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Evaluation of biological activities of silver nanoparticles (AgNPs) synthesized by green nanotechnology from birch (*Betula* spp.) branches extract

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Abstract

Green synthesis of silver nanoparticles (AgNPs) provide superiority due to their usage of various biological applications. The aim of this study is synthesizing the silver nanoparticles by using Birch (*Betula* spp.) branches extract with an ecofriendly, cost-effective, simple, and cheap green method. Even *Betula* is wide-spread tree with rich in phenolic compounds, data on the use of *Betula* branches is limited. Within this scope, this study is the first for using *Betula* branche extracts which take part as reducing and capping agent to synthesize the silver nanoparticles to evaluate the antimicrobial activity and antiproliferative efficiency. The biosynthesized AgNPs were characterized by various characterization methods such as UV−visible spectroscopy, dynamic light scattering (DLS), Fourier transform infrared (FTIR) spectroscopy and scanning electron microscopy (SEM). The characterization analysis has revealed the phenolic compounds of *Betula* extract acted as reducing and capping agent for formation of AgNPs.

The synthesized selected AgNPs were exhibited spherical shape with 103.2 ± 5.2 and 69.2 ±12.7 nm according to DLS and SEM analysis, respectively. Also, the biological activity of biosynthesized AgNPs were evaluated by antimicrobial and antiproliferative tests on selected microorganisms and cell line, respectively. The IC_{50} values of B3-4 AgNPs was determined as 64.27 µg/mL on HT29 colorectal cancer cells. And also, the antimicrobial activity results of AgNPs have revealed the dose-dependent inhibition for all studied test microorganisms.

In conclusion, this study evidently suggests the use of silver nanoparticles biosynthesized from *Betula* branches extract as a potential agent for antimicrobial and anticancer studies.

Keywords: *Betula* spp., silver nanoparticles, green synthesis, antiproliferative, colorectal cancer, antimicrobial

1. Introduction

Nanotechnology is a multidisciplinary field of science and engineering that deals with the design, manufacture and control of nanometer-scale materials, the smallest building blocks of matter. A nanometer is a unit of measurement that is one billionth of a meter, and this scale is an ideal size for understanding and manipulating the properties of materials at the atomic and molecular level. The main goal of nanotechnology is to enable the production of new and advanced products by utilizing the unique properties of materials of these small sizes. The properties of nanomaterials differ from those of large-scale materials and offer several advantages in their electrical, mechanical, thermal and optical properties. These properties allow nanotechnology to be used in a range of industrial, scientific and medical applications [1–5]. The applications of nanotechnology in the field of health aim to treat diseases more effectively, increase the rate of early diagnosis and overcome limitations in the field of medicine. Nanotechnology enables the development of drug carrier systems that enable more effective transport and delivery of drugs to targeted sites in the body, the development of high-resolution imaging and diagnostic tools, the development of nano-sized sensors that allow the detection of biological analytes at low concentrations, and the design of nanorobots or nanomachines used in surgical operations [6–11]. Among the nanotechnological products that have application areas in many sectors, silver nanoparticles play an important role in many applications, especially in the field of medicine due to their unique properties. One of the most remarkable properties of silver nanoparticles is their strong antimicrobial effects. Silver disrupts the cell membrane of microorganisms and prevents them from multiplying. This property provides antibacterial, antiviral and antifungal effects, which

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makes silver nanoparticles preferred in many applications such as wound healing, medical devices and antibacterial coatings. In addition, silver nanoparticles are generally biocompatible, meaning that they can be used in a compatible manner with biological systems. This property allows them to cause minimal toxic effects in the body and optimize their interaction with biological tissues. This allows silver nanoparticles to be used safely in medical applications. The surface plasmon resonance (SPR) properties of silver nanoparticles enable their use in imaging and sensor technologies. Silver nanoparticles can be well dispersed in various solvents. This property allows nanoparticles to be homogeneously used and dispersed in various medical applications. Nano-sized silver particles have large surface areas, which increases their reactivity. This feature enables silver nanoparticles to be effective in antimicrobial and catalytic applications. The high surface area enhances the antibacterial effect by increasing the interaction with microorganisms. In addition, these properties allow them to be used in drug transport and targeting studies. Moreover, silver nanoparticles attract attention with their anticancer activities. Silver nanoparticles have the capacity to stop the cell cycle of cancer cells. It has been reported that with this property, they stop the uncontrolled growth of cancer cells by preventing the division and proliferation of cells. Silver nanoparticles can increase oxidative stress in cancer cells. This may cause damage to cancer cells by increasing the production of free radicals in the cells. Silver nanoparticles can trigger the degradation of the genetic material of cancer cells and the process of apoptosis. Considering all these features, silver nanoparticles are widely used in studies such as imaging and diagnostics, drug delivery and targeting, tissue regeneration and rehabilitation, anti-microbial applications, development of sensitive and unique nanosensors that detect tumor markers [12–21].

The methods used for the preparation of silver nanoparticles include chemical reduction, thermal reduction, microwave heating, and electrochemical synthesis methods. In these methods, silver salts are reduced from solutions under special conditions to obtain nanoparticles. However, these traditional methods have problems with the use of toxic chemicals, energy intensity, high cost, long response times and scalability. Chemical reduction and thermal reduction methods are generally not environmentally friendly and the use of toxic agents is contrary to the principles of green chemistry. The microwave heating method may involve disadvantages in terms of equipment cost and energy consumption. Electrochemical synthesis requires specialized equipment and can sometimes result in uncontrolled particle growth. In recent years, green synthesis method has been used to synthesize silver nanoparticles. Green synthesis represents an eco-friendly and sustainable approach and involves the use of herbal extracts, microorganisms or substances of natural origin for the synthesis of silver nanoparticles. In this method, environmentally friendly reducing agents are used. Green synthesis reduces the environmental impact of conventional synthesis methods, minimizes the use of toxic chemicals and enables medical and biological applications of silver nanoparticles with their biocompatibility properties. Green synthesis is an important step in the development of sustainable nanotechnology [14, 21–28].

Colorectal cancer is a type of cancer that develops in the large intestine (colon) or rectum. This type of cancer is recognized as a major health problem worldwide. Its incidence is increasing and it is a condition where early diagnosis is vital. Colorectal cancer is usually caused by abnormal cell masses called polyps, which can become cancerous over time. Risk factors include age, genetic predisposition, family history, inflammatory bowel diseases, poor eating habits and obesity. Methods used in the treatment of colorectal cancer include surgical intervention, chemotherapy, radiotherapy and targeted therapies. Surgery involves an operation to remove tumoral masses. Chemotherapy involves drugs used to kill cancer cells or control their growth. Radiotherapy is a treatment method that aims to destroy or shrink cancer cells by targeting them with high-energy rays. However, traditional treatment methods have various disadvantages. Disadvantages of surgery include the invasiveness of surgery, the patient's long recovery period, the serious side effects of chemotherapy and radiotherapy, and damage normal tissues. These methods often have a broad range of action and can also affect healthy cells. Additionally, in some cases, resistance to treatment may develop. Therefore, the development of new and more specific treatment strategies is an important area of research [29–31].

Betula is a genus of plants belonging to the Betulaceae family and includes tree and shrub species native to temperate climate regions. *Betula* tree has very valuable components, and the components may vary depending on the type of plant, growing conditions and extraction methods. *Betula* extracts contain triterpenoid components such as betulin and betulinic acid, flavonoids, phenolic acids, essential oils, minerals and vitamins. *Betula* tree extracts, through their various components, are an important biological resource in terms of reducing silver nitrate and stabilizing the silver nanoparticles formed by the green synthesis method [32, 34].

Taking all this into account, the aim of this study was to synthesize and to characterize silver nanoparticles,

which may have potential use in the treatment of infectious diseases and colorectal cancer, using green synthesis. It was also aimed to evaluate the antimicrobial and anticancer effects of silver nanoparticles synthesized and characterized by the green synthesis method.

2. Materials and Methods

2.1. Materials

Silver nitrate ($AgNO_3$), Muller Hilton Broth, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St Louis, MO, USA). Dulbecco's Modified Eagle's Medium (DMEM), high glucose, and pyruvate were purchased from Gibco (Waltham, MA, USA). *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), gram-positive bacteria, *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212) and a yeast, *Candida albicans* (ATCC 10231) strains were purchased for antimicrobial studies. HT29 (ATCC HTB-38) colorectal cancer cell line was purchased for antiproliferative studies and L929 mouse fibroblast cell line was also purchased for *in vitro* biocompatibility from ATCC Cell Bank (USA). All other chemicals which used in study were analytical grade.

2.2. Methods

2.2.1. Preparation of Betula spp. extract from branches

Betula tree branches were collected from Van province, Türkiye. The purification of branches was done under the distilled water by washing twice and dried under room temperature in the shade. The dried samples were grounded in an electric grinder and weighed. After that dried ground samples were weighed to 25 g and mixed with 250 mL distilled water for extraction. The extraction was carried out in Soxhlet Extractor at 80 °C for 8 hours. Then the extract was filtered by using Whatman No. 1 filter paper and stored at 4 °C in amber colored bottle for further use [34]. The experiment was carried out in duplicate.

2.2.2. Synthesis of silver nanoparticles (AgNPs)

The synthesis of silver nanoparticles from branches was carried out with some modifications as reported before [35]. Silver nitrate ($AgNO_3$) was used as precursor salts to obtain silver nanoparticles. To prepare silver nanoparticles, various concentrations (1 mM, 3 mM, and 5 mM) of $AgNO_3$ solutions were incubated with *Betula* extracts in the ratio 1:10 for 24 hours. Also, the effect of temperature on the formation of silver nanoparticles was performed by changing the temperature from room temperature to 80 °C. Finally, the formation of silver nanoparticles was confirmed by color changes on different reaction solutions. After color change, the reaction was stopped and silver nanoparticles were purified by centrifugation (Sigma 3-30KS, Germany). Therefore, the mixtures were centrifuged at 13500 rpm for 20 minutes twice to remove unreacted silver ions. Then the supernatant was discarded and the pellet was air dried in incubator overnight to obtain powder. The experiment was carried out in duplicate.

2.2.3. Characterization of AgNPs

The obtained silver nanoparticles were characterized by UV-Vis spectrophotometer (Rayto,RT, 2100C, China), particle size analyzer (Zetasizer Nano ZS, Malvern, UK), Fourier transform infrared (FTIR) spectrophotometer (Thermo Scientific / Nicolet IS50, China), Scanning electron microscopy (SEM) (FEI / Quanta 450 FEG, Japan).

2.2.3.1. UV-Vis spectroscopy

The reduction of silver ions in the presence of *Betula* extract was determined by measuring the spectrum of the reaction medium. The green synthesized silver nanoparticles were characterized by UV-Vis spectroscopy. UV-Vis spectrum of obtained silver nanoparticles were measured in the range of 200–800 nm by using UV-visible spectrometer. Three replicates of experiments were carried out.

2.2.3.2. Particle size analysis

The particle size, zeta potential and distribution of silver nanoparticles were analyzed by dynamic light scattering using Zetasizer Nano ZS (Malvern). The samples for measurement were prepared fresh each time and were analyzed at 25 °C. Three replicates of experiments were carried out.

2.2.3.3. FT-IR Analysis

Analysis of synthesized silver nanoparticles by FT-IR (Fourier Transform Infrared Spectroscopy) spectrometer provided information about structural properties of these nanoparticles. The measurements were carried out in the range of 400–4000 cm^{-1} at a resolution of 4 cm^{-1} by using KBr pellet method to introduce the bioreduced silver nanoparticles.

2.2.3.4. Scanning Electron Microscopy (SEM)

The synthesized silver nanoparticles from *Betula* spp. branches were analyzed by scanning electron microscopy to determine the formation, stability and surface morphology of nanoparticles.

2.2.4. Antiproliferative activity of AgNPs against HT-29 colorectal cancer cell

Antiproliferative activity of (AgNPs) against HT-29 colorectal cell (CRC) was evaluated using a MTT (3-(4,5-

dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. 1 × 10^4 cells/well were plated in 96-well plates and were incubated in DMEM medium supplemented with 10% FBS, and 1% antibiotics (100 U/mL penicillin, 100 µg/mL streptomycin) at 37 °C in a humidified atmosphere containing 5% CO_2. Medium was changed every 48 h. When the cells reached about 70–80% confluency, they were serum-starved over-night before AgNPs exposure and medium was changed with serum supplemented medium (100 µL). Then, cells were exposed to 100 µL of AgNPs which dispersed in serum free medium at different concentrations (20–100 µg/mL) for 24 and 48 h. Serum free medium was used as a control. After the incubating period, 20 µL of 5 mg/mL MTT solution was added to each well and incubated at 37 °C and 5% CO_2 for 4 h. Then, the culture media was aspirated and 200 µl DMSO was added to solubilize the formazan crystals. The plate was read in a plate reader at 570 nm. Results are standardized using control group values. The viability (%) of the exposed cells was defined as the percentage of absorbance compared to control unexposed cells. Three replicates of experiments were carried out.

2.2.5. Biocompatibility of AgNPs

Healthy fibroblast cells were used to determine the biocompatibility of synthesized nanoparticles in living cells. For this purpose, L929 (Mouse Fibroblast cell line) cells in DMEM containing 10% FBS were grown in 96-well plates. After 2 days, the cells were washed and AgNPs obtained at different concentrations were applied to the cells. Cell culture medium not treated with AgNP was used as a control. After 4 hours, the cells were washed twice and the MTT test was performed according to the manufacturer's instructions. The absorbance value was measured in a microplate reader at 570 nm. Three replicates of experiments were carried out.

2.2.6. Antibacterial and antifungal activity of AgNPs

The antibacterial and antifungal activities of AgNPs were investigated using gram-negative bacteria, *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), gram-positive bacteria, *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212) and a yeast, *Candida albicans* (ATCC 10231) as test microorganisms by the Kirby–Bauer Disk method. In brief, bacterial and fungal suspensions were inoculated at 10^6 cfu/spot on Mueller–Hinton Agar (MHA) and Sabouraud Dextrose Agar plates, respectively. Next, sterile paper disks were placed on the agar surface and different volumes of AgNPs stock solution (1 mg/mL) were added to disks to get the concentration of 5–20 µg/disk prior to microdilution method. 10 µg/disk gentamicin and 10 µg/disk of fluconazole were used as positive controls for bacteria and fungi, respectively. The negative control consisted of disk treated with serum free medium. After incubation at 37 °C for 24 h for bacteria and at 30 °C for 48 h for yeast, the diameters (in mm) of the inhibition zone of were determined. Three replicates of experiments were carried out.

2.2.7. Minimal inhibitory concentration (MIC) of AgNPs against test microorganisms

The microdilution method is a widely used method to systematically and sensitively evaluate the antimicrobial properties and MIC values of AgNPs. MIC of the AgNPs against test microorganisms was evaluated using broth microdilution method. Briefly, the test microorganisms (1 × 10^4 CFU/mL) were incubated with AgNPs diluted in MHB with the concentration gradients from 0.046–60 µg/mL. The wells not treated with AgNP was used as a control. The 96 micro-well plates were incubated at 37 ± 1 °C for 24 h. The first well without microbial growth was defined as the MIC, expressed in µg /mL. The tests were carried out in triplicate for sampling.

2.2.8. Statistical analysis

All data were given as mean ± standard deviation and the results were expressed by repeated analyses. Cell culture analysis results were evaluated by one-way ANOVA (*$p<0.05$) and considered statistically significant.

3. Results and Discussion

Firstly, the formation of silver nanoparticles was confirmed based on color change of the solution that usually occurs as a result of a chemical reaction. These reactions are usually based on a redox reaction and lead to the reduction of silver ions of $AgNO_3$ to form silver nanoparticles. The phenolic compound of *Betula* branches act as a reducing agent which resulted with formation of silver nanoparticles [34]. During these reduction reactions, the size, shape and arrangement of the nanoparticles change, leading to a marked change in their optical properties.

In this study we used three different $AgNO_3$ concentrations (1 mM, 3mM and 5 mM) to determine the color changes depending on the formation of silver nanoparticles. When the *Betula* branches extract was added to various $AgNO_3$ solutions color has changed from yellow to dark upon to $AgNO_3$ solution for 24 h at room temperature (Fig. 1A).

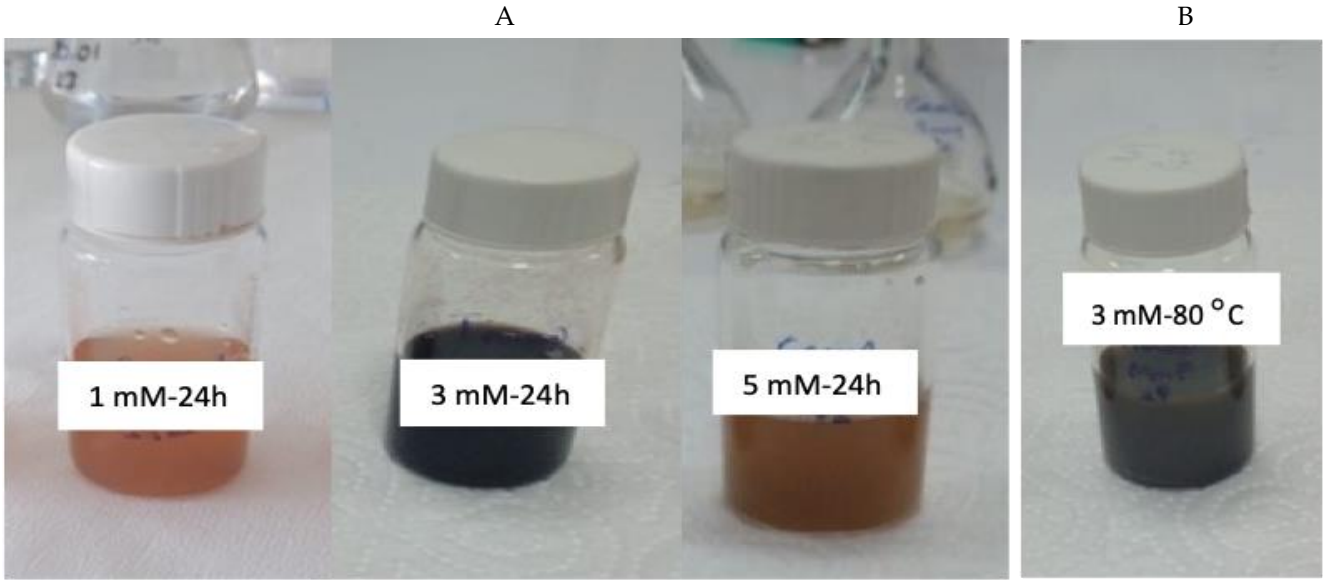

Figure 1. A. Color changes of *Betula* branches extract in the presence of various $AgNO_3$ concentrations (1 mM, 3 mM and 5mM) for 24 h at room temperature **B.** 3 mM $AgNO_3$ at 80°C (B3-4).

As shown in Fig. 1 the darker brown color was obtained by 3 mM $AgNO_3$ concentration (B3-4 formulation) at 24 h. The obtained darker color between the different concentrations indicated the biosynthesis of AgNPs by more reduction-induced silver nanoparticle formation by reducing silver ions to Ag0 nanoparticles [36,37].

Also, the effect of the temperature on formation of AgNPs, the reaction was carried at 80 ° C which observed by also color changes. These results consistent with the previous reports that increasing temperature of the reaction mixture decreases largely the reaction times to 4 h (Fig. 1B) [38]. And this outcome suggesting that the nanoparticle size would be decrease to the same extent. These findings revealed that the phenolic compounds of the *Betula* branches exhibits reducing agent.

Formulations (B3-4 and B3-24) in which color change occurred depending on different temperature and silver nitrate concentrations were selected and continued with the following characterization method.

3.1. UV-Vis spectroscopy

Selected AgNPs were characterized for their structural analysis by UV-vis spectroscopy which is most commonly used analytical method. In this study, the reduction of $AgNO_3$ silver ions to the AgNPs in existence of *Betula* extract was analyzed by UV-Vis spectroscopy in the range of 200–800 nm. While the UV-Vis profile of *Betula* plant branches extract did not show any absorbance peak, the maximum absorbance peak was obtained at 425 nm with B3-4 formulation (Fig. 2). Among the various $AgNO_3$ concentrations, 3mM $AgNO_3$ treated extracts exhibited higher reduction activity. This may be due to the fact that only fixed *Betula* extract can reduce the sufficient amount of $AgNO_3$. These findings compatible with the literature that higher concentration of plant extracts introduce more reducing activity in the presence of higher $AgNO_3$ concentration [39].

The color changes and the presence of a sharp absorption peak at 425 nm also revealed the formation of AgNPs which related with the surface plasmon resonance (SPR) because of the release of free electrons [40]. Our results were also compatible with the previous reports that silver nanoparticles were formed in the range 420–430 nm according to UV-Vis spectroscopy technique [41–43].The formation of selected AgNPs were verified depending on color changes by also UV-Vis spectroscopy.

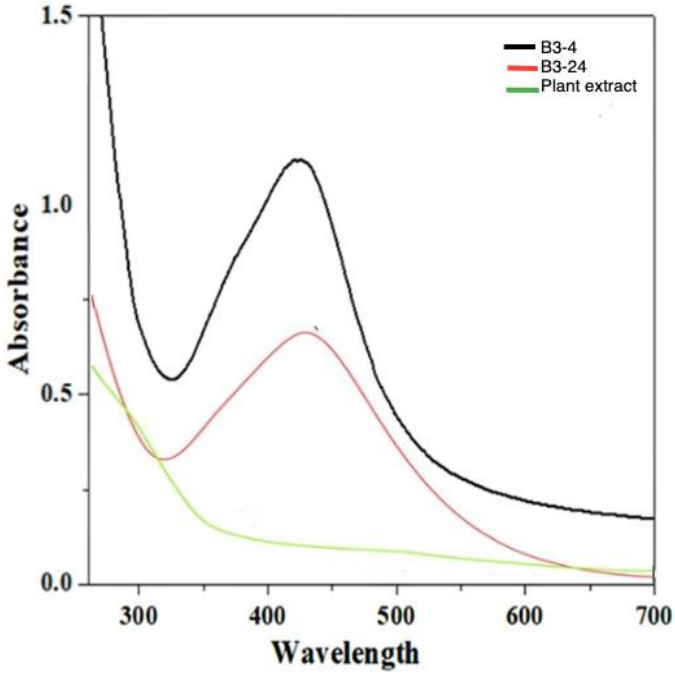

Figure 2. UV spectrum of the different *Betula* extracts according to synthesis temperature and AgNPs synthesized plant extract

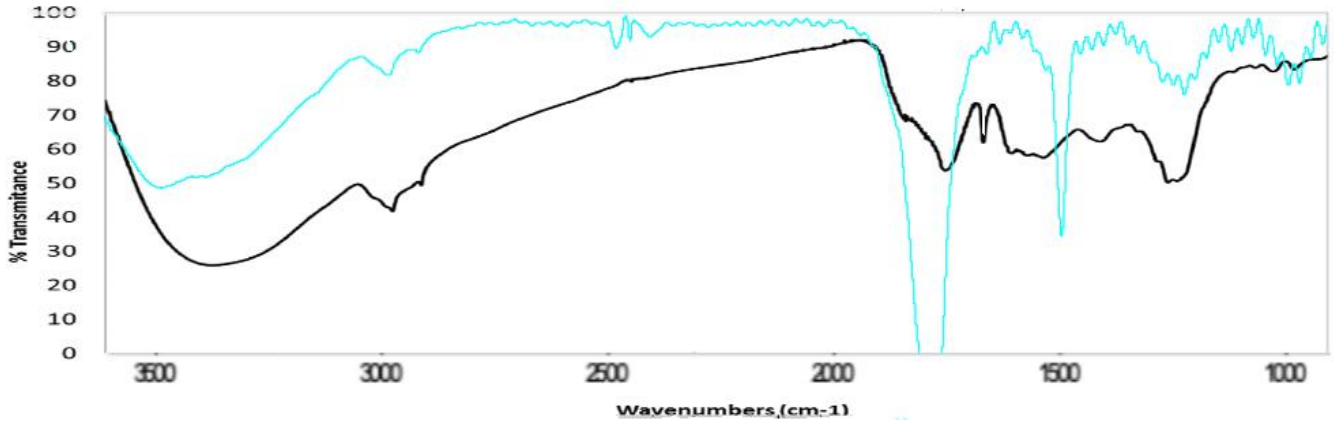

Figure 3. FTIR spectrum of *Betula* branches extract (Blank line) and AgNPs (Blue line)

3.2. Particle size analysis

In this study, AgNPs were synthesized based on the reducing power of the plant extract. However, there are various parameters that affect the physicochemical properties of nanoparticles synthesized with this method. In this study, the effect of initial $AgNO_3$ concentration on the mean particle size, zeta potential and distribution of AgNPs was evaluated. Next, the effect of reaction temperature on the size and preparation time was evaluated. Once the formation of colloidal AgNPs was confirmed by UV–vis spectroscopy, physicochemical properties of synthesized nanoparticles were analyzed by dynamic light scattering (DLS) method. Table 1 shows the synthesis conditions and physicochemical properties of AgNPs. As it can be clearly seen from the results, mean particle size and reaction time was significantly decreased by the increasement in reaction temperature. Therefore, formulation B3-4 was selected for further studies. Our results differed from the study that synthesized silver nanoparticles using aqueous extract of *Acacia cyanophylla*. In the study, relatively large nanoparticles were obtained when the reaction temperature increased from 35 °C to 100 °C [37]. Our results revealed that this synthesis method led to formation of monodispersed nanoparticles. The AgNPs demonstrated an acceptable particle uniformity indicating homogeneity. No shifts in dispersity of nanoparticles were observed with the increase of reaction temperature. The results were in agreement with those reported by Salvioni *et al* [44]. Nanoparticles were also evaluated in terms of zeta potential to determine the surface charge and the stability of nanoparticles. Results revealed that all formulations were negatively charged and reaction temperature had no significant effect on zeta potential.

3.3. FT-IR analysis

Functional groups contained in phytocomponents in plant extracts are responsible for the reduction of silver ions to AgNP [41,42]. In this context, *Betula* is wealthy plant that contains tannis and other phenols with hydroxyl groups (-OH) which is responsible for bioformation of silver nanoparticles [35].

In present work, we used Fourier transform infrared (FTIR) spectroscopy analysis to identify the functional groups present in the *Betula* branches extract involved in the reduction and capping of silver nanoparticles. The FTIR spectrum of the *Betula* branches extract exhibited distinctive absorption peaks in the range of 800–1650 cm^{-1}. The determined characteristic fingerprint absorption peaks of *Betula* extract at 1628–1640 cm^{-1} are related with the phenolic components of plant extract such as lignin, as indicated previous literatures [45,46]. Also, the fingerprint peaks at 1440 cm^{-1} indicated the methyl groups and 1200 cm^{-1} indicated the alcohol groups. These alcohol groups which found in plant extract presents the bioreduction of silver ions to AgNPs.

The main absorption peaks which determined at 3400, 2934, 1728 and 1008 cm^{-1} has revealed the formation of AgNPs (Fig. 3). Our reported results were also compatible with earlier publications [47,48].

3.4. Scanning electron microscopy (SEM)

Morphological characterization of the biosynthesized AgNPs was also determined by using SEM technique. Fig. 4 showed SEM images of the B3-4 with particle sizes ranging between 60–90 nm. When the results were

Table 1 Physicochemical properties of AgNPs synthesized at different conditions. Data was represented as the mean±SEM values of three independent batches

Formulation code	$AgNO_3$ concentration	Reaction temperature	Reaction time	Size (nm)	Polydispersity index	Zeta potential (mV)
B3-24	3 mM	23 °C	24 h	117.7 ± 14.4	0.28 ±0.09	-21.0 ± 2.7
B3-4	3 mM	80 °C	4 h	103.2 ± 5.2	0.26 ± 0.01	-21.5 ± 4.8

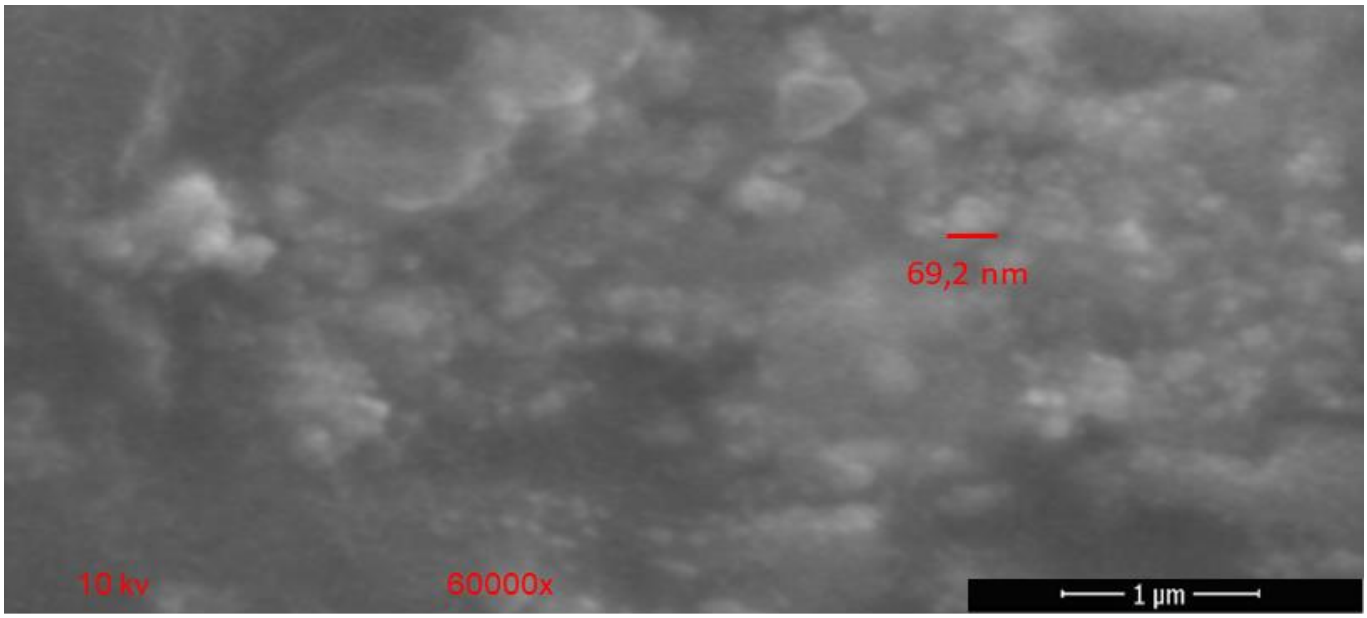

Figure 4. Scanning electron microphotographs of B3-4

compared to DLS method, the mean size of both biosynthesized nanoparticles were considerably smaller as a result of SEM analysis. In general, the size of nanoparticles determined by SEM analysis is approximately 20% smaller than those determined by the DLS method. The difference between the results regarding the size of the nanoparticles is due to the different analysis conditions of the two techniques. Through the DLS method, the average sizes of nanoparticles dispersed in liquid are analyzed. The presence of dispersants causes an increase in the size of the particles. SEM analysis is generally performed under vacuum and the sample must be dried during this process. Additionally, during SEM analysis, samples are usually coated with a thin metal layer. This coating process can lead to particle size reduction by sealing and flattening the surface of the particles [49]. Furthermore, when the morphology of the AgNPs was examined, it was seen that they exhibited spherical morphology. It also could be clearly observed that Ag-NPs partly aggregated.

3.5. Antiproliferative activity of AgNPs against HT-29 colorectal cancer cell

After characterization of the biosynthesized AgNPs, B3-4 formulation was evaluated for its cytotoxicity on malignant cells. For this purpose, HT-29 CRC cells were exposed to various concentrations of B3-4 AgNPs for 24 h and 48 h. After the indicated incubation period, MTT assay was performed to determine the half-maximal inhibitory concentration (IC_{50}). As it can be clearly seen in the Fig. 5, B3-4 AgNPs exhibited a dose and time-dependent cytotoxicity on HT-29 CRC cells. There was significant difference in cell viability between 24 hours and 48 hours incubation periods ($p<0.05$). The IC_{50} value of B3-4 AgNPs was 64.27 µg/mL after 48 h of treatment. Our results corresponded well with the study, in which biosynthesized AgNPs were evaluated for the antiproliferative activity against HT-29 CRC cell [50]. Our results were also in agreement with the antiproliferative activity of AgNPs biosynthesized using *Acacia nilotica* extract against two colon cancer cell line [51]. Similar results were reported by Shathviha *et al* [52]. They reported that β-sitosterol mediated AgNPs induced concentration-dependent cytotoxicity in HT-29 cells. Our results are in contrast to research conducted by Durai *et al* [53]. They reported that lower concentration of AgNPs had an antiproliferative effect on both HT-29 and HCT15 CRC cells (IC_{50}: 8 µg/mL).

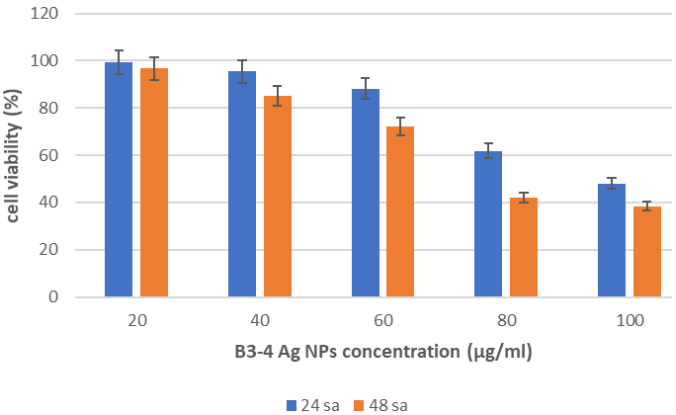

Figure 5. Determination of % cell viability of HT-29 CRC cells following treatment with different concentrations of B3-4 AgNPs

3.6. Biocompatibility of AgNPs

To evaluate the biocompatibility of B3-4 AgNPs, L929 mouse fibroblast cell line were exposed to various concentrations of nanoparticles. The effect of different concentrations of nanoparticles on L929 cell viability was determined with MTT assay. Fig. 6 showed the cell viability of L929 cells following 24 and 48 h incubation with B3-4 AgNPs.

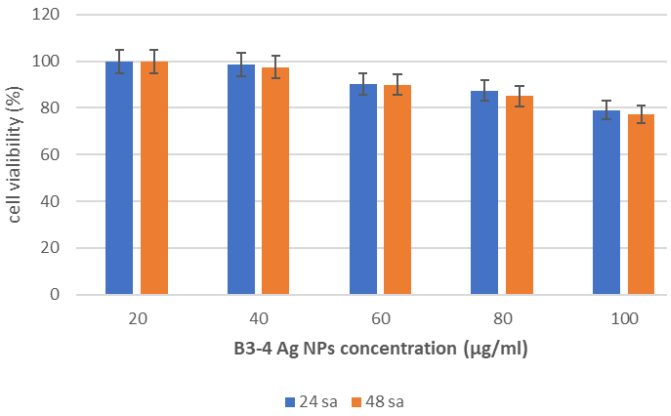

Figure 6. Determination of % cell viability of L929 cells following treatment with different concentrations of B3-4 AgNPs

The results clearly demonstrated that nanoformulations showed dose dependent toxicity. The IC_{50} value of B3-4 AgNPs was 142.67 µg/mL after 48 h of treatment. Survival rate of L929 cells did not show significant difference between 24 h and 48 h incubation periods ($p>0.05$). Our results was similar to the study in which biosynthesized AgNPs did not exhibit any harmful interaction to L929 mouse fibroblast cell line and the IC_{50} value was established as 64.5 µg/mL [54].

3.7. Antibacterial and antifungal activity of AgNPs

Silver nanoparticles are important nanosized materials that exhibit a strong antimicrobial effect against both gram-positive and gram-negative bacteria. Various studies show that the antimicrobial potential of silver nanoparticles is effective on different types of bacteria. Previous studies showed that silver nanoparticles were effective on important Gram-positive pathogens such as *Staphylococcus aureus* and *Streptococcus pneumoniae* [55]. On the other hand, there are many studies showed that silver nanoparticles are effective on gram-negative species such as *Escherichia coli* and *Pseudomonas aeruginosa* [56]. Another striking feature about silver nanoparticles is that these nanoparticles have an antimicrobial effect even at low concentrations. For these reasons, silver nanoparticles synthesized by the green synthesis method were first screened by the disk-diffusion method for their antimicrobial activity. For this purpose, different concentrations of AgNPs were evaluated for inhibitory activity. As seen in Table 2, AgNPs showed significant zones of inhibition against all test organisms. AgNPs showed maximum inhibition zones of 18 mm, 19 mm, 11 mm, 20 mm and 20 mm in *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Candida albicans*, respectively. The results revealed that *Enterococcus faecalis* and *Candida albicans* were the most susceptible strains against AgNPs whereas *Staphylococcus aureus* is the most resistant strain. AgNPs exhibited greater inhibition zones against *Pseudomonas aeruginosa* and *Staphylococcus aureus* compared to the reference antibiotic gentamicin. Our results were in agreement with the studies in which inhibitory effect of AgNPs was evaluated against *Escherichia coli* and *Pseudomonas aeruginosa* [57,58].

Table 2. The mean zone diameters of AgNPs as measured by disk diffusion method

Test microorganisms	Average zonesof inhibition (mm)					
	AgNPs (µg/disk)				Gentamicin (µg/disk)	Fluconazole (µg/disk)
	5	10	15	20	10	10
Escherichia coli	10	12	13	18	18	—
Pseudomonas aeruginosa	15	17	19	19	12	—
Staphylococcus aureus	3	8	9	11	20	—
Enterococcus faecalis	15	19	20	20	19	—
Candida albicans	12	14	17	20	—	15

3.8. Minimal inhibitory concentration (MIC Minimal inhibitory concentration (MIC) of AgNPs against test microorganisms

Minimum Inhibitory Concentration (MIC), the lowest inhibitor concentration of B3-4 AgNPs, was determined by the microdilution method for the test microorganisms. Serial dilutions of the B3-4 AgNPs solution were prepared to adjust the concentrations in the range of 0.046–60 µg/mL. As seen in Table 3 MIC values for *Escherichia coli* and *Pseudomonas aeruginosa* were 3.75 µg/mL and 1.875 µg/mL, respectively. A similar results were found in the research carried out by Panacek *et al*, in which *Escherichia coli* and *Pseudomonas aeruginosa* exposed to AgNPs with a mean size of 28 nm and MIC values was found 3.38 µg/mL and 1.69 µg/mL, respectively [59]. The results were also in agreement with the study determined the MIC values of AgNPs against foodborne pathogens [60]. *Staphylococcus aureus* was found to be most tolerant isolate to B3-4 AgNPs with MIC value of 60 µg/mL. Our results were in contrast to previous study in which MIC value of AgNPs biosynthesized from lyophilized hydroalcoholic extract of *Syzygium cumini* against *Staphylococcus aureus* was 250 µg/mL [61]. The result for *Candida albicans* showed that B3-4 AgNPs caused a slight decrease in yeast cell growth rates at low concentrations (0.12 µg/mL). A significant inhibitory effect was observed at high concentrations (15 µg/mL). Similar result was found in the research conducted by Arsène *et al*, in which MIC values of AgNPs ranged from 16 µg/mL to 32 µg/mL for clinical isolates of *Candida albicans* [62].

Table 3. The MIC values for the AgNPs prepared using *Betula*

Test microorganisms	MIC values (µg/mL)
Escherichia coli	3.75
Pseudomonas aeroginosa	1.875
Staphylococcus aureus	60
Enterococcus faecalis	0.94
Candida albicans	3.75

4. Conclusion

Green nanotechnology is an ecofriendly, low-cost and efficient method to synthesize the silver nanoparticles from biological components such as plants. In this scope, the silver nanoparticles were synthesized from the extract of *Betula* branches by bioreduction of silver ions. This is the first study for synthesis of AgNPs from *Betula* branches extract which collected from Van province, Türkiye. In this study *Betula* branch extract which is reachable in all seasons and rich in phenolic compounds, successfully reduced silver ions to silver nanoparticles and the presence of nanoparticles was confirmed by various characterization methods such as color change, UV-vis spectroscopy and FTIR analysis, particle size analysis and morphological analysis. The effect of $AgNO_3$ concentration and the temperature changes was evaluated in the scope of contribution to the formation of silver nanoparticles. Additionally, the antimicrobial activity and the anticancer efficiency studies have revealed that the green synthesized silver nanoparticles exhibit dose-dependent biological activity.

As a conclusion, the efficiency of silver nanoparticles obtained from a poorly studied plant part has been successfully demonstrated which can be used for further studies in terms of antimicrobial and anticancer properties.

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