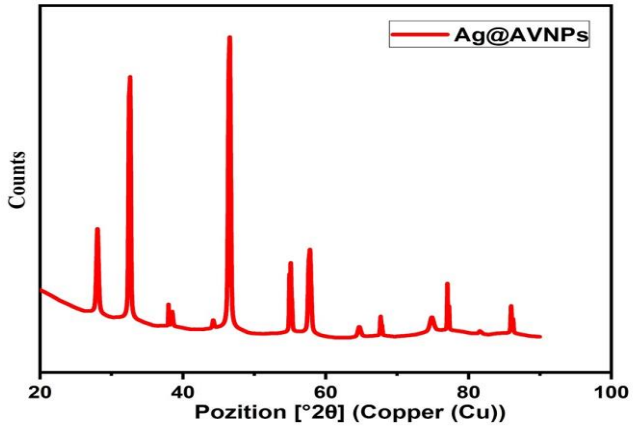

Figure 5. XRD image of Ag@AVNPs

3.2. Antimicrobial Effects of Silver Nanoparticles

In our study, MIC values were determined by using the antimicrobial activity of AgNPs on 4 different pathogens using microdilution method (Table 1). AgNP values were higher for *Pseudomonas aeruginosa* and *Acinetobacter baumanii*, while lower concentrations were obtained for *Staphylococcus aureus* and *Escherichia coli.*

Table 1. MIC values of Aloe vera AgNPs and Aloe vera extract (µg/ml)

Microorganisms	AgNPs	Aloe Vera extract	$AgNO_3$
S. aureus	64	256>	0.25
E. coli	64	256>	0.5
P. aeruginosa	32	128	0.5
A. baumannii	32	256	0.5

Antimicrobial activity of Aloe vera extract has been found to be negligible in our pathogens. When we compared AgNPs, $AgNO_3$ and Aloe vera extract, the most effective ratio was seen in silver nanoparticles.

3.3. Antibiofilm Effects of Silver Nanoparticles

Antibiofilm activity of silver nanoparticles was assayed. for biofilm-forming bacteria. In the present study, the in vitro anti-biofilm activity of AgNPs was analyzed in a dose-dependent manner against for *E. coli*, *S. aureus*, *P. aeruginosa* and *A. baumannii*. Biofilm formation in resistant bacteria is a challenge in treatment. Inhibition of exopolysaccharide formation greatly reduces biofilm formation. The efficiency of green synthesis on silver nanoparticles was evaluated. The biofilm inhibition rate was calculated by calculating the measured OD values with the following formula (Formula 1). Biofilm eradication was found to be higher in *A.baumanii* than in other bacteria. The dose-dependent variation of biofilm inhibition is plotted (Figure 6) (Fan et al., 2018).

$$Inhibition\ (\%) = [1- OD_{sample}/OD_{control}] \times 100 \quad (1)$$

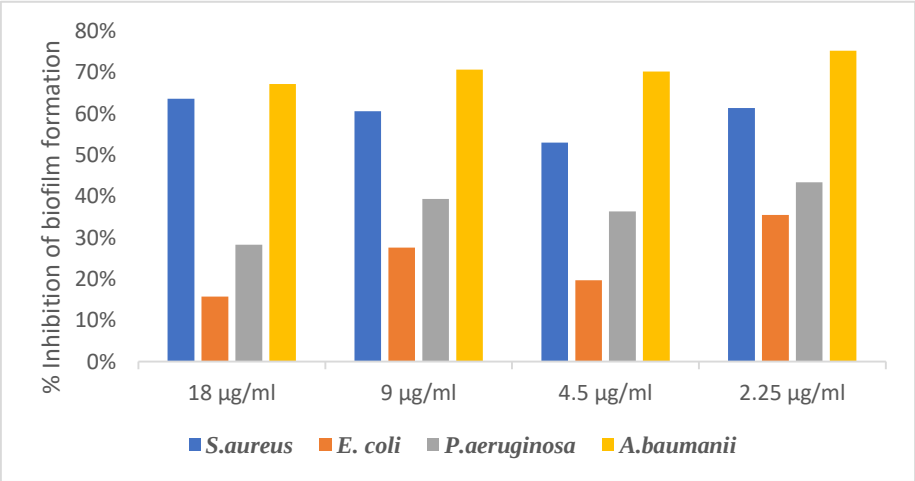

Figure 6. Inhibition of biofilm formation

4. Discussion

The growing case of multidrug resistance in bacteria has become a global concern due to the misuse of antibiotics. It has been observed that the antimicrobial and antibiofilm effect of AgNPs synthesized from the aloe vera plant has a stronger effect than $AgNO_3$ and aloe vera extract. The antimicrobial effect may be due to the synergistic effects of AgNPs and natural compounds in the structure of aloe vera. Biofilm, which leads to antibiotic resistance in bacteria, draws attention. Biofilms are microbial consortia embedded in self-produced exopolymer matrices composed mainly of exopolysaccharides (EPS) (Arsene et al., 2022). It is necessary to seek ways to combat biofilm formation. For this reason, new products are sought as an alternative to antibiotics all over the world. In this context, it is important the antimicrobial and antibiofilm properties of AgNPs, which have the potential to be used instead of antibiotics. It has a bactericidal effect especially on pathogenic microorganisms *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Acinetobacter baumannii our study*. There are studies showing that the MIC and MBC values of silver nanoparticles are effective at 0.625 µg/ml by the macrodilution method against *S. aureus* (Parvekar et al., 2020). Ellis et al. concluded that *P. aeruginosa* could develop resistance to silver nanoparticles, while *S. aureus* and *A. baumannii* did not develop resistance to silver nanoparticles (Ellis et al., 2018).

In the study of Ahmad et al. evaluating the MIC results of silver nanoparticles synthesized by various plant extracts, the MIC values of aloe vera silver nanoparticles against *E. coli* and *S. aureus* bacteria were found to be 8 µg/mL and 7.8 µg/mL, respectively (Ahmad et al., 2022).

Antibiotic resistance of microorganisms is increasing day by day. Therefore, finding an alternative for antibiotics is an urgent need which has focused attention on natural products. In the study, the antimicrobial activity of AgNPs was found to be more effective than 1 mM silver nitrate solution and antibiotics, similar to ours. They reported that it is effective at concentrations of 12.25 µg/ml for gram negatives and 6.25 µg/ml for gram positives (Baran, 2019).

Using the crystal violet attachment assay *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Acinetobacter baumannii* were retained as biofilm producers in the antibiofilm activity investigation. Biofilm eradication was found to be higher in *A. baumannii* than in other bacteria. Biofilm inhibition was about <40% in case of *E. coli* but was more than 40% for other bacterial isolates. According to the data of Mehrishi P. et al., it was observed that the highest MIC effect was in *S. aureus* and the biofilm reduction was 50% in standard strains. Although the values related to *S. aureus* are close to our study data, the highest effect was determined in A.baumanni. Also, clinical isolates with antibiotic resistance were preferred in our study. Aloe vera showed the highest zone of inhibition for *S. aureus* (Mehrishi et al., 2022). Mbarga M. J. Arsene determined the antibiofilm effect of gram negatives at different concentrations of aloe vera extract (Arsene et al., 2022).

Bacterial biofilms are found about 60% of chronic wounds and delay the healing process due to their resistance to many antibiotics. The biofilm inhibition activity of *E.coli* showed a dose-dependent decrease effect. Biofilm inhibition activity at the highest concentration of 150 µg/mL tested was

reported as the highest for *E. coli*. In our study, the highest antibiofilm (40 %) effect was observed for *E. coli* at 2.25 μg/mL (Naveen et al., 2021).

In addition, Aloe vera and AgNPs, have already been reported antimicrobial and antibiofilm activity on several microorganisms, including against Gram (–) bacteria such as *P. aeruginosa*. In addition, AgNPs is that they are known to antimicrobial effect (Rai et al., 2012). However, AgNPs must have a bioactive function during the formation of AgNPs@AVs. It is especially important to understand the functional effects on microorganisms in order to develop novel antibacterial agents.

5. Conclusion and Suggestions

AgNPs have a wide field of study such as catalysis, biosensing, imaging and antimicrobial activity. It has become the focus of attention, especially with its environmental friendliness away from the toxicity of chemicals. Nanoparticles attract a lot of attention as they prevent antibiotic resistance and are an alternative to antibiotics. Green synthesis is an alternative method developed to produce silver nanoparticles using natural plant components. Plant extracts: they contain many functional substances, including cyclic peptides, sorbic acid, citric acid, euphol, polyhydroxy limonoids, ascorbic acid, retinoic acid, tannins, ellagic acid and gallic acid. It is believed to play a crucial role in the biological reduction and stabilization of nanoparticles. Therefore, our study focuses on the biosynthesis of AgNPs with plant extracts of aloe vera leaves (Tippayawat et al., 2016). In addition, we see the effects of AgNPs on antibiotic resistant bacteria in our study.

Statement of Conflict of Interest

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The study is complies with research and publication ethics.

Author's contributions

The contribution of the authors is equal.

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