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# Ölmez Çiçek Ekstresi Kullanılarak Gümüş Nanoparçacıkların Yeşil Sentezi, Karakterizasyonu ve Antioksidan Özellikleri

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#### Araştırma Makalesi

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## ÖZ

Gümüs nanoparçacıklar, benzersiz kimyasal ve fiziksel özellikleri sayesinde ilaç taşıma sistemleri, biyomedikal uygulamalar, kanser tedavileri, kozmetikler ve gıdalar gibi birçok alanda kullanılmaktadır. Gümüş nanoparçacıklar fiziksel, kimyasal ve biyolojik olmak üzere üç farklı şekilde sentezlenebilir. Son yıllarda araştırmacılar, çevre dostu, güvenilir ve biyouyumlu olması nedeniyle yeşil senteze yönelmiştir. Bu bağlamda, bu çalışmada doğal bir fitokimyasal kaynak olan Helichrysum sp. ekstresi kullanılarak gümüş nanoparçacıkların sentezi için yeni bir yöntem önerilmiştir. Nanoparçacık oluşumu üzerinde gümüş iyon konsantrasyonunun, Helichrysum sp. ekstresi konsantrasyonunun ve reaksiyon süresinin etkileri arastırılmış ve optimum reaksiyon kosulları belirlenmiştir. Helichrysum sp. ekstresi varlığında sentezlenen gümüş nanoparçacıklar, ultraviyole (UV) spektrofotometri, dinamik ışık saçılması (DLS), Fourier Dönüşümlü Kızılötesi Spektroskopisi (FTIR), wet-STEM (taramalı geçirimli elektron mikroskobu), and termogravimetrik analiz (TGA) kullanılarak karakterize edilmiştir. Karakterizasyon sonuçlarına göre, ortalama 54 nm boyuta sahip homojen ve küresel nanoparcacıklar basarıvla sentezlendi. Sentezlenen gümüs nanoparçacıkların antioksidan kapasiteleri/aktiviteleri Bakır İndirgeyici Antioksidan Kapasite (CUPRAC) ve DPPH (2,2-diphenyl-1-picrylhydrazyl) yöntemleri kullanılarak belirlendi. Önerilen yesil nanoteknoloji esaslı sentez yöntemi yeni, basit, hızlı, düşük maliyetli, sürdürülebilir ve çevre dostudur.

# Green Synthesis, Characterization, and Antioxidant Properties of Silver Nanoparticles Using Helichrysum sp. Extract

#### Research Article

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#### ABSTRACT

Silver nanoparticles are used in many fields such as drug delivery systems, biomedical applications, cancer treatments, cosmetics and food thanks to their uniqe chemical and physical properties. Silver nanoparticles can be synthesized in 3 different ways: physical, chemical and biological. In recent years, researchers have turned to green synthesis because it is environmentally friendly, reliable and biocompatible. In this context, a new method for the synthesis of silver nanoparticles was proposed in this study using Helichrysum sp. extract, a natural phytochemical source. The effects of silver ion concentration, Helichrysum sp. extract concentration and reaction time on nanoparticle formation were investigated, and optimum reaction conditions were determined. Silver nanoparticles synthesized in the presence of Helichrysum sp. extract were characterized using ultraviole (UV) spectrophotometry, the dynamic light scattering (DLS) analysis, Fourier transform infrared spectroscopy (FTIR), dynamic light scattering (DLS), wet-STEM (scanning transmission electron microscope), and thermogravimetric

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analysis (TGA). According to characterization results, homogeneous and spherical nanoparticles with an average size of 54 nm were successfully synthesized. The antioxidant capacities/activities of the synthesized silver nanoparticles were determined using Cupric Reducing Antioxidant Capacity (CUPRAC) and DPPH (2,2-diphenyl-1-picrylhydrazyl) assays. The proposed green nanotechnology-based synthesis method is novel, simple, rapid, low-cost, sustainable, and eco-friendly.

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The genus *Helichrysum* Mill. is a member of the plant family Asteraceae and consists of about 600 species worldwide (Akaberi et al., 2019). Plants of this genus exhibit various biological properties,

#### 1. Introduction

including antimicrobial, antiallergic, antioxidant, anti-inflammatory, and healing effects for coughs, colds, and wounds. These biological activities are depend of compounds like flavonoids, α-pyrones, coumarins, and terpenoids, which are found in various parts of Helichrysum plants. Helichrysum species (sp.) are extraordinarily rich in phenolic compounds and flavonoids that plays an important role in defense mechanisms (Gouveia and Castilho, 2010). Helichrysum sp. has been used for centuries as flavoring spices in various foods, for cosmetic purposes and in folk remedies (Viegas et al., 2014). Nanotechnology is recognized as one of the most important milestones of science in the last decade. Nanomaterials have applications in many fields including electronics, agriculture, pharmacy, and medicine. The increasing demand for nanomaterial applications has also created the need for new synthesis methods (Huston et al., 2021). Noble metal nanoparticles such as silver, gold and palladium are in the size range of 1-100 nm and have a high surface-to-volume ratio. These properties enable the use of noble metal nanoparticles for a wide variety of purposes (Apak et al., 2018). Silver nanoparticles are widely used in many fields such as medicine, pharmacy, cosmetics and food due to their unique chemical and physical properties (Gurunathan et al., 2015; Zhang et al., 2016; Bamal et al., 2021). Metal nanoparticles are synthesized by physical, chemical and biological methods. Due to the disadvantages of traditional synthesis methods such as being toxic, harmful to the environment and expensive, researchers have turned to greener methods and natural resources (Hutchison, 2008; Zhang et al., 2016). The synthesis of silver nanoparticles by biological reduction is environmentally harmless, rapid, simple and inexpensive (Gurunathan et al., 2009). In addition, silver nanoparticles produced by green synthesis have high yield and high stability (Gurunathan et al., 2015). Therefore, green chemistry is an important approach for the synthesis of silver nanoparticles.

The biogenic synthesis of nanoparticles is called green chemistry (Singh et al., 2018). Green synthesis requires harmless solvent/reagent consumption and the use of renewable natural resources (Singh et al., 2016). The green synthesis of nanoparticles can be realized using plant extracts or microorganisms. However, the use of plant sources is more preferred by researchers in terms of reaction time, process ease and simplicity (Khandel et al., 2018). Phytochemicals such as flavonoids, flavones, phenolic compounds, carbohydrates, vitamins in the composition of plant extracts enable the reduction of metal

ions to metal nanoparticles (Singh et al., 2018). Helichrysum sp. has phytochemicals such as flavonoids, phenolic acids, terpenes, chalcones, essential oils, and pyrones (Akaberi et al., 2019). In this context, Helichrysum sp. was selected as a bioreducing agent to synthesize safe and environmentally friendly silver nanoparticles in this study. The method enables the production of pure and safe nanoparticles by eliminating some of the drawbacks, such as the toxicity of strong chemical reductants and the accumulation of these harmful reductants on the nanoparticles during synthesis. In addition, this method replaces conventional solvents with distilled water, which is a safer and environmentally friendly alternative. It is also a simple and inexpensive method that researchers can easily reproduce in any laboratory. In this work, the effect of silver concentration, concentration of *Helichrysum* sp. extract and reaction time on the synthesis of silver nanoparticles was investigated. The synthesized silver nanoparticles were characterized by ultraviole (UV) spectrophotometer, the dynamic light scattering (DLS) analysis, Fourier transform infrared spectroscopy (FTIR), and scanning transmission electron microscope (wet-STEM). The thermal stability of the synthesized silver nanoparticles was investigated by thermogravimetric analysis (TGA). Finally, the antioxidant properties of the synthesized nanoparticles were evaluated using the CUPRAC and DPPH assays. Thus, environmentally friendly, safe, and biocompatible silver nanoparticles were produced that can be used in many fields such as food, materials science, and health.

#### 2. Material and Methods

#### 2.1. Chemicals and Instruments

Silver nitrate (AgNO<sub>3</sub>), copper (II) chloride dehydrate (CuCl<sub>2</sub>), ammonium acetate (NH<sub>4</sub>Ac): Merck (Darmstadt, Germany); neocuproine (2,9-dimethyl-1,10-phenanthroline) (Nc), DPPH (2,2-diphenyl-1-picrylhydrazyl), ethanol, methanol: Sigma-Aldrich (Steinheim, Germany). Dried *Helichrysum* sp. was provided by seller of the medicinal herbs.

Inesa L7 Double Beam UV–Vis spectrophotometer (Hinotek, China) was used for absorption measurements. Anton-Paar Litesizer 500 analyzer (Graz, Austria) was used for particle size distribution (DLS) analysis. HE-SNPs were imaged with the Thermo Scientific Quattro S (ThermoFisher Scientific, Waltham, MA, USA). Fourier transform infrared (FTIR) spectra of HE-SNPs were recorded via using a Shimadzu IRTracer100 (Kyoto, Japan) spectrometer. Telstar Cryodos freeze dryer (Terrassa, Spain) was used to dry silver nanoparticles and *Helichrysum* sp. extract for FTIR analysis. Thermogravimetric analysis (TGA) of HE-SNPs was carried out using a Netszch TG 209- F3 Tarsus testing device.

#### 2.2. Preparation of Solutions

#### 2.2.1. Preparation of Ag<sup>+</sup> solution

A stock solution of silver nitrate in distilled water was 10 mM. Silver nitrate solutions were diluted to different concentrations (1.0; 2.5; 5.0; and 7.5 mM) for the optimization study of the synthesis of silver nanoparticles.

### 2.2.2. Preparation of Helichrysum sp. extract (HE)

0.2 g of dried *Helichrysum* sp. was weighed into the beaker. 70 mL of boiled distilled water was added and incubated for 30 minutes at room temperature. It was filtered through filter paper into a 100 mL volumetric flask. The extract was made up to 100 mL with distilled water and cooled. To study the effect of the concentration of *Helichrysum* sp. extract on the production of silver nanoparticles, HE was diluted to different concentrations (1:2 diluted, 1:5 diluted, 1:10 diluted and 1:20 diluted).

# 2.3. Optimization Study for the Synthesis of Helichrysum sp. Extract Capped Silver Nanoparticles (HE-SNPs)

Effects of concentration of Ag<sup>+</sup> ion, concentration of *Helichrysum* sp. extract, and reaction time were investigated on the synthesis of silver nanoparticles using *Helichrysum* sp. extract. In order to observe the effect of Ag<sup>+</sup> ion concentration, various Ag<sup>+</sup> concentrations (1.0; 2.5; 5.0; 7.5; and 10.0 mM) were studied in the presence of *Helichrysum* sp. extract diluted 1:2 (v:v) for 60 min incubation at room temperature. Whereas, to observe the effect of the concentration of *Helichrysum* sp. extract, different dilutions of *Helichrysum* sp. extracts (1:2, 1:5, 1:10, and 1:20 (v:v)) were studied in the presence of 5.0 mM Ag<sup>+</sup> for 60 min incubation at room temperature. In order to observe the duration of the reaction, the mixture containing 5.0 mM Ag<sup>+</sup> and diluted 1:2 (v:v) *Helichrysum* sp. extract was incubated at room temperature for 60 minutes. Finally, to observe the effect of pH on the HE-SNPs formed with *Helichrysum* sp. extract, the mixtures containing 5.0 mM Ag<sup>+</sup> and diluted 1:2 (v:v) *Helichrysum* extract were incubated in the range pH 2.0–9.0 at room temperature for 40 minutes.

# 2.4. Synthesis of HE-SNPs

HE-SNPs were synthesized using *Helichrysum* sp. extract by green synthesis. *Helichrysum* sp. extract was used as a bioreducing and stabilizing reagent. According to the results of the optimization studies, 1 mL of 1:2 diluted *Helichrysum* sp. extract was added to 2 mL of 5.0 mM AgNO<sub>3</sub> for the synthesis of HE-SNPs. The mixture solution was kept at room temperature for 40 minutes and light-yellow nanoparticles were obtained.

## 2.5. Characterization of Synthesized HE-SNPs

In the optimization and characterization of HE-SNPs, absorption spectras of silver nanoparticle solutions synthesized in the presence of silver ions and Helichrysum sp. extract were obtained by L7 Dual Beam UV-Vis spectrophotometer. The particle size distribution of the synthesized HE-SNPs was determined by Anton-Paar Litesizer 500 analyzer. Synthesized HE-SNPs were imaged using wet-STEM. The chemical properties of HE-SNPs synthesized in the presence of the *Helichrysum* sp. extract were investigated by FTIR spectroscopy. Thermogravimetric analysis (TGA) of HE-SNPs was conducted.

## 2.6. Antioxidant Properties of Synthesized HE-SNPs

#### 2.6.1. CUPRAC method

The total antioxidant capacity of HE-SNPs was determined by the CUPRAC method (Apak et al., 2004). In this method, CUPRAC reagent consists of 1 mL of 10 mM CuCl<sub>2</sub>, 1 mL of 7.5 mM Nc, and 1 mL of 1.0 M NH<sub>4</sub>Ac. 0.6 mL of HE-SNP solution and 0.5 mL distilled water were added to this reagent mixture. The absorbance of the nanoparticle solution was recorded at 450 nm against a reagent blank after 30 min. The TAC was expressed as a Trolox equivalent (mmol TR/g-dried sample (DS)) based on the standard curve of TR standard.

## 2.6.2. DPPH assay

The radical scavenging activity (RSA) of HE-SNPs on DPPH free radicals was determined using the DPPH assay (Sánchez-Moreno et al., 1998). In this method, 2 mL of HE-SNP solution was added to 2 mL of 0.2 mM DPPH solution in a test tube. The tubes were shaken, and kept in the dark for 30 min. The absorbance of HE-SNP solution at 515 nm was recorded against methanol.

The absorbance values were used to calculate the RSA of the HE-SNPs. It was calculated from the following Equation (1):

$$\Delta A = A_{DPPH} - (A_s - A_0) \tag{1}$$

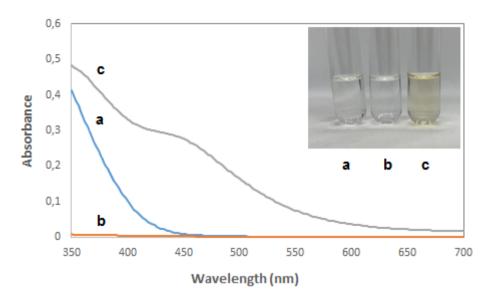
where  $\Delta A$  gives the corrected absorbance of the HE-SNPs,  $A_{DPPH}$  indicates the absorbance of DPPH in the absence of the HE-SNPs,  $A_s$  indicates the absorbance of the HE-SNPs with DPPH,  $A_0$  indicates the absorbance of the HE-SNPs without DPPH.

#### 2.7. Statistical Analysis

Mean values, their standard deviations and relative standard deviations were obtained using Excel software (Microsoft Office 365).

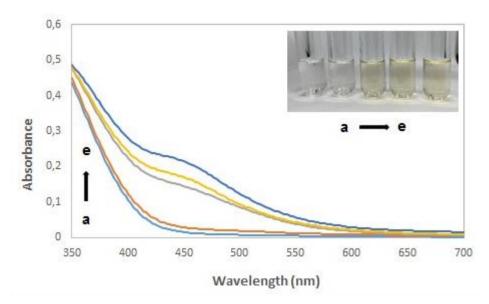
#### 3. Results and Discussion

In the green synthesis of silver nanoparticles, the phenolic groups (Ar-OH) present in the *Helichrysum* sp. extract are oxidized to the corresponding quinones, while Ag<sup>+</sup> ions are reduced to Ag<sup>0</sup>. Thus, light yellow HE-SNPs are produced, showing maximum plasmon absorption at 440 nm.



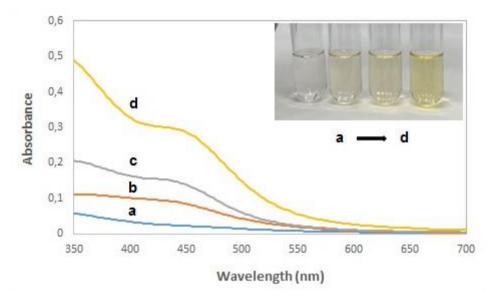
**Figure 1.** UV–Vis spectra of **a**) *Helichrysum* sp. extract (alone), **b**) AgNO<sub>3</sub> (alone), and **c**) HE-SNPs synthesized using *Helichrysum* sp. extract

Figure 1 shows the absorption spectra of HE-SNPs the synthesized using *Helichrysum* sp. extract, AgNO<sub>3</sub> alone, and *Helichrysum* sp. extract alone. Figure 1a is the absorption spectrum of *Helichrysum* sp. extract. Figure 1b is the absorption spectrum of AgNO<sub>3</sub> (2.5 mM) solution. Figure 1c shows the absorption spectrum of silver nanoparticles synthesized in the presence of *Helichrysum* sp. extract (1:2 diluted). The synthesized silver nanoparticles are light yellow and give maximum absorption at a wavelength of 440 nm. Silver nanoparticles have the surface plasmon resonance (SPR) band in the proximity of 420 nm in the visible region (Amendola et al. 2017). As seen in Figure 1a, and 1b, *Helichrysum* sp. extract (1:2 diluted) and AgNO<sub>3</sub> at 2.5 mM initial concentration did not give any absorption at 440 nm.



**Figure 2.** UV-Vis spectra of HE-SNPs with various concentrations of Ag<sup>+</sup> (initial concentration): **a)** 1.0 mM, **b)** 2.5 mM, **c)** 5.0 mM **d)** 7.5 mM, and **e)** 10.0 mM

Figure 2 gives the effect of Ag<sup>+</sup> ion concentration on silver nanoparticle production using *Helichrysum* sp. extract. The optimization study of concentration of Ag<sup>+</sup> ion was performed in the presence 1:2 (v:v) diluted *Helichrysum* sp. extract within the concentration range 1.0–10.0 mM of AgNO<sub>3</sub> for 60 min at room temperature. As shown in Figure 2a and 2b, no silver nanoparticle formation was observed in the presence of 1.0 mM and 2.5 mM AgNO<sub>3</sub> concentration. In the concentration range of 5.0-10.0 mM AgNO<sub>3</sub>, silver nanoparticle formation with increasing yellow color was observed with increasing concentration. The light-yellow silver nanoparticles were obtained (Figure 2c-2e). Hence, the optimal concentration of AgNO<sub>3</sub> was determined to be 5.0 mM.



**Figure 3.** UV-Vis spectra of HE-SNPs with varying dilutions of *Helichrysum* sp. extract (initial concentration): **a)** 1:20 diluted, **b)** 1:10 diluted, **c)** 1:5 diluted, and **d)** 1:2 diluted

The effect of *Helichrysum* sp. extracts at different dilutions on silver nanoparticle synthesis by biogenic reduction is presented in Figure 3. The optimization study of concentration *of Helichrysum* sp. extract was carried out in the presence of *Helichrysum* sp. extract diluted 1:2, 1:5, 1:10, and 1:20 (v:v) and 5.0 mM Ag<sup>+</sup> ion concentration for 60 min incubation at room temperature. In the presence of 1:20 diluted *Helichrysum* sp. extract, no silver nanoparticles were formed. In the presence of 1:10 and 1:5 dilution ratios, respectively, seeds of silver nanoparticles were formed. However, a distinct maximum absorption was observed at 440 nm in the diluted 1:2 *Helichrysum* sp. extract medium. Accordingly, the *Helichrysum* sp. extract concentration was determined as 1:2 diluted *Helichrysum* sp. extract.

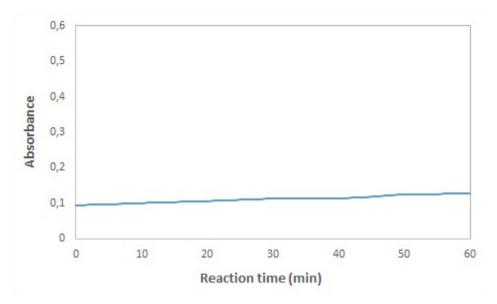


Figure 4. Reaction kinetic of HE-SNPs synthesized using *Helichrysum* sp. extract

Figure 4 shows the reaction kinetic of silver nanoparticles synthesized using *Helichrysum* sp. extract at room temperature for 60 minutes. A rapid increase in SPR absorbance was observed within the first few minutes. The increase in surface plasmon absorbance of HE-SNPs continued for 30 minutes. The absorbance stabilized after 30 minutes. Therefore, the optimum reaction time for synthesis HE-SNPs was determined as 40 min. In a study conducted with *Helichrysum arenarium*, the extract was prepared in 24 hours in the first stage and silver nanoparticles were synthesized by continuous shaking in the second stage (Kahraman, 2024a). However, in the proposed method, silver nanoparticles were prepared in 40 minutes. In this context, the proposed method is a simple and fast method that does not require additional processing.

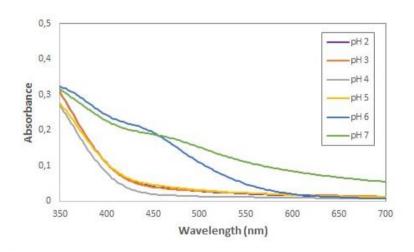


Figure 5. UV–Vis spectra of HE-SNPs formed in the range of pH 2.0–7.0

The effect of pH on the HE-SNPs formed with *Helichrysum* sp. extract was studied in the range pH 2.0–9.0 under the same experimental conditions (the mixture containing 5.0 mM Ag<sup>+</sup> and diluted 1:2 (v:v) *Helichrysum* sp. extract). Observable from Figure 5, HE-SNPs were not formed at pH 2.0-5.0. HE-SNPs

were formed at pH 6.0, and had maximum absorption at 440 nm. The HE-SNP solution formed at pH 7 was slightly turbid. Therefore, it had a spread absorption spectrum, and the maximum absorption wavelength was 465 nm. Silver nanoparticles have an absorption maximum at approximately 400 nm, and aggregation of metal nanoparticles causes a shift in plasmon absorption toward longer wavelengths (i.e., redshift) (Moores and Goettmann, 2006; Siegel et al., 2012). At pH 8.0 and 9.0, Ag<sup>+</sup> ions precipitated. Undesired oxidation of Ag<sup>+</sup> to Ag<sub>2</sub>O is most likely at these pHs. Consequently, pH 6.0 was selected as the most suitable pH for the formation of HE-SNPs. Similarly, the appropriate pH value was found to be 5 in the study with *Helichrysum arenarium* (Kahraman, 2024a). However, in another study, silver nanoparticles could be synthesized at pH 8 in the presence of *Helichrysum arenarium* (Ozdemir et al., 2023).

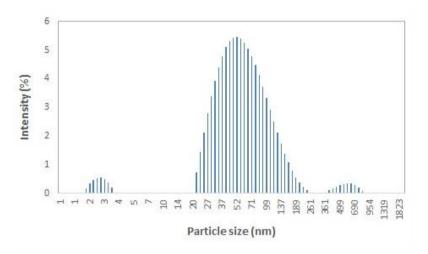
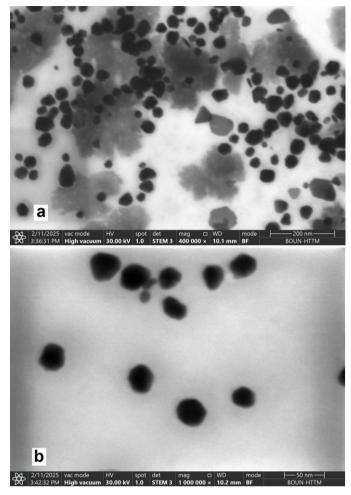


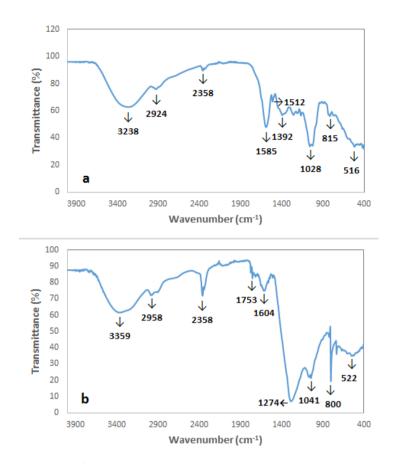
Figure 6. Particle size distribution of the HE-SNPs synthesized using *Helichrysum* sp. extract

The particle size distribution diagram of silver nanoparticles synthesized using *Helichrysum* sp. extract was given in Figure 6. According to dynamic light scattering (DLS) analysis of the HE-SNPs, hydrodynamic diameter was measured to be 54 nm. Polydispersity index (PI) of the HE-SNPs synthesized was determined to be 0.25. The polydispersity index varies depending on the size of the sample and PI is a measure of the heterogeneity of the sample (Mudalige et al., 2019). The value of PI below 0.200 indicates monodisperse nanoparticles, indicating homogeneity (Ledet et al., 2013).



**Figure 7.** The wet-STEM images of the HE-SNPs synthesized using *Helichrysum* sp. extract at varying magnifications a) 200 nm, b) 50 nm

Figure 7 shows wet STEM images at varying magnifications of silver nanoparticles synthesized using *Helichrysum* sp. extract. According to these images, HE-SNPs synthesized by bioreducing are spherical, monodisperse and homogeneous. The spherical silver nanoparticles give intense surface plasmon absorption bands around 400 nm in the visible region (Liz-Marzán, 2006). In this context, the STEM images are supported by the UV visible region spectra (λmax: 440 nm) of the synthesized HE-SNPs. According to the STEM results, the average size of the synthesized HE-SNPs is 31 nm. However, there are nanoparticles larger and smaller than 31 nm. According to DLS results, the % area density of HE-SNPs with an average size of 54 nm was found to be 94.7%. In this respect, wet-STEM images are consistent with particle size distribution analysis results.



**Figure 8.** FTIR spectra of **a**) dried *Helichrysum* sp. extract and **b**) HE-SNPs synthesized using *Helichrysum* sp. extract

Figure 8a represents the FTIR spectra of dried *Helichrysum* sp. extract. The hydroxyl (- OH) groups have an absorption band of 3291 cm<sup>-1</sup> due to vibrational stretching. The absorption band at 2924 cm<sup>-1</sup> corresponds to aromatic C – H stretching vibration. The absorption band at 1585 cm<sup>-1</sup> is due to amine (-NH) stretching vibration. The absorption band at 1512 cm<sup>-1</sup> is assigned to the C = C stretching vibration. The C-N stretching vibration of aromatic and aliphatic amines is responsible for the absorption peak at 1392 cm<sup>-1</sup>. The absorption band at 1028 cm<sup>-1</sup> is assigned to the O – H bond and the C – OH stretch in phenolic groups. Aromatic hydroxyl (– OH) groups, C – OH groups, C–N stretching vibration of aromatic and aliphatic amines, and amine (- NH) stretching vibrations are mainly responsible for the reduction of Ag (I) ion to Ag (0). Figure 8b shows the FTIR spectra of the dried HE-SNPs. When the FTIR spectra of HE-SNPs was compared with the FTIR spectra of HE, it was observed that some of the functional groups present in *Helichrysum* sp. extract disappeared. Further, carbonyl C = O double-bond stretching with an absorption band at 1753 cm<sup>-1</sup> indicated that the hydroxyl groups in the phenolic and flavonide compounds of the Helichrysum sp. extract were oxidized to the relevant quinones during the formation of HE-SNPs. Similarly, the FTIR spectra of silver nanoparticle synthesis with H. arenarium showed that most of the functional groups were disappeared or there were large shifts in the positions of the existing peaks and it was stated that the reduction and stabilization of silver nanoparticles depend on aromatic carbonyl, amide, flavanone, protein, ether and terpenoid components (Ozdemir et al., 2023).

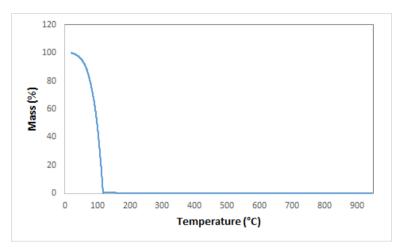


Figure 9. TGA thermogram of the HE-SNP synthesized using *Helichrysum* sp. extract

Figure 9 illustrates the TGA curve of the HE-SNP synthesized by *Helichrysum* sp. extract. It is seen that a thermal mass removal resulting in a temperature of 117 °C removes 86% of the total mass and the remaining mass at the end of 950 °C is 0.1%. It is observed that there is a gradual mass decrease of 0.28% in the range of 117-950 °C. The mass loss occurs in a single and sharp step. These decreases in the mass can be attributed to the loss of absorbed water on the HE-SNPs (De Canha et al., 2021; Kahraman, 2024b).

**Table 1.** Antioxidant properties of the HE-SNPs synthesized using *Helichrysum* sp. extract

	TAC for CUPRAC (mmol TR/g-DS)	RSA for DPPH (mmol TR/g-DS)
HE-SNPs	$0.024 \pm 0.001$	0.005±0.001
HE	$0.027 \pm 0.001$	$0.018 \pm 0.001$
(N=3)		

The antioxidant capacity/activity values of HE-SNPs synthesized in the presence of *Helichrysum* sp. extract are presented in Table 1. The TAC values of the synthesized HE-SNPs and HE were found to be 0.024 and 0.027 mmol-TR according to the CUPRAC method, respectively. The RSA values of the synthesized HE-SNPs and HE were found to be 0.005 and 0.018 mmol-TR according to the DPPH assay, respectively. In the present work, the antioxidant capacity/activity of HE-SNPs can be attributed to the high phenolic and flavonoid content in *Helichrysum* sp. The antioxidant capabilities of silver nanoparticles are mostly influenced by the chemical composition of the plant extract and improve with increasing silver nanoparticle concentration. The nanoparticles synthesized with plant extracts abundant in phytochemicals show high scavenging activity (Bedlovičová et al., 2020). As expected, HE-SNPs exhibit lower total antioxidant capacity and activity compared to HE. The hydroxyl groups of flavonoids and phenolic compounds in *Helichrysum* sp. extract reduce Ag<sup>+</sup> ions to Ag<sup>0</sup>, while they themselves are oxidized to their respective quinones, thus; silver nanoparticles are produced. Since the number of phenolic compounds present in *Helichrysum* sp. extract decreases during the synthesis of HE-SNPs, the antioxidant capacity/activity value decreases. This is evidence that silver nanoparticles are produced in

the proposed green synthesis method. Similarly, the reduced levels of phenolic and flavonoid measured in the nanoparticles compared to the plant extract are shown as evidence for nanoparticle production (Phull et al., 2016).

#### 4. Conclusion

In this study, a reliable and green silver nanoparticle synthesis method that can be used in many application areas was proposed. *Helichrysum* sp. extract, which was selected as a natural source, was used as both a bioreducing and a stabilizing agent in the proposed method. Distilled water was used as a solvent in the synthesis of silver nanoparticles with *Helichrysum* sp. extract. Thus, the basic principles of green synthesis, such as reducing the consumption of harmful solvents and using renewable natural resources, have been fulfilled. The proposed method is as effective as traditional synthesis methods. In fact, the disadvantage that biological reductants are weaker reductants than chemical reductants have also turned into an advantage. Therefore, green synthesis allowed the synthesis of nanoparticles in a monolayer and homogeneously without aggregation. Thanks to the proposed extraction process of *Helichrysum* sp., an extract rich in polyphenolic compounds was obtained. In this way, silver nanoparticles were synthesized quickly at room temperature without the need for an additional process such as heating in the production of nanoparticles. HE-SNPs showed positive results in terms of total antioxidant capacity and radical scavenging activity. Characterization findings demonstrated the sustainable production of pure nanoparticles below 100 nm that exhibit antioxidant properties using an environmentally friendly process for various applications.

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**Conflict of interest** The author declares no conflict of interest.

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