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Dimrit Kuru Üzümü Kullanılarak Elde Edilen Gümüş Nanopartiküllere Optimizasyon Etkisi ve Nanopartiküllerin Antimikrobiyal Aktivitesi

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Öne Çıkanlar:

- Gümüş Nanopartiküllerin (AgNP'ler) sentezi, öncü olarak AgNO₃ ve dengeleyici ajan olarak Dimrit kuru üzüm özü kullanılarak elde edildi.
- AgNO₃ konsantrasyon oranları, sıcaklık ve zamanın AgNP'lerin sentezi üzerindeki etkisi incelenmiştir.
- Sentezlenen AgNP'ler antibakteriyel potansiyelleri açısından değerlendirilmiştir

ÖZET:

Bu makale, Burdur'da yetişen Dimrit kuru üzüm özü kullanılarak AgNP'lerin basit ve çevre dostu üretimini anlatmaktadır. AgNP'lerin sentezini optimize etmek için ekstrakt konsantrasyonu, gümüş solüsyonu konsantrasyonu, sentez süresi ve sentez sıcaklığı dahil olmak üzere bir dizi değişken araştırılmıştır. AgNP'ler, %1'lik ekstrakt konsantrasyonu ve 10⁻¹ M gümüş konsantrasyonunda 173 saat sonunda elde edilmiştir. Sentezlenen AgNP'lerin yapısı, Geçirgen elektron mikroskobu (TEM), X-ışını kırınımı (XRD), UV-görünür spektroskopi ve Fourier dönüşümü kızılötesi spektroskopisi (FT-IR) ile incelenmiştir. TEM analizi, AgNP'lerin çoğunluğunun küresel bir şekle sahip olduğunu ve ortalama parçacık boyutunun 30 nm olduğunu göstermiştir. *Staphylococcus aureus* ATTC43300, *Enterococcus faecalis* ATTC29212, *Bacillus subtilis*, *Listeria monocytogenes*, *Klebsiella pneumoniae* ve Gram-negatif *Salmonella enterocolitis*, *E. coli* 0157:H7 ATTC 43895 ve *Escherichia coli* ATTC 35150'ye karşı antimikrobiyal aktivite görülmüştür.

Anahtar Kelimeler:

- Yeşil sentez
- Gümüş
- Dimrit üzümü
- Nanopartikül
- Spektroskopi

Optimization Effect on Green Synthesis of Silver Nanoparticles (AgNPs) Using Dimrit Raisin Extract and Their Antimicrobial Activity

Highlights:

- Synthesis of Silver Nanoparticles (AgNPs) was achieved using AgNO₃ as precursor and Dimrit raisin extract as stabilizing agent.
- The effect of concentration ratios of AgNO₃, temperature, and time was examined on the synthesis of AgNPs.
- The synthesized AgNPs were evaluated for their antibacterial potential

ABSTRACT:

This paper describes the simple and environmentally friendly production of AgNPs using Dimrit raisin, grown in Burdur, extract. To optimize the synthesis of AgNPs, a number of variables, including extract concentration, silver solution concentration, synthesis time, and synthesis temperature, were investigated. AgNPs were obtained after 173 h at 1% extract concentration and 10⁻¹ M silver concentration. The structure of the synthesised AgNPs was investigated by Transmission electron microscopy (TEM), X-ray diffraction (XRD), UV-visible spectroscopy, and Fourier transform infrared spectroscopy (FT-IR). TEM analysis showed that the majority of the AgNPs had a spherical shape, and the average particle size was 30 nm. Antimicrobial activity was seen against *Staphylococcus aureus* ATTC43300, *Enterococcus faecalis* ATTC29212, *Bacillus subtilis*, *Listeria monocytogenes*, *Klebsiella pneumoniae* and Gram-negative *Salmonella enterocolitis*, *E. coli* 0157:H7 ATTC 43895, and *Escherichia coli* ATTC 35150.

Keywords:

- Green synthesis
- Silver
- Dimrit raisin
- Nanoparticle
- Spectroscopy

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INTRODUCTION

The study of nanotechnology is the field of material science that is the most dynamic, and nanoparticle (NP) manufacturing is rising quickly globally. Due to special characteristics including size (1-100 nm), shape, and structure, NPs exhibit entirely new or better properties (Alabdallah et al., 2021). In recent years, metal nanoparticles have drawn attention to the different fields of study because of their superior properties such as antibacterial, electronic, magnetic, catalytic, optical activity etc. These properties have arisen from their significantly small size and very high surface/volume ratio (Aboelfetoh et al., 2017). Usually two strategies are used in the synthesis of nanoparticles such as bottom-up and top-down. The bottom-up approach is a preferred method for the green synthesis and chemical synthesis of nanoparticles (Hoseinpour et al., 2018; Shaikh & Ghaemi, 2019). AgNPs are one of the most widely synthesized metallic nanoparticles by many researchers due to their notable applications in various fields such as biosensor (Bollella et al., 2017; Meng et al., 2019), drug delivery (Praphakar et al., 2018; Sakr et al., 2018), anticancer therapy (Saber et al., 2018; Valsalam et al., 2019), antimicrobial activity (Hemmati et al., 2019; Kirtiwar et al., 2019; Sana et al., 2018), anti-inflammatory activity (Moldovan et al., 2017; Govindappa et al., 2018), catalytic activity (Rokade et al., 2017; You et al., 2018). The antibacterial applications of silver nanoparticles particularly remark among the above-mentioned study areas. Because, AgNPs harm the cell membrane and the cellular content of the organism by inhibiting cell division (Sana et al., 2018; Paosen et al., 2017). Several methods for the synthesis of AgNPs have been reported which includes thermal decomposition (da Silva Pereira et al., 2015), wet chemical reduction (Wang et al., 2018), electrochemical (Abudabbus et al., 2018), microwave (Yüksel et al., 2016) ball milling method (Salarian et al., 2017) and laser ablation method (Arakcheev et al., 2018; Sportelli et al., 2019). The disadvantages of these methods are expensive, high temperature and pressure requirements, and the formation of toxic by-products including carcinogenic and/or highly active radicals resulting from the use of harmful solvents (sodium borohydride, hydrazine, ethylenediamine tetraacetic acid and N, N-dimethyl formamide etc.) (Shanmuganathan et al., 2017; Khatami et al., 2019; Kumar et al., 2019).

Recently, green synthesis has been used as an alternative to these methods in order to eliminate these problems. The green synthesis of nanoparticles can be accomplished by combining an environmentally friendly reducing agent and stabilizing agent with a solvent that is environmentally acceptable. In this method, chemical reducing agents are substituted by natural antioxidant compounds in biological sources such as microorganisms, plants and fruits (Hemmati et al., 2019; Rivera-Rangel et al., 2019; Abdel-Raouf et al., 2017). Although approximately 4000 phytochemicals have been discovered in plants to date, the process involving the synthesis of AgNPs is still poorly understood, especially given the diversity from plant to plant, species to species, and plant part to plant part. In addition, AgNPs of appropriate size and shape can be produced by varying parameters such as time, temperature, pH, precursor and stabilizer concentrations (plant extracts) (Hasan et al., 2022).

In this study, we report the green synthesis of AgNPs using the aqueous plant extract of Dimrit raisin, grown in Burdur, as biosource for reducing and stabilizing agents. Review of the literature revealed that the synthesis of nanoparticles utilizing raisin has been exceptionally rare, which attracted our interest for this study. The AgNPs were characterized by FTIR, TEM, XRD, and UV-visible spectroscopy. Moreover, the AgNPs displayed antibacterial activity against *E. faecalis*, *L. monocytogenes*, *S. aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *E. coli*, *E. coli*, *Salmonella enterocolitis*.

MATERIALS AND METHODS

Materials

All of the chemicals that were utilized in this study were of an analytical grade and did not require any additional purification before usage. Silver nitrate (AgNO_3 99.5%) was purchased from Fluka, UK. The Dried Dimrit raisin (*Vitis vinifera L.*) used in this study which specific to Burdur region was obtained from a local bazaar located in the city center of Burdur. In antibacterial studies, *Staphylococcus aureus* ATTC43300, *Enterococcus faecalis* ATTC29212, *Bacillus subtilis*, *Listeria monocytogenes*, *Klebsiella pneumoniae* and Gram-negative *Salmonella enterocolitis*, *E. coli* 0157:H7 ATTC 43895, and *Escherichia coli* ATTC 35150 bacteria were used.

Preparation of dimrit raisin extract and synthesis of AgNPs

At the preliminary stage, the dried Dimrit raisin, which was supplied in order to remove the possible impurities that may occur on these grapes, was washed several times with ultrapure water and then dried at room temperature. The dried Dimrit raisin was chopped into little pieces, generating a homogenous mixture that represented the full product. The mixture was kept at +4 °C for use in experimental studies. Four different concentrations (1.0%, 1.5%, 2.0%, and 2.5% (m/v)) of the same procedure were utilized to obtain the Dimrit raisin extracts used in the synthesis studies. To prepare the aqueous extract of Dimrit raisin, 100 mL of deionized water was boiled to 100°C under reflux conditions. After that, the required amount of Dimrit raisin was added and mixed for 5 min. Then, the mixture cooled down at room temperature and filtered using blue band filter paper.

The initial concentrations of Dimrit raisin and AgNO_3 are the main factors that play a role in the preparation of AgNPs. Different concentrations of Dimrit raisin extract (1.0%-2.5%) were mixed with different silver solutions concentrations (10^{-1} M- 10^{-6} M) in order to acquire appropriate surface plasmon resonance (SPR) band. Then, various synthesis times (0-222 h) were utilized to determine the optimal synthesis time for AgNPs. In addition, AgNPs synthesis studies were carried out at different temperatures to evaluate the effect of temperature on synthesis efficiency.

Characterization methods

All experimental solutions were prepared using ultrapure water from a PURIS pure water system (PURIS, Expe-UP Series). Centrifugation procedures were performed using a Hettich Universal 320 centrifuge. A UV-Vis spectrometer (PG Instruments TG 60) was used to record the absorption spectra of the AgNPs in the range of 300–800 nm. Memmert UN 110 model drying oven were used to dry the produced AgNPs. The morphologies and particle sizes of the AgNPs were observed using TEM (Zeiss Leo 906E). FTIR spectroscopy (Perkin Elmer Frontier) was used to identify the functional groups found in the Dimrit raisin and AgNPs. The crystal structure of the AgNPs was determined by XRD (Bruker D8 Advance).

The antibacterial test

Antibacterial activity of the Dimrit raisin extract and AgNPs was determined using well diffusion method (Balouiri et al., 2016). Gram-positive *Staphylococcus aureus* ATTC43300, *Enterococcus faecalis* ATTC29212, *Bacillus subtilis*, *Listeria monocytogenes*, *Klebsiella pneumoniae* and Gram-negative *Salmonella enterocolitis*, *E. coli* 0157:H7 ATTC 43895, and *Escherichia coli* ATTC 35150 bacteria were used for this purpose. The number of cells was adjusted to a McFarland standard of 0.5; adjusted cultures were inoculated into soft nutrient agar, and culture-inoculated soft agar was overlaid on nutrient agar. Holes with a diameter of 9 mm were made aseptically using a sterile cork borer, and 50 μL of biosynthesized AgNPs (1.0 mg/mL) was added to the wells. Each plate was incubated at 37

°C for 2-day. Subsequently, the size of the inhibition zone was measured to determine whether or not the samples had an inhibiting effect on bacterial growth. Dimrit raisin extract was used as a negative control.

RESULTS AND DISCUSSION

Surface plasmon resonance (SPR), which is dependent on the size and shape of the particles, predominates the optical absorption spectra of AgNPs. During this experiment, a number of variables, including Dimrit raisin extract concentration, AgNO₃ concentration, synthesis temperature, and time were examined in an effort to determine the best conditions for the synthesis of AgNPs.

Effect of Extract and AgNO₃ Solutions Concentrations

Dimrit raisin extract was used as a reducing and capping agent because of its good biodegradability and biocompatibility, and AgNPs were obtained by the reduction of AgNO₃ with Dimrit raisin extract. The initial concentrations of Dimrit raisin and AgNO₃ are the main factors that play a role in the preparation of AgNPs. Different concentrations of Dimrit raisin extract (1.0%-2.5%) were mixed with different silver solutions concentrations (10⁻¹ M-10⁻⁶ M) in order to acquire appropriate SPR band.

Dubey et al.(2010) prepared 0.5-1.0-1.8-2.8-3.8 and 4.8% (m/V) extract concentrations to investigate the effect of extract concentration on AgNP synthesis. They reported that the absorbance values of AgNPs increased with increasing extract concentrations (Dubey et al., 2010). In this study, no significant difference in absorbance values was observed when the concentration of Dimrit raisin extract was increased from 1% to higher extract concentration. Because the peaks obtained at 1% extract concentration were sharper and the signal/noise ratios were lower, this concentration was used in subsequent studies (Fig 1a).

When the UV-Vis spectra in Figure 1b were analyzed, the increase in absorbance with increasing AgNO₃ concentration indicates that the concentration of AgNPs increased. As the highest SPR band was observed in the 10⁻¹ M AgNO₃ solution, this concentration was chosen as the optimum AgNO₃ solution concentration.

Effect of synthesis temperature

To evaluate the effect of temperature on synthesis efficiency, AgNP synthesis was carried out at 4°C, 25°C, 40°C, and 50°C using 1% Dimrit raisin extract and 0.1 M AgNO₃ solution. Increasing the temperature of the reaction mixture increased the kinetic energy of the reactants, leading to an increase in the reaction rate. This results in faster reduction of Ag ions to form AgNPs (Song & Kim, 2009). However, increasing the temperature may lead to a decrease in the stability of AgNPs because higher temperatures may increase the rate of oxidation and agglomeration of nanoparticles (Abbasi et al., 2015).

As shown in Figure 1c, a small increase in the absorbance of the AgNPs was observed with increasing temperature. Because the difference between the absorbance values of AgNPs at 25°C and 40°C is quite similar, there is no additional advantage of working at 40°C; therefore, room temperature was chosen as the optimum temperature.

Effect of synthesis time

The time taken for the synthesis of AgNPs is an important parameter that can influence the size, shape, and stability of the nanoparticles. Longer reaction times allow for more nucleation and growth

of the nanoparticles, leading to the formation of larger particles (Li et al., 2007; Song & Kim, 2008; Dwivedi & Gopal, 2010).

To examine the effect of synthesis time, UV-vis spectroscopy was used to measure the SPR of AgNPs at varied reaction times ranging from 0 to 222 hours. As the synthesis time increases from 0 h to 173 h, the corresponding SPR peak intensity increased. As shown in Figure 1d, no considerable changes in the SPR of AgNPs were observed with increasing the synthesis time higher than 173 h. Therefore, 173 h was selected as the optimum synthesis time.

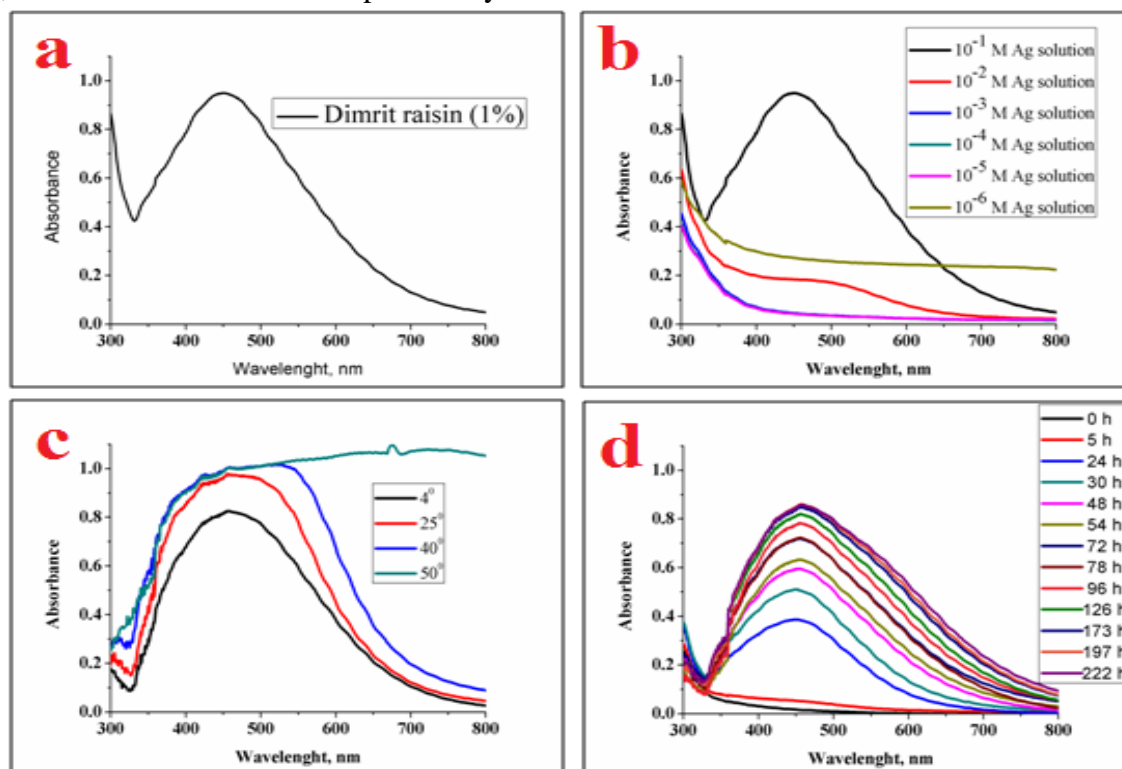


Figure 1. Effect of (a) Dimrit Raisin Extract Concentration (%1), (b) AgNO_3 Solutions Concentration (%1 Extract Concentration), (c) Synthesis Temperature (%1 Extract Concentration; 10^{-1} M AgNO_3 Concentration), (d) Synthesis Time (%1 Extract Concentration; 10^{-1} M AgNO_3 Concentration; 25°C)

Characterization Studies of the AgNPs

TEM analysis was performed using grids prepared from AgNPs solution. In order to accomplish this, a drop of AgNPs solutions that were produced under ideal circumstances was placed on a carbon-coated copper grid with a mesh size of 200, and it was then allowed to dry at 25°C for one day. Then, using these grids, TEM images were taken to determine both the size and surface morphology of AgNPs in a TEM instrument. Average particle diameters were calculated from the TEM images using the image proplus 6 program. For this, 160 particles were counted from TEM images (Figure 2a-c). As a result of TEM analysis, the average size of silver nanoparticles was determined as 30 ± 11 nm. In addition, it was observed that the particle size showed a homogeneous distribution. It has been determined that the synthesized silver nanoparticles are generally in spherical shapes, but also in triangular and tetragonal shapes.

The TEM data were compared to studies in the literature, and it was determined that the results obtained were consistent with the literature. For example, Song and Kim stated that from the TEM images of silver nanoparticles obtained by green synthesis, the nanoparticles have a spherical structure and the particle size is formed with an average diameter of 32 nm (Song & Kim, 2009). Dubey et al., stated that the particles were mostly triangular, spherical and hexagonal shapes from the TEM images

of silver nanoparticles obtained by green synthesis. They also reported that the particle sizes of AgNPs were in the range of 10-40 nm on average (Dubey et al., 2010). Qidwai et al., synthesized AgNPs using *Phoenix sylvestris L.* seed extract by green synthesis method. They stated from the TEM images that the nanoparticles have a spherical shape and have an average size of 40-50 nm (Qidwai et al., 2018).

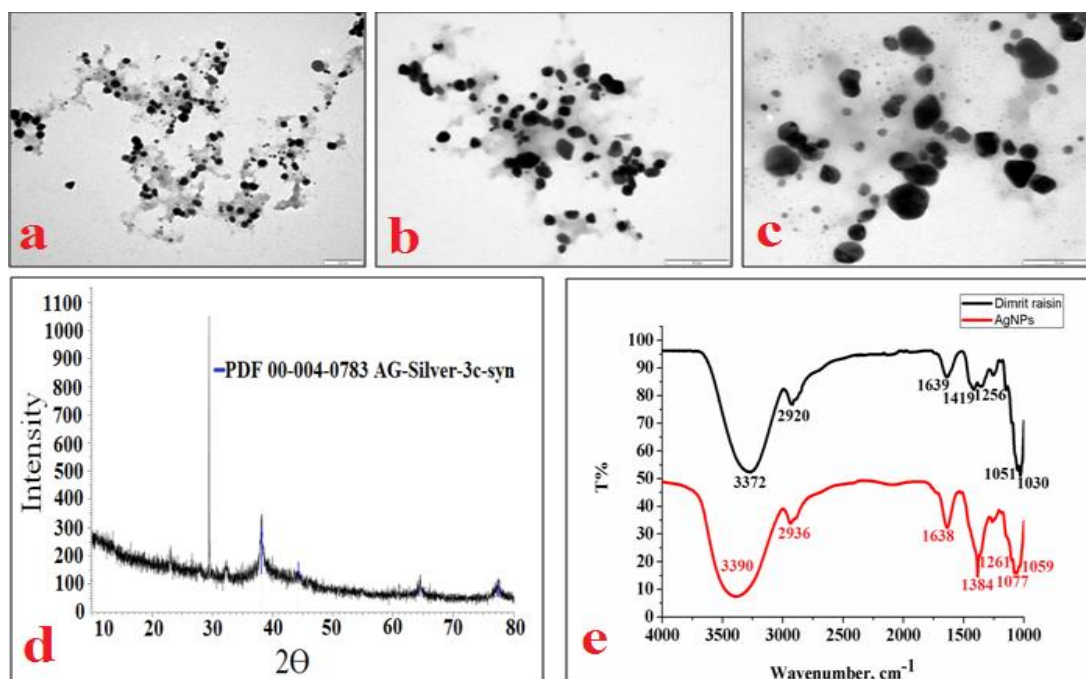


Figure 2. (a-c) TEM Images of AgNPs, (d) XRD Pattern of AgNPs, (e) FTIR Spectra of AgNPs and Dimrit Raisin Extract

The crystal structure of the produced AgNPs was determined by XRD analysis. For this purpose, an AgNPs solution was centrifuged (4500 rpm) and then dried in a 35 ° C oven for 12 hours. The XRD examination was carried out following the homogenization of the produced silver nanopowder with the use of grinding in a mortar. According to the results of XRD diffraction patterns given in Figure 2d, silver nanoparticles synthesized with raisin extract exhibited four diffraction peaks at, 38°, 44°, 65° and 77°. The measured diffraction peaks correspond to the JCPDS card file number 00-004-0783. The XRD results of the synthesized AgNPs were similar to those from previous studies in the literature (Budi et al., 2021; Zaki et al., 2011; Sauthier et al., 2011).

The FT-IR absorption spectra of extract as well as those obtained following the reduction of silver ions are presented in (Figure 2e). The FTIR is helpful for identifying the interactions between silver ions and bimolecular compounds responsible for capping and stabilizing silver ions throughout the nanoparticle formation process. At a frequency of 3272 cm⁻¹, the extract spectrum displayed a wide band that corresponded to the -OH group. The position of this band was moved to 3390 cm⁻¹ for the AgNPs. The occurrence of bands for extract at 2920 cm⁻¹ and 1639 cm⁻¹ could be attributed to stretching of C-H and C=C groups, respectively. For AgNPs, these bands occurred at 2936 cm⁻¹ and 1638 cm⁻¹. The C-O-H group was responsible for the band that was discovered at 1419 cm⁻¹ for extract; however, this band was found to be displaced to 1384 cm⁻¹ for AgNPs. The bands at 1256 cm⁻¹ and 1051 cm⁻¹ were due to C-O group for extract. For AgNPs, these bands moved to higher wave numbers, specifically 1261 cm⁻¹ and 1077 cm⁻¹. The =C-O-C group was identified as being responsible for the band at 1030 cm⁻¹ for extract, whereas for AgNPs, this band was found to be at 1059 cm⁻¹ (Moteriya et al., 2014; Fagbayigbo et al., 2017; Doan et al., 2020; Razavi et al., 2019).

Antibacterial Activity

Analysis of the antibacterial activity of the synthesized AgNPs against pathogenic Gram positive (*Staphylococcus aureus* ATTC43300, *Enterococcus faecalis* ATTC29212, *Bacillus subtilis*, *Listeria monocytogenes*, *Klebsiella pneumoniae*) and Gram negative (*Salmonella enterocolitis*, *E. coli* 0157:H7 ATTC 43895, *Escherichia coli* ATTC 35150) bacteria was performed using the disk diffusion method.

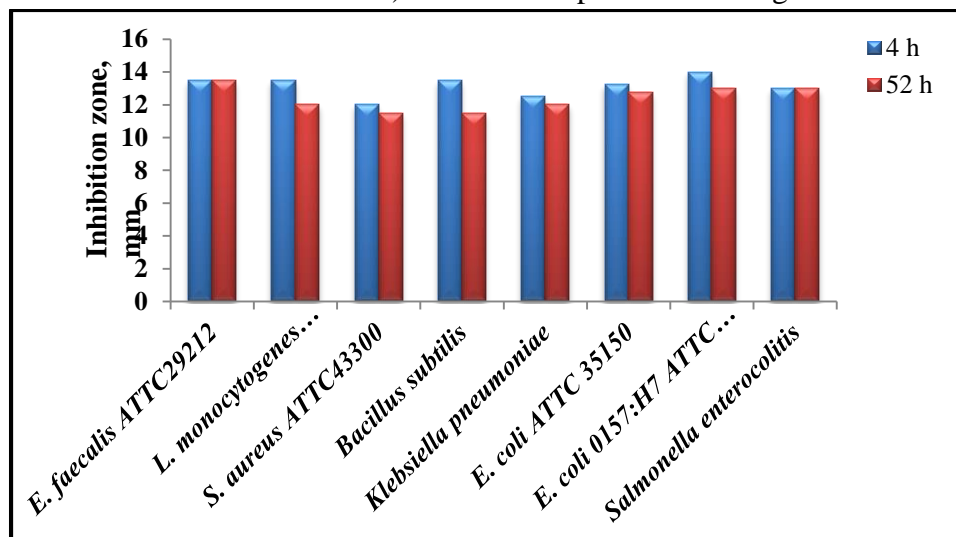


Figure 3. The Antibacterial Activity of AgNPs (the Blue Column Indicates the Zone Diameters Measured After 4 h, and the Red Column Indicates the Zone Diameters Measured After 52 h)

In order to determine the effects of AgNPs on bacterial growth inhibition, the inhibitory sites were evaluated. When the findings of the disk diffusion method were analyzed, it was determined that the bacteria *E. faecalis* ATTC29212 (13.5 mm), *E. coli* 0157:H7 ATTC (13 mm), and *Salmonella Enterocolitis* (13 mm), exhibited the highest levels of antibacterial activity. Other results are shown in Figure 3, and the lowest zone was obtained against *S. aureus* ATTC43300 (11.5 mm) and *Bacillus subtilis* (11.5 mm). In general, AgNPs were most effective in this study against gram-negative bacteria than gram-positive bacteria. Gram-negative bacteria consist of an inner plasma membrane, a thin peptidoglycan layer, and an outer lipopolysaccharide layer. Contrarily, gram-positive bacteria have a cytoplasmic membrane and a cell wall that is considerably thicker and constituted of many layers of peptidoglycan than gram-negative bacteria (Trak & Arslan, 2021). While the precise mechanism of nanoparticles' antibacterial effect is unknown, it is commonly defined by the way they interact with bacterial cell membranes, allowing positively charged metal nanoparticles to enter. As a Lewis acid, silver interacts with reactive protein, phosphorus, sulfur, and oxygen sites inside bacterial DNA. This enhances protein leakage and inhibits the normal transit of protoplasm (Tang & Zheng, 2018).

CONCLUSION

Green synthesis was successfully employed to synthesize AgNPs using Dimrit raisin as reducing agent and capping agent in aqueous solution at room temperature and avoided using any hazardous or toxic materials. The effects of certain synthesis conditions, including synthesis temperature and time, concentrations of Ag solutions, and concentrations of Dimrit raisin extracts were optimized in order to get the best possible results. The antibacterial activity of the biologically synthesized AgNPs was evaluated against eight pathogenic bacteria (*E. faecalis* ATTC29212, *E. coli* 0157:H7 ATTC, and *Salmonella Enterocolitis*), showing effective bactericidal activity. The findings of the characterization of the AgNPs that were prepared showed that this plant can be utilized as a reducing and stabilizing

agent. In addition, it is thought that the prepared nanoparticles can be used as an effective antibacterial agent in various medical applications.

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Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

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