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# Biosynthesis of Silver Nanoparticles Using White Propolis Extract as a Reduction Agent and Optimized by Box-Behnken Design

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## ABSTRACT
In this study, it was aimed to optimize the biosynthesis of silver nanoparticles with a Box-Behnken design. The white propolis extract was utilized as the reduction and stabilizing agent. The synthesized silver nanoparticles using white propolis extract solution were optimized by Box-Behnken design considering the effect of certain independent variables such as microwave power, time, and concentration of $AgNO_3$ (silver nitrate). A quadratic polynomial model was used in mathematical modeling and response surface analysis was performed to determine the independent variable-response relationship. The optimum synthesis conditions were determined as 10 mM of $AgNO_3$ concentration, 0.3 of VExt/VAg, 150 watts of microwave power, and 35 seconds. The optimized silver nanoparticles were characterized using FTIR (Fourier Infrared) spectroscopy, UV-VIS (Ultraviolent visible) spectrophotometry, and DLS (Dynamic Light Scattering). In addition, the antibacterial activity of the optimized silver nanoparticles was tested against *Staphylococcus aureus (S. aureus), Klebsiella pneumonia (K. pneumoniae), Pseudomonas aeruginosa (P. aeruginosa),* and *Enterococcus faecalis (E. faecalis)* strains. It was observed that synthesized silver nanoparticles had higher antibacterial activity compared to propolis extract.

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# Gümüş Nanopartiküllerin Beyaz Propolis Ekstresi Kullanılarak Biyosentezi ve Box-Behnken Yöntemi ile Optimizasyonu

## ÖZET
Bu çalışmada gümüş nanopartiküllerin biyosentezinin Box-Behnken yöntemi ile optimize edilmesi amaçlandı. Beyaz propolis özütü, bir indirgeme ve stabilize edici ajan olarak kullanıldı. Beyaz propolis özüt çözeltisi kullanılarak sentezlenen gümüş nanopartiküller, mikrodalganın gücü, zaman, $AgNO_3$ (gümüş nitrat) konsantrasyonu ve beyaz propolis özüt çözeltisinin hacminin $AgNO_3$ hacmine oranı gibi farklı faktörlerin etkisi dikkate alınarak Box-Behnken yöntemi ile optimize edildi. Matematiksel modellemede ikinci dereceden polinom modeli kullanıldı ve bağımsız değişken-yanıt ilişkisini belirlemek için yanıt yüzey analizi yapıldı. Optimum koşullar 10 mM $AgNO_3$ konsantrasyonu, oran: 0.3, 150-watt mikrodalga gücü ve 35 saniye olarak belirlendi. Optimize edilmiş gümüş nanopartiküller, FTIR (Kızılötesi) spektroskopisi, UV-VIS (Ultraviyole görünür bölge) spektrofotometri ve DLS (Dinamik ışık saçılımı) kullanılarak karakterize edildi. Ek olarak, optimize edilmiş gümüş nanopartiküllerin antibakteriyel aktivitesi Staphylococcus aureus (S. aureus), *Klebsiella pneumoniae (K. pneumoniae), Pseudomonas aeruginosa (P. aeruginosa)* ve *Enterococcus faecalis (E. faecalis)* suşlarına karşı test edildi. Gümüş nanopartiküllerin bakteriler üzerinde propolis ekstraktına göre daha etkili olduğu gözlendi.

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## INTRODUCTION

The nanoparticle-based investigation has been sustained widely because of its potential applications, especially in the interdisciplinary sciences and nanotechnology field (Antoine et al., 2016). Metallic nanoparticles have attracted great interest in nanomedicine due to their unique properties such as surface area, particle size, and effective biological activities. Metallic nanoparticles can be synthesized using physical, chemical, and biological methods. All these methods have advantages and disadvantages. For example, chemical methods are expensive because they require special chemical agents, and most of them are very toxic. (Mohanpuria, Rana, and Yadav, 2008). The physical methods often require high temperature or pressure. On the other hand, natural resources used in biological methods have low toxicity and the reaction can occur at room temperature. The biological methods can be applied in microbiology because natural resources are safe, eco-friendly, and cheap (Padalia, Moteriya, and Chanda, 2015). In this context, numerous plant extracts have been utilized as a biological reduction agent in the synthesis of silver nanoparticles (Cho et al., 2005; Khadri et al., 2013; Joy Prabu and Johnson, 2015; Garibo et al., 2020). As an antimicrobial and anti-cancer agent, silver nanoparticles are utilized in several applications such as in cosmetics, water purification, medical diagnostic (Kaplan et al., 2021), and textile (Li, Mathew, and Mao, 2012). Silver nanoparticles are important biomaterials thanks to their antimicrobial activity and can also help overcome antimicrobial resistance (Khatoon et al., 2018).

Propolis, which is produced and used by bees to build honeybee comb, is used by humans as a supplementary food (Burdock, 1998; Banskota, Tezuka, and Kadota, 2001). Silver nanoparticles synthesized using Brazilian propolis have been reported to exhibit effective antimicrobial activity (Barbosa et al., 2019). On the other hand, synthesized silver nanoparticles using Brazilian green propolis have been executed to show antifungal activity and lower cytotoxic effects (Kischkel et al., 2020). Propolis extract produced in Romania has been used to reduce Ag+ and synthesized silver nanoparticles have been shown to exhibit an antibacterial effect on S. aureus and P. aeruginosa (Corciovă et al., 2019). Silver nanoparticles synthesized using propolis produced by Apis mellifera honey have been revealed to inhibit some bacterial strains (Ghramh et al., 2019). The white propolis is Turkish propolis and has been used for years as a medicine in folk medicine (Katircio and Mercan, 2006). White propolis has various biological properties such as antimicrobial and antioxidant activity (Ozdal et al., 2019).

This study aims to optimize the biosynthesis of silver nanoparticles by using white propolis extract solution. In the literature, some researchers have reported the use of the Box-Behnken design (BBD) to optimize the synthesis of silver nanoparticles (Gökşen Tosun and Kaplan, 2021; Yadav, Gupta and Sharma, 2021). The BBD has been widely used in many scientific fields such as formulation development and analytical chemistry (Kumar Nayak, Saquib Hasnain and Malakar, 2013; Nayak, Kalia and Hasnain, 2013; Malakar, Das and Nayak, 2014; Hasnain et al., 2016). In this study, the synthesis of silver nanoparticles was optimized using BBD and the produced silver nanoparticles were characterized. In addition, antimicrobial activities of nanoparticles produced under optimized conditions were tested on gram-negative and gram-positive bacteria. The obtained results are comprehensively presented to support the hypothesis of the study based on the green synthesis of nanoparticles using white propolis extract solutions.

## MATERIAL and METHOD

### Materials

The white propolis was purchased from MADEVA (Ankara, Turkey). Silver nitrate ($AgNO_3$) (Carlo Erbaa), sodium hydroxide (NaOH), the filter discs, and nutrient agar were obtained from Sigma Aldrich Chemicals (Istanbul, Turkey). Deionized water was used in all stages, from the nanoparticle's synthesis to its purification. The sterilized water was used for antimicrobial studies.

### Preparation of White Propolis Extract

The white propolis (15 mL) was dissolved in 15 mL of distilled water. After that, the obtained extract was stored at 4 °C for further studies.

### Synthesis of Silver Nanoparticles Using White Propolis Extract

For the synthesis of silver nanoparticles, a certain volume of $AgNO_3$ (5-10 mM) solution was added to a certain volume of extract so that the VExt/VAg was within a certain range (0.1 - 0.5) and the solution was stirred using a magnetic stirrer. Finally, the solution was diluted with 0.1 M NaOH solution to adjust the pH to 10.6 (Kischkel et al., 2020). The solution was heated by microwave (10 s-60 s) and in a certain power range of 150 - 800 watts. The reduction process of silver ions was observed by the color change from yellowish to brownish or deep brown depending on the reaction conditions. The synthesized silver nanoparticles were centrifuged at 15000 rpm for 15 minutes and washed twice with deionized water, dried, and stored at 4 °C in the dark.

### Optimization of Silver Nanoparticles

The optimization of silver nanoparticles was realized via BBD by using Design Expert® ver. 12.0 software. Four effective factors including the power of microwave (power), time, $AgNO_3$ concentration, and the ratio of the volume of white propolis extract solution to a volume of $AgNO_3$ (VExt/VAg) were selected as independent factors for optimization at three different levels which were changed dependent of the factors. A total of 29 experiments calculated by the design were recommended as shown in Table 1.

The formation of silver nanoparticles was confirmed by the observed peak between 350-450 nm (Kischkel et al., 2020). The absorbance data of the synthesized silver nanoparticles were measured by a UV-vis spectrophotometer. The % yield was determined as the maximum measured absorbance value of 100 and the other data were calculated accordingly, and the particle formation was defined as the % yield at a range of 350-450 nm, and these values were placed in BBD as responses. After placing the data in the BBD, mathematical modeling was created to evaluate the results. The quadratic model was determined by ANOVA along with other parameters such as correlation coefficient ($r^2$), adjusted $r^2$, predicted $r^2$, and predicted residual squares. Ideal circumstances for the synthesis of silver nanoparticles have been optimized by numerical desirability function and graphical optimization technique.

Table 1. The BBD of matrix illustrating trial runs carried out for optimization of synthesized silver nanoparticles using white propolis extract solution.

*Çizelge 1. Beyaz propolis özü çözeltisi kullanılarak sentezlenmiş gümüş nanopartiküllerin optimizasyonu için gerçekleştirilen deneme çalışmalarını gösteren matrisin Box-Behnken Tasarımı.*

| Run | Concentration of $AgNO_3$ (mM) | The volume of $AgNO_3$/ Volume of extract | Power(Watt) | Time (sec.) | %Yield |
|---|---|---|---|---|---|
| 1 | 5 | 0.3 | 475 | 10 | 28.2114 |
| 2 | 7.5 | 0.1 | 475 | 10 | 62.6016 |
| 3 | 7.5 | 0.5 | 800 | 35 | 64.065 |
| 4 | 5 | 0.3 | 150 | 35 | 30.813 |
| 5 | 7.5 | 0.1 | 800 | 35 | 51.3821 |
| 6 | 5 | 0.1 | 475 | 35 | 48.6179 |
| 7 | 10 | 0.5 | 475 | 35 | 65.6098 |
| 8 | 7.5 | 0.3 | 475 | 35 | 89.9187 |
| 9 | 7.5 | 0.1 | 475 | 60 | 54.4715 |
| 10 | 10 | 0.1 | 475 | 35 | 63.8211 |
| 11 | 7.5 | 0.3 | 475 | 35 | 89.9187 |
| 12 | 7.5 | 0.3 | 150 | 10 | 61.1382 |
| 13 | 10 | 0.3 | 475 | 60 | 68.6179 |
| 14 | 7.5 | 0.3 | 800 | 60 | 51.7073 |
| 15 | 10 | 0.3 | 150 | 35 | 100 |
| 16 | 7.5 | 0.1 | 150 | 35 | 86.9919 |
| 17 | 7.5 | 0.3 | 475 | 35 | 89.9187 |
| 18 | 7.5 | 0.5 | 475 | 60 | 55.8537 |
| 19 | 7.5 | 0.5 | 475 | 10 | 43.4146 |
| 20 | 7.5 | 0.3 | 475 | 35 | 89.9187 |
| 21 | 7.5 | 0.3 | 475 | 35 | 89.9187 |
| 22 | 10 | 0.3 | 800 | 35 | 46.9919 |
| 23 | 5 | 0.3 | 475 | 60 | 44.5528 |
| 24 | 10 | 0.3 | 475 | 10 | 66.0976 |
| 25 | 5 | 0.3 | 800 | 35 | 56.9919 |
| 26 | 7.5 | 0.5 | 150 | 35 | 55.2846 |
| 27 | 5 | 0.5 | 475 | 35 | 20.813 |
| 28 | 7.5 | 0.3 | 150 | 60 | 78.4553 |
| 29 | 7.5 | 0.3 | 800 | 10 | 56.4228 |

### Characterization of Optimized Silver Nanoparticles Using White Propolis Extract

The formation of silver nanoparticles was determined by scanning the 200-500 nm range using a Nanodrop DeNoVIX spectrophotometer (Wilmington, USA). The synthesized silver nanoparticles were also chemically characterized by using an FTIR spectroscope (Jasco FT/IR 4700, Germany). Zeta potential and size distribution of optimized silver nanoparticles were measured by dynamic light scattering method using HORIBA SZ-100 Nanoparticle Analyzer.

### Antibacterial Activity of White Propolis Extract And Silver Nanoparticles

To determine the antibacterial activity of the optimized silver nanoparticles, white propolis extract and optimized silver nanoparticles were tested against *S. aureus (ATCC 25923), K. pneumoniae (ATCC 1538and 0), P. aeruginosa (ATCC 27853),* and *E. faecalis (ATCC 29212)* strains using disc diffusion method. Firstly, bacterial strains were seeded in nutrient broth and incubated overnight at 210 rpm, 37 °C. At the end of the incubation time, the cultured bacterial suspension was adjusted to $1x10^8$ CFU / mL and seeded on the medium-loaded petri dish. After those sterile blank discs, which are used generally for the disc diffusion method, were placed on the petri dish. One of the discs was loaded with 20 µL of white propolis extract and the other was loaded with 20 µL of the silver nanoparticles solution then the petri dish was incubated overnight at 37 °C. Subsequently, zone diameters of inhibition were measured. Three replicates were made for each substance.

### Minimum Inhibitory Concentration (MIC) of White Propolis Extract And Silver Nanoparticles

MIC assay of silver nanoparticles and white propolis extract were analyzed as indicated in our previous study (Kaplan and Gökşen Tosun, 2021). Briefly, the nutrient broth was added to each well of the 96-well plate for serial dilution of white propolis and silver nanoparticles. The bacterial suspensions were adjusted to $1x10^8$ CFU / mL and seeded in each well after that the plates were incubated overnight at 37 °C. The plates were measured at 600 nm by using Plate Reader. Three replicates were made for each substance.

### Statistical Analysis

Statistical analysis and comparable data sets were evaluated using GraphPad Prism 8.1 software with a two-way ANOVA test. Probability values of $p < 0.05$ were considered statistically significant.

## RESULTS and DISCUSSION

### Synthesis of silver nanoparticles using white propolis extract

Numerous studies have shown that various plant extracts have been used for the green synthesis of silver nanoparticles in the last few years (Lin et al., 2010; Roy and Barik, 2010; Raghunandan et al., 2011; Bansal et al., 2020). In this paper, the biosynthesis method was preferred to produce silver nanoparticles using white propolis extract. White propolis extracts were used as a reduction agent in the synthesis of silver nanoparticles because of their important biological ingredients used to treat various diseases (Nagai et al., 2003).

### Optimization of Silver Nanoparticles

To optimize the synthesis procedure of silver nanoparticles, the BBD method of the Design Expert® ver. 12.0 software was used (Stat-Ease Inc., Minneapolis, USA). To calculate the percentage yield of particle formation, their absorbance values were measured by UV-Vis at 350-500 nm and optimized as responses. Power, time, $AgNO_3$ concentration, and VExt/VAg were determined as factors at three different levels which were changed depending on the factors. The acquired data coincided with the quadratic polynomial model and various statistical parameters were utilized to fit the analysis. After that, the model demonstrated the existence of interaction, the curvature effect was performed, and the polynomial equation was generated for the response factor. The parameter of coefficient of correlation was calculated as perfectly in the range between 0.9915 along with great values of predicted and adjusted $r^2$, and low values of PRESS. Additionally, model diagnostic graphs for response are shown in Figure 1, showing that the data is parallel to the selected model.

$$\%Yield = 89.92 + 15.09 * A - 5.24 * B - 7.09 * C + 2.98 * D + 7.40 * AB - 19.80 * AC - 3.46 * AD + 11.10 * BC + 5.14 * BD - 5.51 * CD - 21.61 * A^2 - 17.63 * B^2 - 9.22 * C^2 - 17.80 * D^2$$

### Factor-response relationship and response surface mapping

Response surface analysis (RSA) was obtained using 3D response surface plots and 2D contour plots, which elucidated the existence of interactions among the factors and their impacts on the response factor. The RSA plots for a percentage of yields of silver nanoparticles were shown in Figure 2. The relationship between $AgNO_3$ concentration and VExt/VAg was shown in Figure 2(A). In cases where VExt/VAg was particularly above 0.4 and $AgNO_3$ concentration was between 5 mM and 6 mM, the percentage yield of particle formation tends to be minimal. However, at the medium and low levels of VExt/VAg, the increase in $AgNO_3$ concentration showed a sharp rising pattern to high levels. In addition, the effect of VExt/VAg on the particle formation efficiency showed a decreasing trend at around 0.4 to 0.5 levels. The 3D graph and 2D plot of the relationship between $AgNO_3$ concentration and power were indicated in Figure 2(B).

When the relationship between $AgNO_3$ concentration and power was evaluated, it was observed that the effect of these two parameters on the particle formation efficiency was inversely proportional. As the power was at a minimum and $AgNO_3$ concentration was at a maximum level, the percentage of particle formation was determined at its highest. Moreover, the relationship between $AgNO_3$ concentration and the time was similar to the relationship between $AgNO_3$ concentration and VExt/VA,g and its 3D response surface plots and 2D contour plots were depicted in Figure 2(C). The relationship between power and VExt/VAg was indicated in 3D response surface plots and 2D contour plots in Figure 3(A). When VExt/VAg was between 0.1 and 0.4 and power was below 400 watts, it was determined that the particle formation percentage was over 90%. In addition, the relationship between VExt/VAg and time showed that when both were at a medium level, the particle formation percentage was seen to be high (Figure 3(B)). Lastly, the relationship between time and power was determined and the high level of the percentage of particle formation was observed over 25 seconds and less than 670 watts, as shown in Figure 3(C).

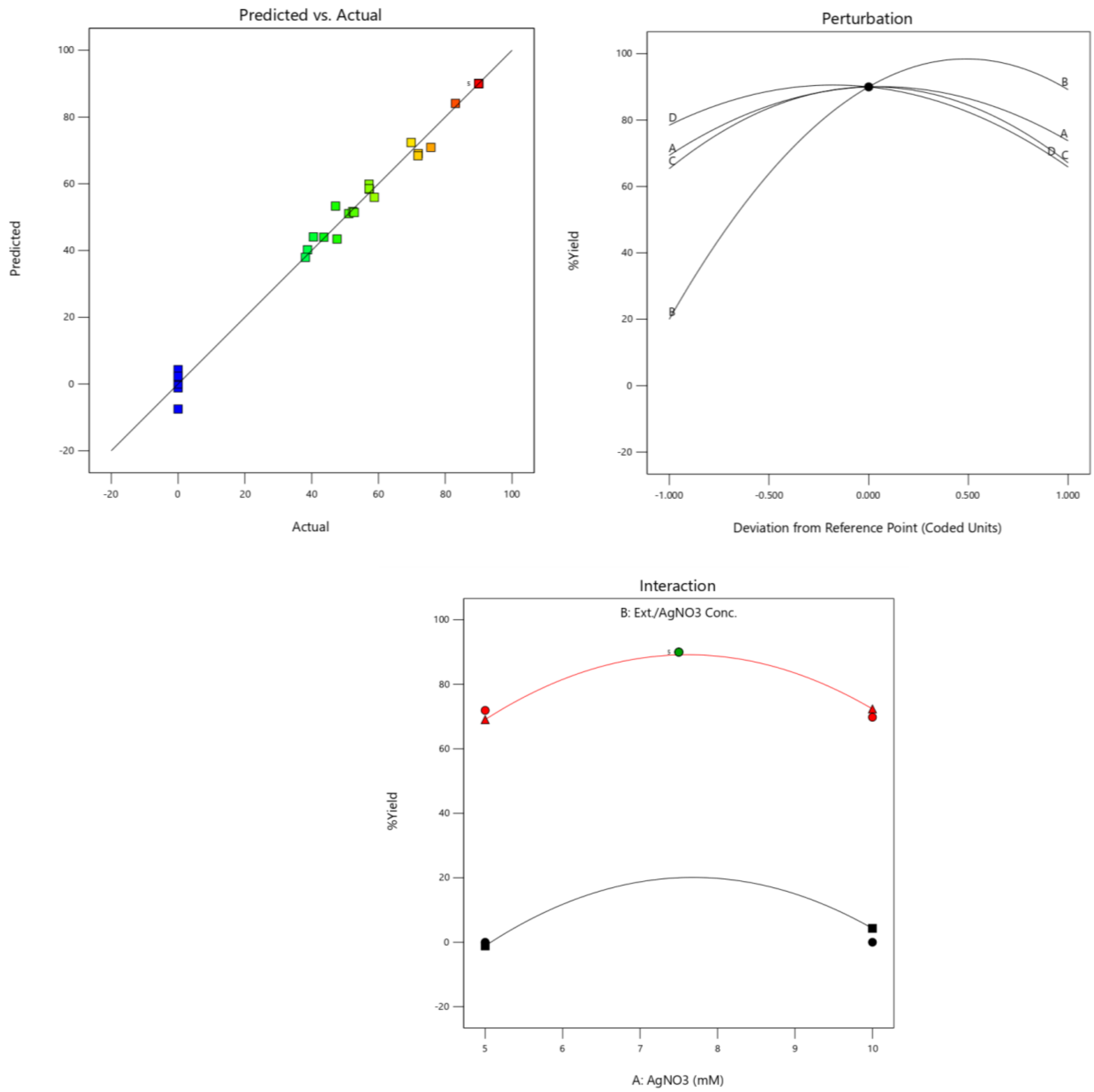


Figure 1. The graphs indicating 'Predicted vs. Actual plot', perturbation chart, and interaction plot for response values

*Şekil 1: Grafikler, elde edilen cevaplar için tahmin edilen değere karşı gerçekleşen değer grafiğini, pertürbasyon tablosu ve etkileşim grafiğini göstermektedir.*

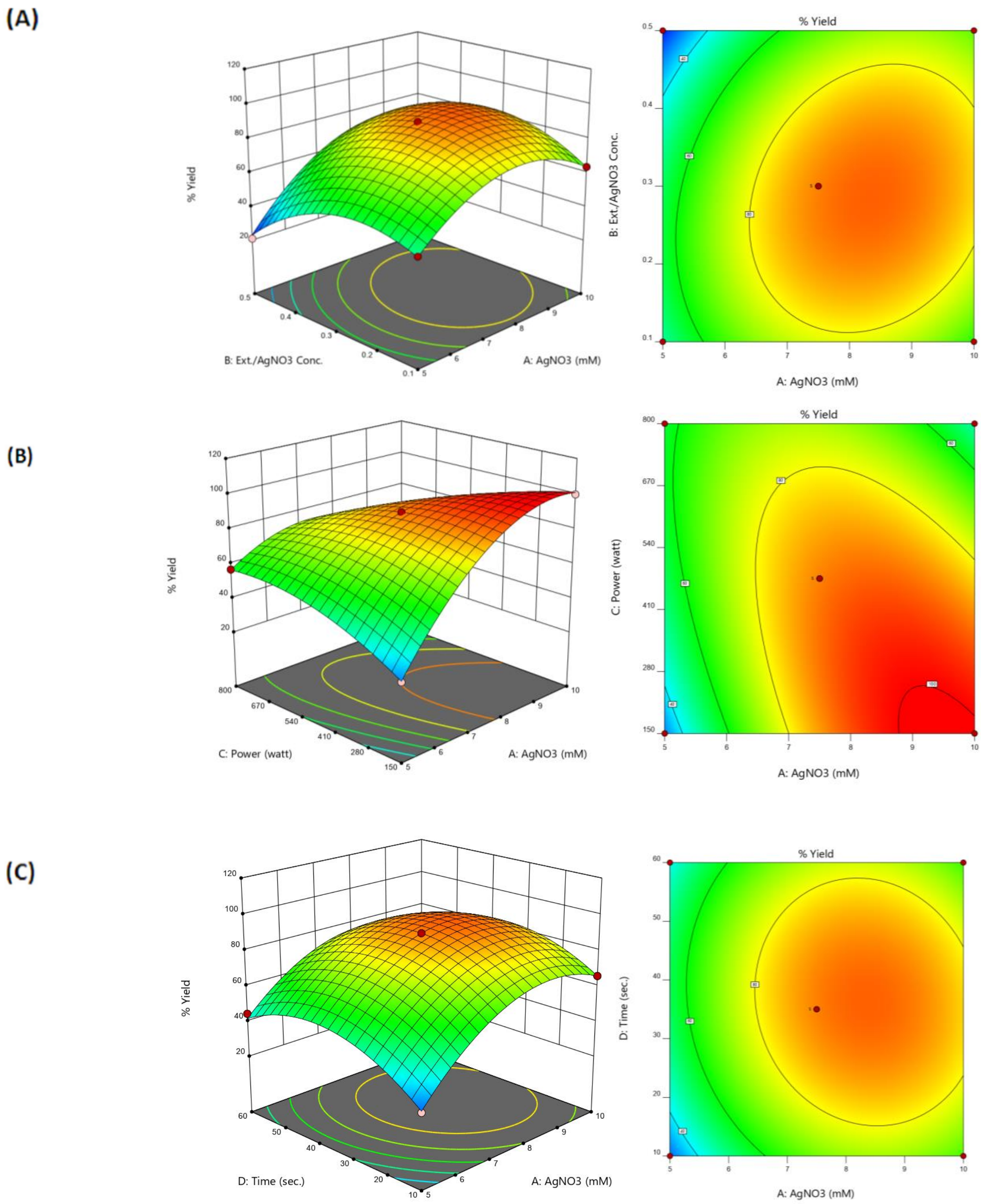


Figure 2. The graphs indicate the impact of factors like A: $AgNO_3$ concentration, B: VExt/VAg, C: Power, and D: time on the percentage of particle formation (%Yield) of synthesized silver nanoparticles using white propolis extract solution as the response factor.

*Şekil 2. A: $AgNO_3$ konsantrasyonu, B: oranı, C: Mikrodalganın gücü ve D: zaman gibi faktörlerin, yanıt faktörü olarak beyaz propolis ekstresi çözeltisi kullanılarak sentezlenen gümş nanopartiküllerin partikül oluşum yüzdesi (% Verim) üzerindeki etkisini gösteren grafikler.*

## Optimum conditions for silver nanoparticle synthesis

To identify with numerical optimization, the silver nanoparticles were optimized and evaluated. It was observed that the desirability function value was close to 1.0 and the goal for the response variable was achieved. As the optimized silver nanoparticles synthesis setting, the overlay plot indicated the yellow color area as the optimized area along with the flagged point displaying 10 mM concentration of $AgNO_3$, power at 150-watt, time at 35 seconds, and 0.3 of the VExt/VAg (Figure 4).

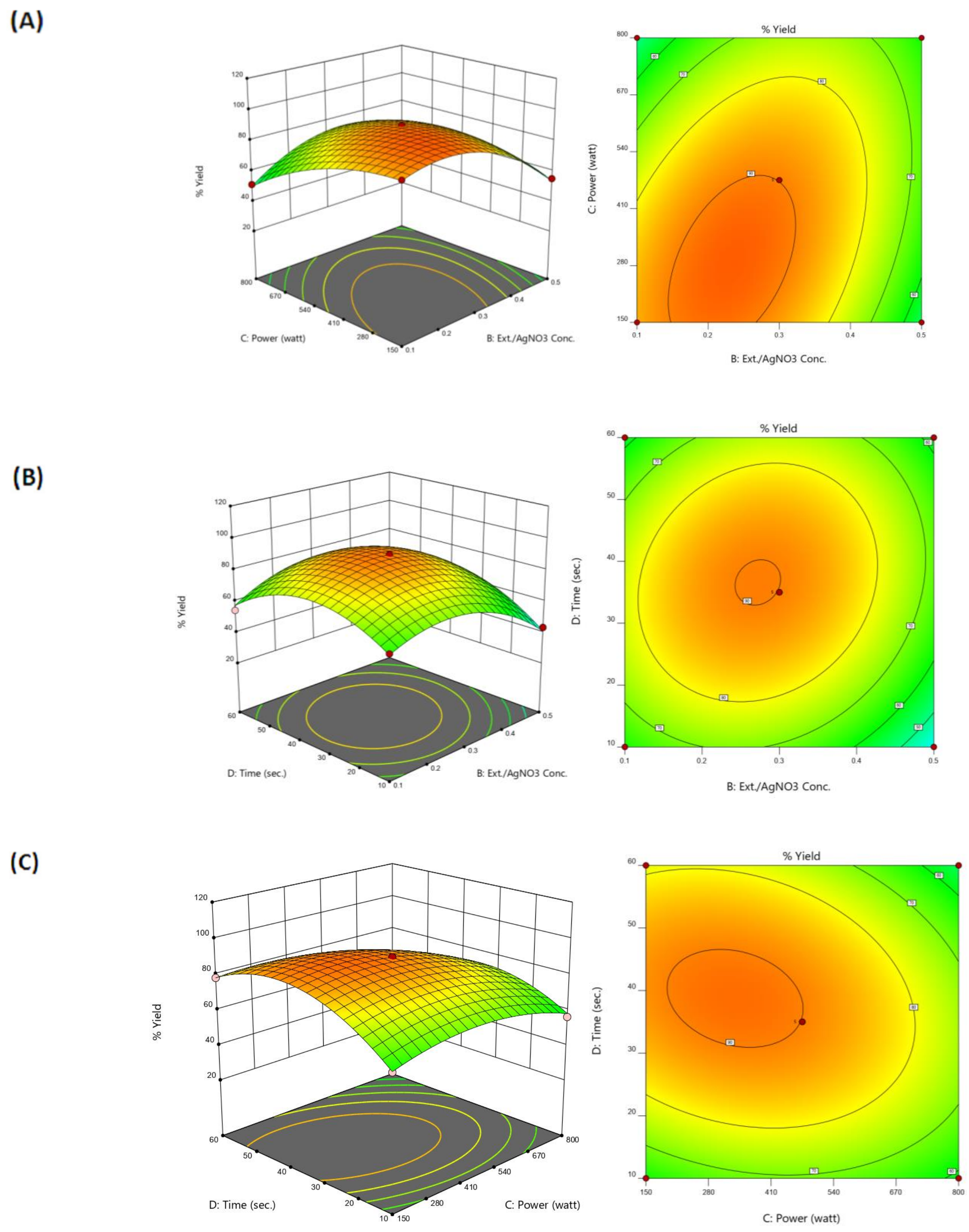


Figure 3. The graphs indicate the impact of factors like B: VExt/VAg, C: Power, and D: time on the percentage of particle formation (%Yield) of synthesized silver nanoparticles using white propolis extract solution as the response variable.

*Şekil 3.* B: VExt/VAg oranı, C: Mikrodalganın gücü ve D: zaman gibi faktörlerin, yanıt faktörü olarak beyaz propolis ekstresi çözeltisi kullanılarak sentezlenen gümüş nanopartiküllerin partikül oluşum yüzdesi (% Verim) üzerindeki etkisini gösteren grafikler.

## Characterization of optimized silver nanoparticles

The synthesized silver nanoparticles were characterized by UV-VIS spectrophotometry and the result was shown in Figure 5.

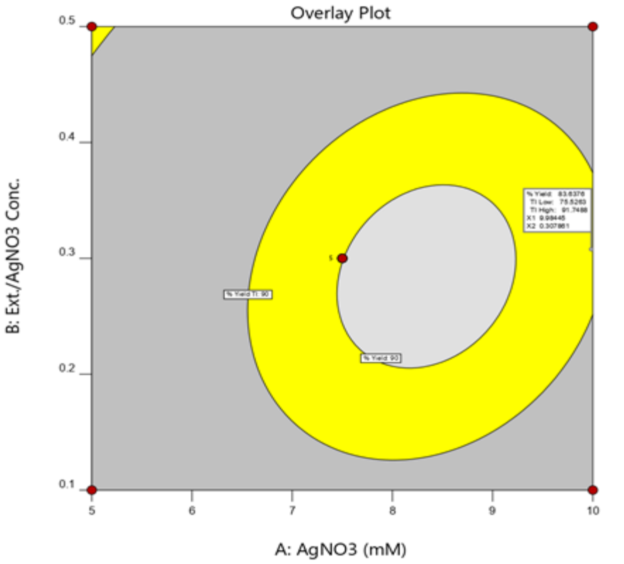


Figure 4. The graphs show a yellow color area as the optimized area and a flagged point as the optimized synthesized silver nanoparticles using white propolis extract.

*Şekil 4. Grafik, sarı renkli alanı, optimize edilmiş alan olarak ve işaretlenmiş noktayı ise beyaz propolis özütü kullanılarak optimize edilmiş sentezlenmiş gümüş nanopartiküller olarak göstermektedir.*

While the spectrum peak of the white propolis extract was determined as 302 nm, the spectrum peak of the silver nanoparticles synthesized under optimum conditions was observed at 420 nm. This result was fitting with the brownish color of the nanoparticles and confirmed the synthesis of silver nanoparticles (Kischkel et al., 2020). At the same time, the study was carried out by changing the volume of white propolis extract solution, $AgNO_3$ concentration, and power. Comparing the literature, the spectrum peaks were observed at nearly the same wavelengths such as 480 nm (Corciovă et al., 2019) and 421 nm (Barbosa et al., 2019) but wavelength ranges were wider. However, when VExt/VAg was greater than 0.3, it was observed that the absorption peaks shifted towards a higher wavelength and the wavelength range of the peak increased.

FTIR spectrum of the optimized silver nanoparticles was shown in Figure 6. The significant absorption bands for silver nanoparticles were observed at 2985.27, 1636.3, and 1330.64. The optimized silver nanoparticles exhibited a wide absorption band of –OH groups at 3279.36. The absorption band at 2985.27 was associated with C–H stretching of aliphatic –CH, –CH2 groups. The absorption peaks at 1636 and 1330 were assigned to the asymmetrical and symmetrical –COO stretching of carboxylate compounds in white propolis. The absorption peak at 1085 was associated with the C–O stretch.

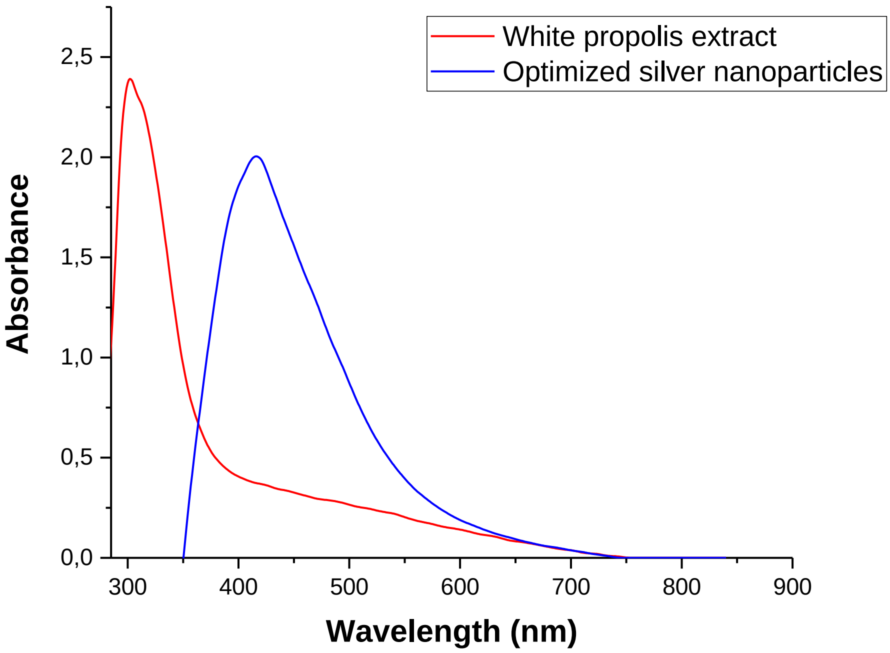


*Figure* .5. UV-Visible spectra of optimized silver nanoparticles prepared using white propolis extract and white propolis extract

*Şekil 5. Beyaz propolis ekstresi kulllanılarak optimize edilmiş gümüş nanopartiküllerin ve beyaz propolis ekstresinin UV-Vis spektrum piki*

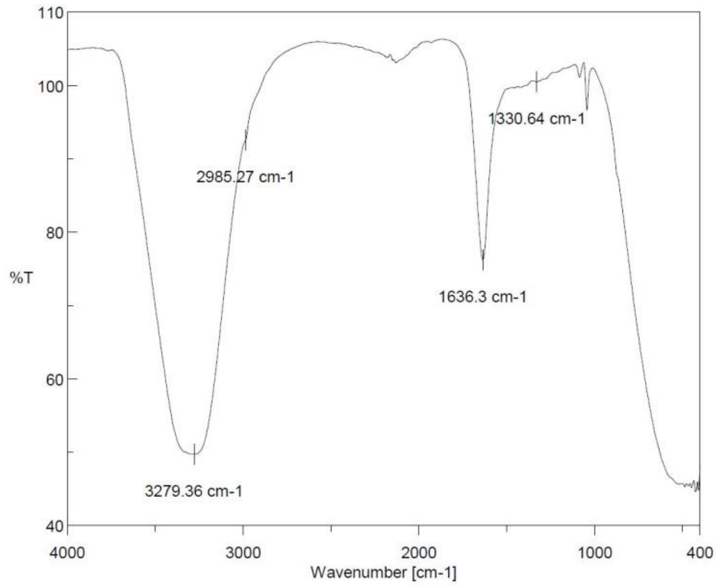


Figure 6. FTIR spectra of optimized silver nanoparticles prepared using white propolis extract
*Şekil 6. Beyaz propolis ekstresi kullanılarak optimize edilmiş gümüş nanopartiküllerin FTIR spektrumu*

The particle size distribution of optimized silver nanoparticles was determined with the DLS method and shown in Figure 7. The size of silver nanoparticles was measured as 108.2 nm. The zeta potential provides information about the charges and stabilities of nanoparticles (Dhiman et al., 2021). The zeta potential of optimized silver nanoparticles was measured as -22.3 mV and PDI (polydispersity index) value was measured as 0.224 which correlates with monodispersity since when the PDI value is bigger than 0.7, the particle size distribution is not uniform (Khorrami et al., 2018).

## Antibacterial activity of silver nanoparticles

It has been known that silver nanoparticles have an antibacterial effect. They affect both Gram-positive bacteria and Gram-negative bacteria (Dada et al., 2018). In this study, the antibacterial effect of optimized silver nanoparticles was examined on *S. aureus (ATCC 25923), K. pneumoniae (ATCC 15380), P. aeruginosa (ATCC 27853) and E. faecalis (ATCC 29212)* by using Disc Diffusion Method (Figure 8).
The antibacterial effects of white propolis extract on the same bacteria were studied to compare with the silver nanoparticle form and inhibition zones were measured and shown in Table 2.
Since the standard antimicrobial blank discs have diameter of 6.0 mm, the inhibition zone value must be higher than 6.0 mm to prove the sample has antimicrobial activity. As the results of DISC assay were evaluated while optimized silver nanoparticles using white propolis extract exhibited antibacterial activity, it was observed that white propolis had no antibacterial effect on any bacteria. Corciovă et al. (2019) reported that inhibition zone values of the synthesized silver nanoparticles using propolis extract on S. aureus and P. aeruginosa were determined as 10.0 mm, and 2.0 mm respectively. However, inhibition zone diameter values of synthesized silver nanoparticles in this study were higher. The MIC method is generally used to determine the minimum inhibition concentration value of the active component. To evaluate MIC values of the optimized silver nanoparticles and white propolis extract, crude data were obtained using Plate Reader and calculated by GraphPad 8.1 Software as shown in Table 3. Moreover, the percentage of inhibition curve of the optimized silver nanoparticles and the percentage of inhibition values of white propolis extract solution were tested to determine the effectiveness of the silver nanoparticle as shown in Figure 9. Both graphs were also plotted by using MIC data.
MIC analysis was also performed to determine the minimum inhibitory concentration of the optimized silver nanoparticles and white propolis extract. While the optimized silver nanoparticles using white propolis extract exhibited antibacterial effect even at minimum concentration, white propolis extract exhibited antibacterial activity at maximum concentration.
The efficacy of silver nanoparticles synthesized under optimized conditions and white propolis extract on bacteria were evaluated and, the difference between MIC values was tested statistically with two-way ANOVA. *p-value* was calculated as $p = 0.0001$ for all bacterial species, and this p-value was considered significant because $p < 0.005$.
MIC values of obtained silver nanoparticles against S. aureus, P. aeruginosa, and E. faecalis were measured as 25.1 µg/mL, 26.2 µg/mL, 18.2 µg/mL, 18.2 µg/mL, respectively (Figure 8). In literature, the MIC value of

the produced silver nanoparticles using propolis hydroalcoholic extract was reported as 8 µg/mL and it was smaller than that using white propolis because this synthesis was carried out with ethanol extract (Barbosa et al., 2019).

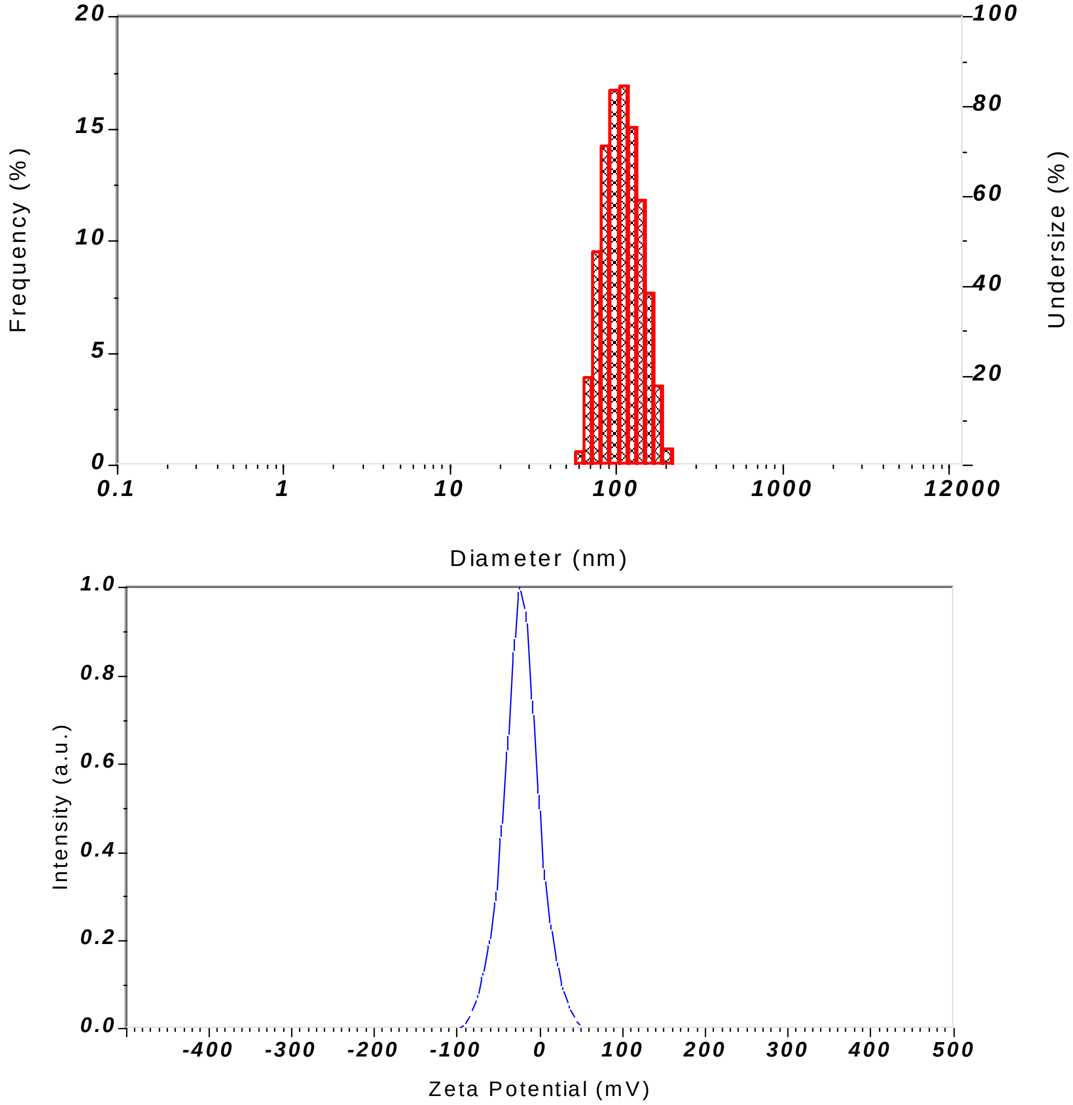


Figure 7. DLS and zeta potential values of optimized silver nanoparticles prepared using white propolis extract, DLS (A) and zeta potential (B)

*Şekil 7. Beyaz propolis ekstresi kullanılarak optimize edilmiş gümüş nanopartiküllerin DLS ve zeta potansiyel değerleri, DLS (A), zeta potansiyeli (B)*

Table 2. The inhibition zone diameter (mm) of silver nanoparticles was determined by the DISC Method.
*Çizelge 2. DISC yöntemi ile gümüş nanopartiküllerin inhibisyon zon çapının (mm) belirlenmesi*

| **Microorganisms** | **Inhibition zones diameter (mm)** |
|---|---|
| *Klebsiella pneumoniae* | 10 |
| *Pseudomonas aeruginosa* | 10 |
| *Enterococcus faecalis* | 11 |
| *Staphylococcus aureus* | 13 |

Table 3. MIC values of the optimized silver nanoparticles and white propolis extract.
*Çizelge 3. Optimize edilen gümüş nanopartiküller ve beyaz propolis ekstresinin MIC değerleri*

| Microorganisms | MIC values (µg/mL) | |
|---|---|---|
| | **Optimized silver nanoparticles** | **White propolis extract** |
| ***S.aureus*** | 25.1 ± 0.0002 | 250 ± 0.0002 |
| ***P. aeruginosa*** | 26.2 ± 0.0001 | 250 ± 0.0002 |
| ***K. pneumaniae*** | 18.2 ± 0.0002 | 250 ± 0.0002 |
| ***E. faecalis*** | 17.9 ± 0.0002 | 500 ± 0.0001 |

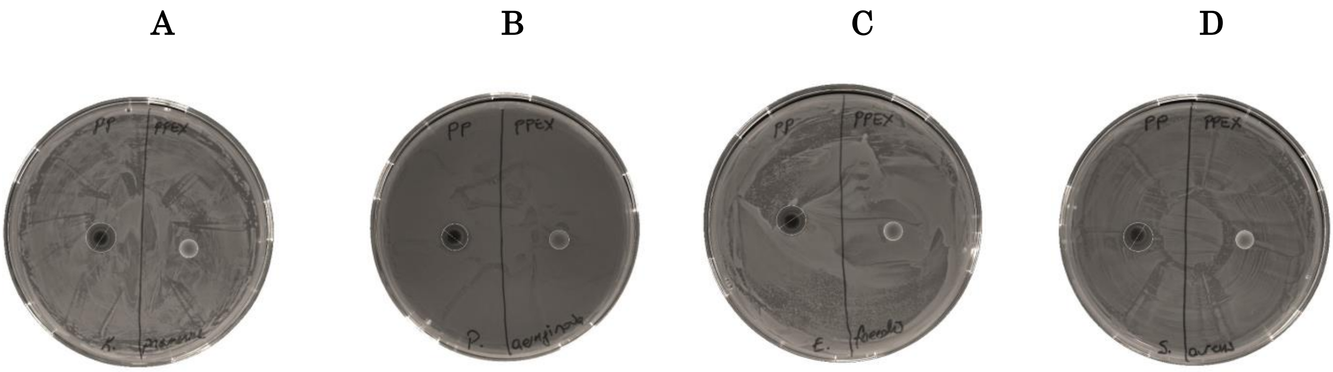


Figure 8. Antibacterial activity assay of white propolis extract and optimized silver nanoparticles against K. pneumoniae (ATCC 15380) (A), P. aeruginosa (ATCC 27853), E. faecalis (ATCC 29212), and S. aureus (ATCC 25923) (D)

*Şekil 8. K. pneumoniae (ATCC 15380) (A), P. aeruginosa (ATCC 27853), E. faecalis (ATCC 29212), and S. aureus (ATCC 25923) (D) bakteri suşlarına karşı beyaz propolis ekstresi kullanılarak optimize edilen gümüş nanopartiküllerin antibakteriyel aktivitesinin analizi*

Comparing the inhibition values of silver nanoparticles and white propolis extract, while white propolis extract at the concentration of 500 µg/mL inhibited nearly 70% of all strains, lower silver nanoparticles amount was enough for inhibition of all strains as shown in Figure 9.

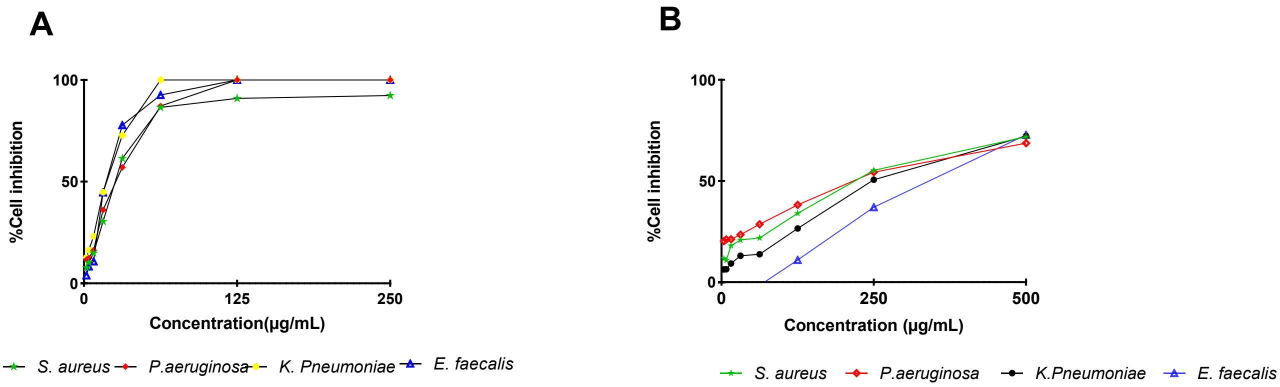


Figure 9. % Cell Inhibition values on the bacteria of optimized silver nanoparticles (A) and white propolis extract (B)

*Şekil 9. Beyaz propolis ekstresi kullanılarak optimize edilen gümüş nanopartiküllerin (A) ve beyaz propolis ekstresinin(B) bakteriler üzerindeki % hücre inhibisyon değerleri*

## CONCLUSION

In presenting this paper, a biological approach was used for the synthesis of silver nanoparticles and white propolis extract solution was preferred as a biological reduction agent. The synthesized silver nanoparticles were systematically optimized by BBD utilizing Design Expert® ver. 12.0 software depending on the influence of different factors. The optimized silver nanoparticles exhibited antimicrobial activities with the proper size, zeta potential, and PDI index. These findings imply that optimized silver nanoparticles using white propolis extract solution may subscribe as a prospective antibacterial agent in cosmetic and therapeutic applications.

## Researchers' Contribution Rate Declaration Summary

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

## Conflicts of Interest Statement

Authors have declared no conflict of interest.

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