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Green Synthesis of Silver Nanoparticles (PA-AgNPs), Characterisation, Antibacterial and Proliferative Effects

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ABSTRACT

Currently, the creation of several novel materials has accelerated the fields of nanoscience and nanotechnology. The production of nanoparticles is being developed using a variety of physical, chemical and even biological processes. The biological approach is becoming increasingly popular among these methods because it is easy to use, requires modest operating conditions, and produces waste and products in a more environmentally friendly manner. Green nanoparticles can be synthesised using a variety of components and biochemicals found in plants, which can act as stabilising and reducing agents. There is great potential for AgNPs, particularly in the medical applications or biomedical field. The most common application of nanoparticles has been as antibacterial agents. In this study, AgNPs were synthesised using the green synthesis method. The antibacterial and proliferative effects of these synthesised nanoparticles were investigated. In this direction, yellow cherry (*Prunus avium* L.) belonging to Konya/Ereğli region was harvested at appropriate time and conditions. The extract of the collected yellow cherry was used as a reducing agent to obtain Ag nanoparticles. The extract obtained from the collected yellow cherry, rich in polyphenols and flavonoids, served as a reducing agent for AgNP synthesis. The Prunus avium-AgNPs (PA-AgNPs) were found to be spherical with an average size of 40.98 ± 12.45 nm based on SEM analysis. The synthesised Ag-NPs were shown to be effective against S. aureus and E. faecalis bacteria based on their antibacterial activity, which was assessed using the disc diffusion technique. At the same time, a healthy cell line (L929) was used to assess the proliferative effect using the MTT technique. The results showed that the IC50 (concentration that inhibits 50%) of the cells was 46 µg mL-1 at 24 hours, 92.5 µg mL-1 at 48 hours and 105 µg mL-1 at 72 hours. In this study, silver nanoparticles were synthesized with an environmentally friendly approach using the extract obtained from the fruits of Prunus avium, which is rich in bioactive content. Compared to AgNPs synthesized by chemical methods, particles synthesized by biosynthesis (green synthesis) method showed much lower cytotoxic effects.

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INTRODUCTION

Nanotechnology is one of the emerging branches of science with a variety of applications in the biomedical field (Mustapha et al., 2022). The aspects that green nanotechnology, a subset of nanotechnology, has recently revealed at the molecular level have attracted much attention (Uyaner et al., 2024). These products continue to be a developing field of study with their use in the treatment of various diseases in many fields, especially in medicine (Ramazanli and Ahmadov 2022). A new class of useful materials are nanoparticles. Among the useful superior properties that NPs offer during synthesis is their resistance to high temperature fluctuations (Syafiuddin et al., 2017). The field of biomedical applications is very interested in metallic NPs. Metallic nanoparticles can be created using a variety of chemical, physical and biological methods (Keskin et al., 2023). Physical and chemical methods are time and energy consuming, expensive and not environmentally friendly. Various enzymes, algae, microbes and plants are used for the biological synthesis approach. Green synthesis of NPs using plant extracts has become a promising technique for the production of metallic NPs, especially in recent years. It offers significant advantages, including the ability to synthesise NPs in a straightforward, rapid, inexpensive and environmentally friendly manner, as well as ease of scaling, a less biohazardous structure and protection of cell lines (Jabir et al., 2021). Nanometals, particularly gold (Au), zinc (Zn), silver (Ag), palladium (Pd) and titanium (Ti), are often preferred in areas such as drug delivery systems, biological markers and the manufacture of optical devices. Furthermore, these metals are not only used in the synthesis of NPs, but also in some processes such as increasing their stability (Emanuel et al., 2015). In addition, NPs are useful substances with applications in food and cosmetics, medical applications, bioremediation research, etc. (Khalilov, 2023). Silver NPs are being studied by scientists in many different fields due to their unusual physicochemical structures among metal nanoparticles (Khan et al., 2023). In a variety of scientific fields, including biomedical research, AgNPs are extensively used in antibacterial, antiviral, anti-inflammatory and anti-cancer treatments. Several biomedical articles that inhibit infection are made with AgNPs (Jumah et al., 2020). Silver was used to heal infected wounds before antibiotics were used in modern medicine, but its use has declined due to its high toxicity and the accessibility of antibiotics (Singh et al., 2018). Antibiotics have become the most preferred group of drugs for the treatment of infections in human and animal health, and even in aquaculture production. However, excessive use of this group of drugs causes antibiotic resistance in organisms and their presence in large quantities in wastewater has been analysed (Aydın et al., 2024). Scientists have recently become interested in AgNPs, partly because of their potential use as antibacterial agents (Gunashova, 2022). Much research has used biological methods and plant sources (leaf, flower, root, fruit or whole plant) to produce AgNPs (Namburi et al., 2021). In particular, the fact that AgNPs produced in synthesis studies using plant sources are environmentally friendly and do not require special conditions, together with the fact that the synthesis process is simple, inexpensive and produces a larger quantity of product, are some of the factors that are driving interest in biological approaches (Rather et al., 2022). Due to its non-toxicity, environmental friendliness, affordability, ease of scalability, and greater yields than chemically synthesized AgNPs, green synthesis AgNPs have gained more attention from researchers in the literatüre (Asif et al., 2022). Plants, especially their fruit parts, are rich sources of potentially bioactive compounds such as phenolic acids, flavonoids, coumarins, and various organic acids. In extracts derived from plant sources, phenolic compounds, alcohols, flavonoids, and phytochemicals with carboxyl groups are substances that decrease the positively valued silver in the aqueous structure, create silver nanoparticles, and also have an impact on stability (Keskinkaya et al., 2023; Srikar et al., 2016). The use of NPs to be obtained using the green synthesis method as reducing agents, such as minimizing carbon footprint, sustainability, and reducing product toxicity, supports environmental protection efforts compared to chemical reducing agents (Kazak, 2024).

In addition to providing nutrients, fruits and vegetables also naturally contain antioxidants through secondary metabolites. Due to their positive impact on human health, antioxidants are gaining attention. They help prevent the damaging free radicals that cause cancer, heart disease, and many other disorders (Shui et al., 2006). Both naturally occurring oxidation and reduction processes in living things and dangerous sources including radiation, viruses, air pollution, and toxic byproducts of cell metabolism can produce free radicals. Lipids, proteins, nucleic acids, and other pathogenic processes are all oxidatively damaged by these reactive species. The relevant reactive species are known to contribute to aging, cell damage, and tissue damage by causing molecular alterations and gene mutations in cells (Ugur et al., 2023). The human body benefits from antioxidants because they neutralize free radicals and stop them from forming by giving up their electrons. They delay or stop the oxidation of the substrate that causes oxidative damage and are present in foods and the body in lower quantities than oxidizable substrates (Buyuktuncel et al., 2013). The fruit of the genus Prunus, which belongs to the Rosaceae family, is *Prunus avium* (Usenik et al., 2008). *P. avium* is one of the most popular temperate climate fruits. According to reports, one of the natural antioxidants is the white cherry, Prunus avium (Pszczola, 2001 It is a fruit with high consumption due to its sweetness, rind color, and sugar content. It has been reported that sweet cherries contain various phenolics, anthocyanins, flavonols and flavan-3-ols, and hydroxycinnamate (Gonçalves et al., 2004; Usenik et al., 2008). Based on this, we hypothesized that *P. avium* fruits containing flavonoids, ellagitannins, and anthocyanins could be applied in the green synthesis of AgNPs.

In this study, non-toxic, environmentally friendly, cost-effective, easily scalable PA-AgNPs were collected during the harvest of Yellow Cherry (Prunus avium L.), extracted and produced by green synthesis method. The functional groups of the synthesized nanoparticles were evaluated by FTIR measurements and their morphological properties were evaluated using SEM. Using this cherry from Ereğli district of Konya province, high bioactive content and efficient PA-AgNPs were obtained. The antibacterial activity of these green nanoparticles (PA-AgNP) was evaluated using disc diffusion method (*E. coli, S. epidermidis, S. aureus and B. Subtilis*). MTT method was applied to evaluate the proliferative effect on the cell line L929 (Mouse Fibroblastic Cell Line) in accordance with ISO standards. In this study, AgNP, which is synthesized for the first time by green synthesis method of Prunus avium extracts, one of the natural foods with high bioactive content, was synthesized and its antibacterial and proliferative effect potential was successfully evaluated. Moreover, this study is an innovative approach in obtaining many products with high bioapplicability in the environmentally friendly medical field thanks to green nanotechnology by using plants with various biocomponents.

MATERIAL and METHOD

Preparation of Prunus avium Extract and Synthesis of PA-AgNP

Prunus avium (yellow cherry) species was obtained from Konya/Ereğli public markets and after the samples were sorted and cleaned, they were stored at -20^{o}C. The fruits were brought to room temperature and squeezed with a press to extract the juice for use in the green synthesis study. The prepared extract was filtered using filter paper. About three hours were spent stirring a 100 mL volume solution with 0.1 M concentration AgNO3 salts (metal nitrate) and 20 mL of cherry extract at 60^{o}C using a magnetic stirrer (Atacan et al., 2023). Then, this precipitate nanoparticle was pyrified and dried at 60 ^{0}C.

Characterization Analysis of PA-AgNPs

The biomolecules' functional groups were identified using FTIR measurements in order to assess how well the produced Ag NPs were stabilized. SEM, or scanning electron microscopy, was used to examine the morphological characteristics of the produced nanoparticles.

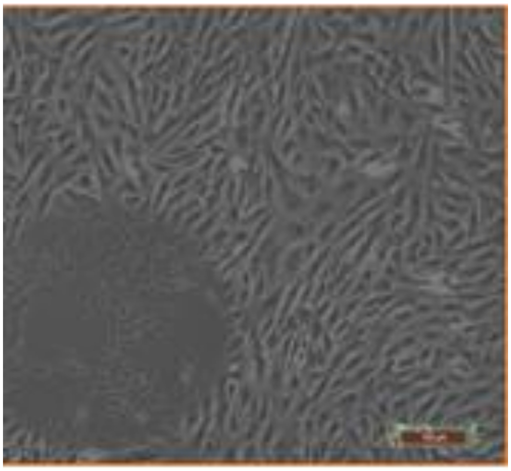
Assessment of PA-AgNPs' Antibacterial Activity

In our study, the disk diffusion method, which is one of the most preferred methods to evaluate the antibacterial activity of the PA-AgNPs, was used. In the study, standard strains of Escherichia coli, Bacillus subtilis, Staphylococcus epidermidis and Staphylococcus aureus, which are commonly found on surfaces and cause hospital infections, were obtained from Sakarya University Microbiology Laboratory. Results were interpreted according to the National Committee for Clinical Laboratory Standards.After dissolving the prepared PA-AgNPs in 1% DMSO, the concentration was adjusted to 1 mg mL-1. Then, as a control group, 30 µL of 1% DMSO, PA-AgNP and yellow cherry extract were impregnated onto sterile discs with a diameter of 6 mm. Using a densitometer, suspensions with a density of 0.5 Mcfarland (108) were prepared from fresh cultures (24 h old). Using a swab, samples from this solution were added to Mueller Hinton agar medium. Pre-prepared sample-impregnated discs were placed in Petri dishes into which bacteria were inoculated. After incubation (24 hours at 37 °C), the inhibition zones formed in the Petri dish were measured using a digital caliper and the results analysed (Semerci et al., 2020). Gentamicin-loaded discs were used as positive controls. All studies were performed in 3 replicates under aseptic conditions and the results were given as mean.

Cell Line and Cell Culture Medium

The cell line L929 (Mouse fibroblastic cell line), which complies with the UNI EN ISO 10993/2009 standard, was used to investigate the proliferative effect of green synthesis PA-AgNPs (Figure 1) (Torabi et al., 2023; Cannella et al., 2019). DMEM High Glucose (2 mM L-glutamine) was used as the culture medium. To avoid contaminating the culture media, Penicillin/Streptomycin was added at a dosage of 1 mg mL-1 in addition to supplementing the culture medium with fetal calf serum (10% (v/v)) (fetal bovine serum, FBS). The mouse fibroblastic cell line was multiplied by incubating in an incubator (at 37°C, 5% CO2). When the monolayer (adherent) cells filled 85% of the medium in which they were grown, they were enzymatically separated from the surface of the culture vessel and passaged. Thus, the storage and continuity of the cell line was ensured.

Figure 1. Inverted microscope image of L929 in (Mouse Fibroblastic Cell Line) that after 24 hours of seeded and after confuluent the flask at 10x.



Investigation of Cytotoxic Effect of PA-AgNPs

MTT is a yellow, water-soluble tetrazolium dye (Cat. No. 32030, Serva, TURKIYE). This dye is reduced to formazan crystals by dehydrogenase enzyme groups active in the mitochondria of living cells and turns purple. The purple colour can be obtained by dissolving the formazan crystals formed with DMSO (dimethylsulfoxide). The purple colour formed by the dissolved formazan crystals is evaluated spectrophotometrically (Korzeniewski and Callewaert, 1983). The MTT method is a colorimetric assay to evaluate metabolically active cells by reducing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide salt to insoluble formazan crystals by dehydrogenase, which can work with mitochondrial activity (Boyraz et al., 2021). In the study, L929 cells (2x105 in 1 ml) were seeded onto the 96-well cell culture dish for analysis and then incubated for 24 hours at 37°C in an incubator with 5% CO2. At the end of the incubation period, PA-AgNPs were prepared at a concentration of 10 mg mL-1 using DMSO at a ratio of 1/1000. Concentrations between 250 µg mL-1 -4 µg mL-1 were applied using the serial dilution method (Concentrations determined by serial dilution method: 4-8-16-32-64-125-250 ug mL-1). To evaluate the proliferative effect, MTT was applied after incubation at these concentrations for 24-48-72 time periods. The proliferative effect of each determined concentration was determined by the MTT method after 24-48-72 hours. Cell proliferation was analysed at 570 nm in a microplate reader (Thermo Scientific, USA) (Kars et al., 2006).

Statistical Analysis

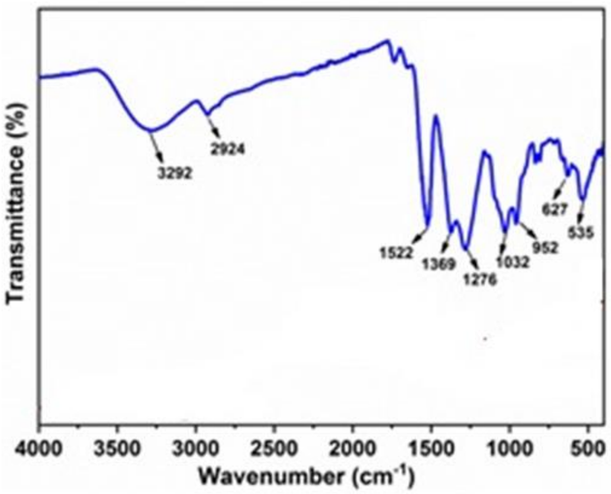
Analyses regarding cell viability were repeated at least three times. Analyses were performed as a result of the proliferation values determined for each concentration using the formula %cell Proliferation=(control/treatment)*100. Significant differences between the concentrations determined at 24, 48 and 72 hours were evaluated as a group for different time periods. Significant differences between proliferative effects at different concentrations were examined using Student's t-test ($p<0.05$) (Selvi, 2024). GraphPad Prism was used to statistically analyze the data (GraphPad Prism version 8.0.2, USA).

RESULTS

Synthesis and Characterization Analyses of PA-AgNPs

The FTIR analysis results of the Ag NPs synthesized by the green synthesis method using yellow cherry fruit extract are shown in Figure 2.

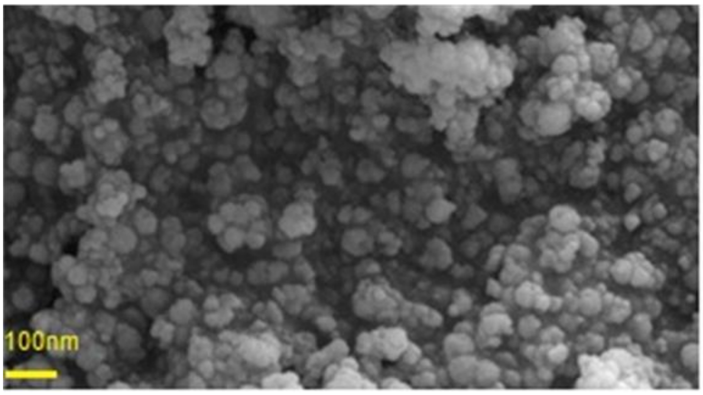
Figure 2. FTIR Spectra of PA-AgNP



The stretching indicated by the peak at 2924 cm-1 is the -CH stretching vibration of alkanes (Saiful et al., 2019). The C=O bond at 1717 cm-1 can be considered as its origin. The CO vibration is responsible for the peak at 1032 cm-1 corresponding to the 952-1276 cm-1 region. The results of the FTIR analysis of this study are in agreement with those of other studies (Albeladi et al., 2020; Bagyalakshmi and Haritha, 2017).

In order to evaluate the surface properties and quantify the size of PA-AgNPs generated at the ideal concentration ratio of white cherry extract and AgNO3 combination, an SEM picture was taken. Figure 3 shows the resulting image and the synthesis of PA-AgNPs was indicated by the white dots present in the image. The average size of the spherical particles was 40.98 ± 12.45 nm**.**

Figure 3. SEM image of PA-AgNP



Evaluation of Antibacterial Activity of PA-AgNPs

The results of the antibacterial activity of AgNPs prepared from yellow cherry extract against Escherichia coli, Bacillus subtilis, Staphylococcus aureus and Staphylococcus epidermidis bacteria are shown in Table 1. In order to determine whether the yellow cherry extract has an effect on the antibacterial activity of AgNPs, their antibacterial activity was also evaluated. It was found that the synthesised AgNPs produced an inhibition zone diameter of 13 mm on S. epidermidis and S. aureus bacteria. It was observed that there was no antibacterial activity in the discs impregnated with 1% DMSO and yellow cherry extract. When PA-Ag NP was compared with the antibiotic gentamicin, it was determined that PA-Ag NPs showed moderate antibacterial properties on E. coli, S. epidermidis and S. aureus.

Table 1. Antibacterial activity of synthesized Ag NPs and yellow cherry extract on test bacteria

	Test bacteria [(Inhibition zone diameter: IZD (mm)] (±SD) (n=3)			
	E. coli	***S. epidermidis***	***S. aureus***	***B. subtilis***
Ag NP	**12±0.1**	**13±0.3**	**13±0.1**	**11±0.1**
Yellow cherry extract	**0**	**0**	**0**	**0**
%1 DMSO	**0**	**0**	**0**	**0**
Gentamicin	**17.5±0.4**	**21±0.1**	**20±0.1**	**20±0.1**

Investigation of Cytotoxic Effect of PA-AgNPs

Using Prunus avium, green synthesised silver nanoparticles were added to L929 cell lines at a dose of 10 mg mL-1 for 24-48-72 hours. Viability analysis (MTT), determined by dehydrogenase enzyme activity dependent on mitochondrial activity, was used for each determined time period. Percentage cell proliferation graphs were plotted according to the results obtained. The graphs showed a decreasing trend between 62.5 µg mL-1 -250 µg mL-1 concentration at 24-48-72 hour periods (Figure 4abc). However, it was observed that the cells showed a proliferative effect between the concentrations of 8 µg mL-1-62.5 µg mL-1. As a result of the proliferation graph prepared, the concentration that inhibited 50% of the cells, IC50, was determined to be 46 µg mL-1 in 24 hours, 92.5 µg mL-1 in 48 hours and 105 µg mL-1 in 72 hours on average (Figure 4d). In addition, the experimental groups whose incubation periods ended as a result of PA-AgNP application were photographed under an inverted microscope as a function of dose and compared with the MTT test results. The % cell viability graph and the inverted microscope images produced as a result of the MTT method used in our research confirm and support each other (Figure 5).

Figure 4. Proliferative effect of green synthesis PA-AgNP. **a)** 24 hour % cell viability analysis, (**b)** 48 hour % cell viability analysis results, **c)** 72 hour cell viability analysis and **d)** concentration that inhibits fifty percent of the cells (24-48-72). The p values were respectively found to be 0.001/0.001/0.001 at 24/48/72 hours GraphPad Prism version 8.0.2 was used to statistically analyze the data. The threshold for statistical significance was set at $p<0.05$.

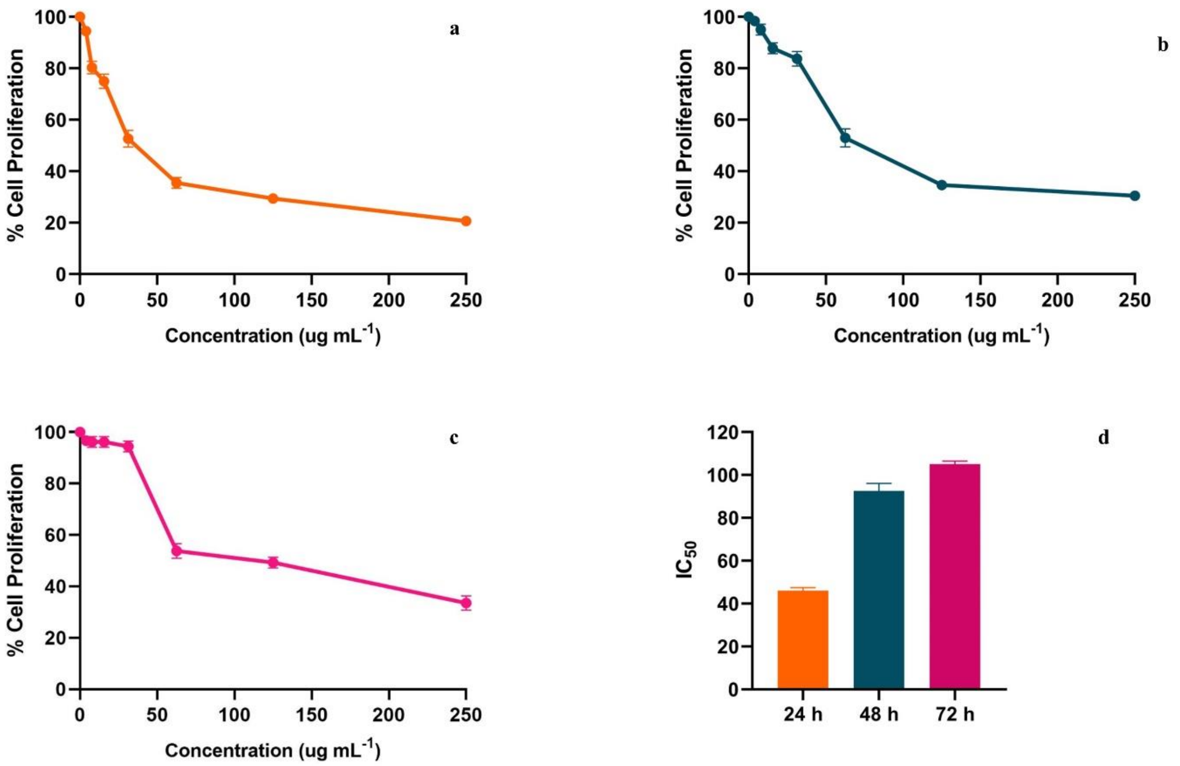
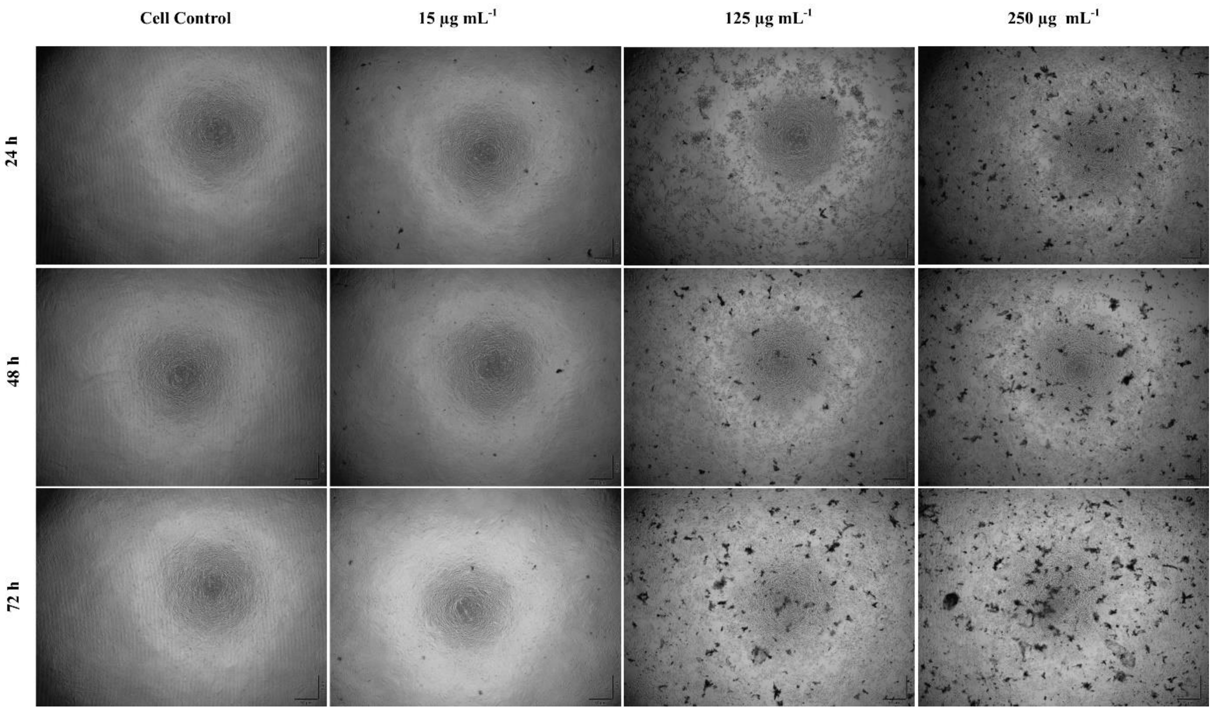


Figure 5. Inverted microscope (10X) images of green synthesis PA-AgNP.



As a result of the statistical analysis, p values were respectively found to be 0.001/0.001/0.001 at 24/48/72 hours (Table 2). The P value less than 0.05 indicates that the analysis results are significant ($p<0.05$).

Table 2. Student's t-test analysis ($p<0,05$)

	t value	**p value (p<0.05)**
24 h	5,635	0,001
48 h	6,115	0,001
72 h	7,078	0,001

DISCUSSION

Silver nanomaterials have great potential to be used directly or indirectly in medical applications (Uygur et al., 2009). However, the research conducted has led to the search for new synthesis methods due to the production of AgNPs by the known classical chemical/physical synthesis method and its toxic effects on living cells. In the age of ecologically friendly development, green synthesis methods for producing metal nanoparticles from plant extracts have drawn attention because of their affordability, ease of use in comparison to chemical and physical methods, and environmental sustainability (Alshameri and Owais, 2022). The reduction of metal ions to zero-valent metals and the stability of metal nanoparticles are caused by terpenoids, flavonoids or alkaloids, polyphenols, phenolic acids, and other secondary metabolites that are present in different plant sections. Furthermore, fatty acids, carbohydrates, or amino acids can be used as reducing agents in the creation of metal oxide nanoparticles (Behravan et al., 2019).

AgNPs have been reported in the literature to exhibit cytotoxicity, which is mostly concentration dependent (Akter et al., 2018). In the study conducted by Park et al. (2010), it was reported that 0.2 µg mL-1 (0.2 ppm) AgNP concentration decreased cell viability by 20% and 1.6 µg mL-1 (1.6 ppm) AgNP concentration decreased cell viability by 40% when AgNPs were applied to the RAW 264.7 cell line (Park et al., 2010). Similarly, 25 µg mL-1 (25 ppm) was reported to be the most toxic concentration of AgNPs applied to the rat liver cell line BRL 3A, and toxicity was observed at concentrations ranging from 1 to 25 µg mL-1 (1-25 ppm) (Akter et al., 2018).

Nowadays, the development of green nanotechnology reduces the toxic effects of highly toxic metals such as Ag, especially by using green synthesis method with plant extracts. Veeraraghavan et al. (2021) obtained green synthesis SB-AgNPs using Scutellaria barbata extract and applied green synthesis AgNPs at concentrations of 2.5, 5, 7.5, 10, 15 µg mL-1 for 24 hours using L929 cell line. In this period, the viability rates of AgNPs at the concentrations used are over 50% compared to the chemical and physical method (Veeraraghavan et al., 2021). A similar study was carried out by Maghimaa and Alharbi. CL-AgNPs obtained from Curcuma longa L. extracts were applied to the L929 cell line for 24 h and it was reported that they showed no toxic effects at concentrations between 5-35 µg mL-1 (Maghima and Alharbi, 2020).

In the study by Ghasemi et al. (2024), RD-AgNP synthesis was carried out using an extract from the leaves of the Rubus discolor plant. It was found that it showed anticancer activity at concentrations between 11.2 µg mL-1 and 49.1 µg mL-1 in different types of cancer cells (HepG2-MCF7-A432), while it showed cytotoxic effect at a concentration of 158 µg mL-1 in the healthy cell line HU02. In comparison, PA-AgNPs were reported to have much lower cytotoxic activity in the healthy cell line (Ghasemi et al., 2024). In another study, DK-AgNPs were obtained by Keskin et al. (2023) by green synthesis method using Diospyros kaki L. The cytotoxic effects of DK-AgNPs were investigated on glioblastoma (U118), human ovarian sarcoma (Skov-3), human colorectal adenocarcinoma (Caco-2) cancer cell lines and healthy human dermal fibroblast (HDF) cell line by MTT technique. Experimental results showed that after 48 hours of incubation with DK-AgNPs for cancer cell lines, it was found to be highly cytotoxic (25-50 µg mL-1), while it was reported that the cytotoxic effect was less in the healthy cell line (25-200 µg mL-1) (Keskin et al., 2023).

In this study, PA-AgNPs obtained from Prunus avium extracts by green synthesis method were applied to the healthy mouse fibroblastic cell line L929 for 24-48-72 hours and 50% cell viability was determined as 24h-46 µg mL-1, 48h-92.5 µg mL-1 and 72h-105 µg mL-1 by MTT method. In the study, concentrations between 250 µg mL-1 -4 µg mL-1 (Concentrations determined by serial dilution method: 4-8-16-32-64-125-250 ug mL-1) were used, which are much higher values compared to the literature. When the obtained IC50 values were compared to the chemical method in the literature, no cytotoxic effect was observed at high concentrations. In the study to evaluate the proliferative effect, PA-AgNPs were evaluated on L929 separately at three different incubation times. IC50 values at different times were also compared within themselves.

Studies in the literature show that AgNPs are mostly spherical and oval in shape and their average diameters are up to 10-50 nm (Rafique et al. 2017). In our study, AgNPs synthesized using yellow cherry fruit extract were found to be in this range. Similarly, AgNPs synthesized using fruit extracts including Carissa macrocarpa and Momordica charantia fruit extracts were determined to be spherical in shape and to have antimicrobial activity (Soman and Ray, 2016; Rashid et al., 2017). Roy et al., (2014) reported that Ag-NPs synthesized by green synthesis method using Malus domestica extract were spherical in appearance and the average diameter of the spheres was 20 nm.

Numerous studies have demonstrated the antibacterial properties of Ag NPs prepared using plant products and the green synthesis approach. Reportedly, Ag NPs made with Abutilon indicum extract exhibited strong antibacterial activity against bacteria such as S. typhi, E. coli, S. aureus and B. subtilis (Ashokkumar et al., 2015). Ag NPs prepared from Aloe vera extract were found to be effective against P. aeroginosa, S. aureus and E. coli bacteria in a related investigation. In our study, Ag NPs synthesised using yellow cherry extract were found to have antibacterial activity against the test bacteria used, similar to studies in the literature.

It is thought that PA-AgNPs exhibit strong antibacterial activity depending on the nanoparticle size (average 41 nm) prepared in our study. The actual mechanisms of the antibacterial and antifungal activities of AgNPs are among the issues that are attempted to be elucidated in detail. The antimicrobial activity of Ag + ions contained in the nanoparticle structure is explained by many mechanisms. The silver ions in the structure have the ability to bind preferentially to the phosphate group of the nucleic acid, rather than to its nucleosides, and to form various complexes with nucleic acids, including DNA and RNA. Some research suggests that positively charged nanoparticles and negatively charged bacterial cells are electrostatically attracted to each other, making them the best proposed bactericidal agent. In addition, they have been shown to not only diffuse within the cell, but also accumulate in the membrane and intercellular space, causing denaturation of the membranes or bacterial wall (Biswas et al., 2019; Abada et al., 2024).

CONCLUSION

In recent years, it has been observed that the bioactivity of metal nanoparticles has been increased by obtaining them by green synthesis method using biological materials. In our study, PA-AgNPs were found to have IC50 values of 46 μg mL^{-1} at 24 h, 92.5 μg mL^{-1} at 48 h and 105 μg mL^{-1} at 72 h in L929 cells, which indicates that the biological (green synthesis) synthesis method has low cytotoxicity when compared to chemical and biological synthesis methods. Thus, in our study, they were found to be much less cytotoxic, even at an average concentration 10 times higher, especially in the healthy cell line L929. In addition, this study suggests that it is a wound healing agent thanks to its bioactive content, especially with metals with high antibacterial properties such as AgNP. Long-term toxicity assessments, anticancer activity, and in vitro and in vivo wound healing models are anticipated in future research to confirm the possible medicinal uses of PA-AgNPs.

Ethical Statement

Local Ethics Committee Approval was not obtained because experimental animals were not used in this study.

Author Contributions

Research Design (CRediT 1) Author 1 (%50) – Author 2 (%40)- Author 2 (%10)
Data Collection (CRediT 2) Author 1 (%50) – Author 2 (%40)- Author 2 (%10)
Research - Data analysis - Validation (CRediT 3-4-6-11) Author 1 (%60) – Author 2 (%40)
Writing the Article (CRediT 12-13) Author 1 (%60) – Author 2 (%40)
Revision and Improvement of the Text (CRediT 14) Author 1 (%60) – Author 2 (%40).

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Conflict of Interest

Authors declare that there is no conflict of interest.

Sustainable Development Goals (SDG)

13 Climate Action

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